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Hypoglycaemic Effectivity of *Brucea javanica* (L) Merr Seed Methanol Extract, Leaf Methanol Extract and Leaf Infusion on Alloxan-induced Diabetic Rats

Candra Dwipayana Hamdin[1,3], Handa Muliasari[1], Muhsinul Ihsan[2], Eka Sunarwidhi Prasedya[3*] and Sunarpi[3]

[1] *Department of Pharmacy, Faculty of Medicine, University of Mataram, Jalan Majapahit 62, Mataram, West Nusa Tenggara 83125, Indonesia*

[2] *Biology Department, Faculty of Education and Teacher Training, Universitas Islam Negeri Mataram, Jalan Pendidikan 35, Mataram, West Nusa Tenggara 81325, Indonesia*

[3] *Bioscience and Biotechnology Research Center, Faculty of Mathematics and Natural Sciences, University of Mataram, Jalan Majapahit 62, Mataram, West Nusa Tenggara 83125, Indonesia*

Brucea javanica (L.) Merr is one of the plants that has been widely used as a traditional medicine for treating diabetes by the local communities in West Nusa Tenggara, Indonesia. However, effectivity of the empirical dose of *B. javanica* seeds and leaves consumed for treating diabetes has never been scientifically validated. Therefore, the aim of this research was to evaluate the hypoglycaemic effectivity of the *B. javanica* seed methanol extract (SME), leaf methanol extract (LME) and leaf infusion (LI) at the same animal-equivalent dose converted from the empirical human dose on alloxan-induced diabetic rats. The rats were administered with SME and LME at a dose of 50 mg/kg body weight (BW), and 5% LI at 10 mL/kg BW. Glibenclamide was used as a reference drug at 0.25 mg/kg BW. The treatments were performed once daily for 15 d. The results showed that different preparations of *B. javanica* seeds and leaves at a dose converted from human use to animal use produced different hypoglycaemic effects without altering the body weight of diabetic animals. Treatment with SME resulted in extreme hypoglycaemia which might have caused the death of test animals; LME did not reduce the fasting blood glucose (FBG) level significantly but the treatment helped with the recovery of pancreatic tissue. The 5% LI treatment was effective both in reducing the FBG level in a constant and gradual manner, and promoting the recovery of damaged pancreatic tissue.

Keywords: hypoglycaemic; *Brucea javanica*; diabetic rats; histology; pancreas

I. INTRODUCTION

Diabetes mellitus (DM) is a metabolism disorder that has become a global and serious disease with an annual mortality rate of about 5.1 million people (Eid & Haddad, 2014). According to the International Diabetes Federation, approximately 285 million people suffered from DM worldwide and this number was estimated to increase to 438 million in 2030 (Chackrewarthy & Thabrew, 2012). The current treatment options for DM include synthetic drugs and insulin therapy, both of which are relatively expensive and can cause various side effects. Therefore, exploration of natural antidiabetic agents from plants is important to develop alternative treatments that are cheaper, simpler and have fewer side effects, as natural products were reported to be safer for use than synthetic drugs (Hamdin *et al.*, 2017).

About 400 plants and compounds are known to have antidiabetic activities *in vivo* or *in vitro* (Chang *et al.*, 2013). One of the plants known to have high antidiabetic

*Corresponding author's e-mail: ekasprasedya@unram.ac.id

activity on Lombok Island is *Brucea javanica* (L.) Merr, which is locally called *makasar* or *wali*. It belongs to the family Simaroubaceae, and is known to have anticancer, antitumor and antidiabetic activities (Ablat *et. al*., 2014; Muliasari *et al*., 2017). Local communities in the Sesaot village (Narmada, West Lombok, West Nusa Tenggara, Indonesia) have long believe that *B. javanica* is effective in treating diseases such as hypercholesterol, hypertension, fever, especially DM. The seeds and leaves of *B. javanica* have been widely used as an antidiabetic drug that significantly reduced the blood glucose level of people who suffer from DM. Empirically, the local communities treat DM using 5–10 dried seeds of *B. javanica* (Muliasari *et al*., 2017). However, effectivity of the empirical doses they use has not been validated by any scientific study.

This study aimed to evaluate the hypoglycaemic effectivity of several preparations of *B. javanica* seeds and leaves on animal model administered at an animal-equivalent dose converted from the empirical human dose established from the villagers' experience in treating diabetes. Although many studies had been conducted on the hypoglycaemic effect of *B. javanica*, our study remains relevant in providing scientific validation to the empirical dose of *B. javanica* seeds used by the local communities on Lombok Island for treating diabetes. We had previously assessed the hypoglycaemic effect of *B. javanica* leaf infusion prepared by steeping the leaves in hot water on the induced-diabetic rats and confirmed that the leaves of *B. javanica* also possess antidiabetic activity (Muliasari *et al*., 2017). As a continuation of the previous research, the hypoglycaemic effects of methanol extracts prepared from *B. javanica* seeds and leaves, as well as the leaf infusion of *B. javanica* on the alloxan-induced diabetic rats were compared and evaluated. An alloxan-induced diabetic animal model was used to evaluate the antidiabetic property of several preparations of *B. javanica* seeds and leaves in this study. This model mimics insulin-dependent diabetes or type 1 DM by causing partial degradation of the pancreatic β cells and subsequent compromised insulin production by the cells (Ighodaro *et al*., 2017). The methanol extracts and leaf infusion of *B. javanica* were expected to reduce the fasting blood glucose of diabetic rats to the normal range; while findings from this study will serve as the baseline data for developing natural antidiabetic product from *B. javanica* in the form of oral pharmaceuticals.

II. MATERIALS AND METHODS

A. Plants and Animals

Plant materials were collected from the cultivated plants at Sesaot village, West Lombok, West Nusa Tenggara, Indonesia (GPS coordinates -8.478723, 116.271566). Prior to sample preparation, the plant was authenticated based on the morphological attributes of the leaves (shape, texture and colour) by the Biology Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram on a voucher specimen numbered 03/HM/Makasar.

Male Wistar rats (*Rattus norvegicus*) of 2–3 months old, weighing between 150 g and 200 g purchased from the Pharmacology Laboratory of Udayana University, Bali, Indonesia were used in this study. All animals were maintained in a standard ventilated room at 20–23°C and 12-h light–dark cycles, with free access to tap water and standard feed pellet. This study was approved by the animal ethics committee of the Medical Faculty, University of Mataram.

B. Preparation of Extract and Infusion

The seeds and leaves of *B. javanica* were air-dried until constant weight was attained for use in preparing methanol extracts and leaf infusion. Methanol extraction was performed separately for the seeds and leaves. The air-dried samples were crushed into small pieces and suspended in 2500 mL 96% methanol (1 g in 5 mL methanol) for 24 h, and the suspension was filtered using general purpose filter paper. The filtrate was collected and stored at room temperature, while the solid residue was soaked in fresh 96% methanol of the same volume for subsequent extraction. The extraction cycle was repeated twice every 24 h. Filtrate collected from 3 cycles of extraction was pooled and dried at 50°C using rotary evaporator. Residual pellet which constituted the crude methanol extract was stored at 4°C until use. Leaf infusion was prepared by steeping 5 g of *B. javanica* leaves in 100 mL of water at 95°C for 15 min, and the volume was made up to 100 mL with distilled water to obtain a 5% leaf infusion. The crude extracts of *B. javanica* seeds [seed methanol extract (SME)] and leaves [leaf methanol extract (LME)], as well as the leaf infusion were then subjected to phytochemical analyses and used for antidiabetic assay.

C. Phytochemical Studies

Qualitative analyses of the methanol extracts and leaf infusion were carried out for the following phytochemical groups: alkaloids, flavonoids, terpenoids, steroids, tannins and saponins (Trease & Evans, 2002). Each test was conducted using 1 mg of crude methanol extracts prepared from *B. javanica* seeds and leaves.

To test for the presence of alkaloids, crude methanol extract was mixed with 2 mL of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity from the resulting precipitate was taken as evidence for the presence of alkaloids. Shinoda test was conducted to check for the presence of flavonoids. The sample was mixed with a few fragments of magnesium ribbon and concentrated HCl was added dropwise. The presence of flavonoids was indicated by the appearance of a pink scarlet colouration after a few min. The alkaloids and flavonoids tests for leaf infusion were carried out in the same way using 3 mL of sample.

The terpenoids test was conducted using 1 mg of crude extract which was dissolved in 2 mL of chloroform and then evaporated to dryness. To this, 2 mL of concentrated H_2SO_4 was added and the solution was heated for about 2 min. A greyish colour indicated the presence of terpenoids. For leaf infusion, the terpenoids test was carried out using 5 mL of sample to which 5 mL of 95% ethanol was added and heated for 30 s at 90°C, followed by the addition of 1 mL of each anhydrous H_2SO_4 and concentrated H_2SO_4. The blue precipitation confirmed the presence of terpenoids.

Both Salkowski and Liebermann-Burchard tests were conducted for determining the presence of steroids. For the Salkowski test where 1 mg of crude extract was dissolved in 2 mL of chloroform and concentrated H_2SO_4 was added sidewise, a red colour produced in the lower chloroform layer indicated the presence of steroids. The Liebermann-Burchard test confirmed the presence of steroids by the development of a greenish colouration upon the addition of 2 mL of each concentrated H_2SO_4 and acetic acid to 1 mg of extract dissolved in 2 mL of chloroform. For leaf infusion, the steroids test was carried out using 3 mL of sample added to 1 mL of each anhydrous H_2SO_4 and concentrated H_2SO_4. The blue-green precipitate confirmed the presence of steroids.

Tannins test was carried out by adding 1 mg of methanol extract or 3 mL of leaf infusion to 2 mL of 96% methanol and 1 mL of $FeCl_3$. The formation of brown precipitate confirmed the presence of tannins. The presence of saponins was tested by mixing *B. javanica* crude extract or 3 mL of leaf infusion with 5 mL of distilled water in a test tube and shaking the tube vigorously. The formation of stable foam was taken as an indication of the presence of saponins.

D. Antidiabetic Assay

Twenty-four test animals were weighed and randomly divided into six groups: positive control, negative control, normal control, seed methanol extract group (SME), leaf methanol extract group (LME) and leaf infusion group (LI). Except for the normal control, diabetes was induced in rats used in all control and treatment groups by intravenous administration of alloxan at a dose of 125 mg/kg body weight (BW). Antidiabetic assay began 3 d after alloxan administration when diabetes was confirmed by a fasting blood glucose (FBG) level above 135 mg/dL. Treatments were performed once daily for 15 d. Diabetic rats in the positive control group were treated with glibenclamide at a dose of 0.25 mg/kg BW; SME group was treated with seed methanol extract at a dose of 50 mg/kg BW; LME group was treated with leaf methanol extract at a dose of 50 mg/kg BW; LI group was treated with 5% leaf infusion at a feed volume of 1% of BW or 10 mL/kg BW. Rats in both negative and normal control groups were untreated. Body weight of the rats was measured at days 1, 5, 10 and 15; blood glucose level of the fasting animals for all treatments was measured every 3 d using GlucoDr™ Test Meter (AGM-2100, Korea). Readings taken at day 1 were considered as the baseline for each treatment group; body weight and FBG were compared to the baseline readings.

E. Histological Observation on Pancreatic Tissue

Histological observation on pancreatic tissue was conducted at the end of the experiment at day 15. Pancreas isolated from the test animals euthanised with chloroform inhalation was cleaned with normal saline, fixed in 10% formalin and then dehydrated in increasing alcohol series using 70, 80, 95 and 100% ethanol for paraffin embedding. The paraffin blocks were then cut into 5 µm

sections and mounted on slides. The slides were allowed to air-dry for 30 min and then dried overnight in an oven at 50°C. The sections were deparaffinised in xylene twice, followed by rehydration in decreasing alcohol series using 100, 95, 80 and 70% ethanol, and washing in water before the sections were stained with haematoxylin followed by eosin. The stained slides were then dehydrated in increasing alcohol series as mentioned earlier and left to dry in the hood overnight, before they were ready for microscopic observation.

F. Statistical Analysis

Changes of the body weight and FBG level from the baseline in each treatment were analysed using ANOVA followed by LSD test. The statistical analyses were performed using SPSS-20 program at a significance level of 0.05 for all tests.

III. RESULTS

A. Phytochemical Analysis of B. javanica *Extracts*

The presence of alkaloids, flavonoids, terpenoids, steroids, tannins and saponins in the preparations of *B. javanica* seeds and leaves was determined. The analyses showed that SME, LME and LI contained different types of chemical compounds (Table 1). SME consisted of all tested groups of compounds, except for terpenoids; LI also consisted of all of phytochemical groups tested but alkaloids. LME contained only alkaloids, terpenoids and tannins.

B. Effect of B. javanica *Extracts on Body Weight*

Weight measurements for the rats were taken every 5 d to monitor the body weight variation in each treatment group. The body weight profile of the rats over 15 d of treatment was presented in Table 2. Body weight loss is a sign of diabetes and this situation is expected to be reversed if an antidiabetic treatment is effective. The results showed that treatments with

Table 1. Qualitative determination of chemical constituents of the seed methanol extract (SME), leaf methanol extract (LME) and leaf infusion (LI) of *Brucea javanica*.

Chemical constituent	**SME**	**LME**	**LI**
Alkaloids	+	+	-
Flavonoids	+	-	+
Terpenoids	-	+	+
Steroids	+	-	+
Tannins	+	+	+
Saponins	+	-	+

(+): presence of component; (-): absence of component

different preparations of *B. javanica* seeds and leaves were able to maintain stable body weight of the rats within the normal range between 150 g and 250 g. Body weight of the rats throughout the treatment in each group was not significantly different from the baseline at day 1 ($p > 0.05$). Interestingly, the negative control group was expected to see a decrease in body weight, but the rats showed body weight within the normal range (150–250 g).

C. Hypoglycaemic Effect of B. javanica

Table 3 and Figure 1 summarised the FBG concentration of the rats, and the reduction of FBG from the baseline every 3 d during treatment. Except for the SME group, the FBG concentration in all groups fluctuated throughout the treatment period (Table 3). Diabetic rats in all control and treatment groups showed a general of FBG reduction from

Table 2. Body weight of alloxan-induced diabetic rats treated with methanol extracts and leaf infusion of *Brucea javanica* for 15 d against the positive, negative and normal controls (mean ± SE, n = 4).

Group	Body weight (g)			
	Day 1	Day 5	Day 10	Day 15
C+	170.5 ± 7.3	166.0 ± 9.3	169.8 ± 9.4	165.0 ± 8.8
C-	158.5 ± 15.1	152.5 ± 14.6	163.5 ± 15.6	163.8 ± 15.0
N	191.0 ± 4.0	197.0 ± 4.8	201.0 ± 4.6	206.8 ± 5.0
SME	171.0 ±12.2	164.0 ± 10.7	157.3 ± 8.6	n.d.
LME	157.0 ± 4.7	157.8 ± 5.2	169.3 ± 4.4	162.8 ± 5.8
LI	192.8 ± 18.6	201.8 ± 16.0	207.3 ± 19.7	207.0 ±19.2

C+: Positive control; C-: negative control; N: normal control; SME: seed methanol extract; LME: leaf methanol extract; LI: leaf infusion; n.d.: not determined

Table 3. Fasting blood glucose level of alloxan-induced diabetic rats treated with methanol extracts and leaf infusion of *Brucea javanica* for 15 d against the positive, negative and normal controls (mean ± SE, n = 4).

Group	Mean fasting blood glucose concentration (mg/dL)					
	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
C+	443.50 ± 37.85	373.50 ± 34.89	358.25 ± 102.61	284.50 ± 95.62	420.75 ± 78.87	345.75 ± 52.36
C-	382.25 ± 52.70	186.50 ± 58.49	304.50 ± 67.07	303.50 ± 54.03	386.00 ± 84.64	283.75 ± 75.99
N	80.25 ± 5.40	84.50 ± 2.63	69.00 ± 5.73	79.00 ± 13.53	89.75 ± 2.06	80.00 ± 2.38
SME	380.50 ± 17.51	131.75 ± 6.79*	86.75 ± 2.68*	81.25 ± 2.06*	n.d.	n.d.
LME	280.25 ± 36.39	280.50 ± 69.44	263.50 ± 104.61	216.50 ± 94.62	218.75 ± 89.91	217.75 ± 71.31
LI	434.00 ± 99.44	172.50 ± 94.79*	220.50 ± 48.01*	278.00 ± 72.30*	259.75 ± 73.41*	215.79 ± 32.19*

Asterisk indicates treatment that shows significant difference in the fasting blood glucose level compared to the baseline at day 1 ($p < 0.05$). C+: Positive control; C-: negative control; N: normal control; SME: seed methanol extract; LME: leaf methanol extract; LI: leaf infusion; n.d.: not determined.

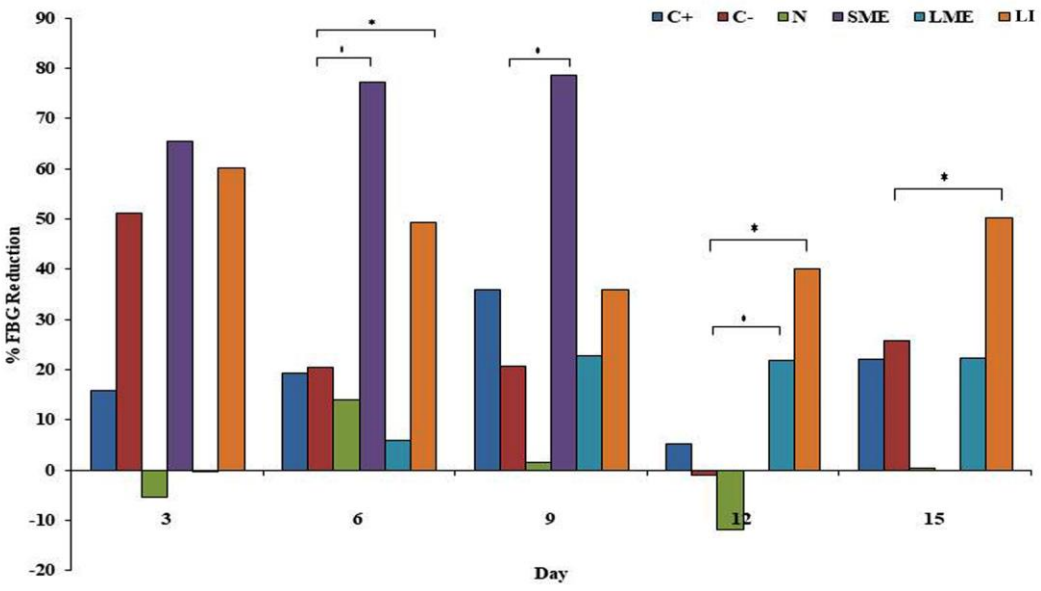

Figure 1. Hypoglycaemic effect indicated as the percentage of fasting blood glucose (FBG) reduction from the baseline (FBG at day 1) in each group during the 15-day treatment. Rats treated with glibenclamide at a dose of 0.25 mg/kg BW in the positive control (C+) only experienced low level of FBG reduction. Negative control (C-) showed a low level of FBG reduction. The non-diabetic normal control (N) showed fluctuation in FBG reduction. Rats treated with the seed methanol extract (SME) and leaf infusion (LI), but not leaf methanol extract (LME), experienced significant reduction in the FBG level compared to the negative control (C-). Asterisk indicates treatment with significant hypoglycaemic effect compared to the negative control.

the baseline throughout the treatment period (Figure 1). The negative control showed a low level of FBG reduction, except for day 3 where a reduction up to 50% was observed, with the FBG concentration still within the range expected of a diabetic control. FBG concentration of the non-diabetic control fluctuated within the normal range. Effectiveness of treatment with the standard drug glibenclamide at a dose of 0.25 mg/kg BW in reducing the FBG level was not significant compared to the negative control. The FBG reduction in the positive control barely exceeded 25%, except on day 9 which saw a larger reduction in FBG level to about 36% (Figure 1). A significant decrease in FBG level up to more than 75% from the baseline ($p < 0.05$) was observed in the SME group from day 3 until day 9 (Figure 1). The unexpected death of test animals in the SME group at day 11 was associated with the extreme hypoglycaemic effect of the seed methanol extract. On the other hand, the LI group generally showed a regular gradual FBG reduction that was significantly different from that in the negative control. The LME group experienced a slight increase in FBG concentration at day 3, but in general the treatment brought about relatively low level of FBG reduction from the baseline compared to treatments with other *B. javanica* preparations. Although the standard drug glibenclamide and all preparations of *B. javanica* resulted in a reduction in FBG from the baseline at the end of the experiment ($p < 0.05$), these treatments did not reduce the FBG concentration to the normal range as seen in the non-diabetic normal control throughout the treatment period.

C. Histological Observations of Pancreatic Tissue

Histological observations of the pancreatic tissue isolated from the rats after treatment were shown in Figure 2. In the positive control (C+), damage in the islets of Langerhans was reduced and limited to pyknosis, the irreversible condensation of chromatin in the nuclei. More advanced damage in islets of Langerhans in the negative control (C-) was observed based on the presence of oedema and karyorrhexis. In the LI group, the islets of Langerhans were mostly recovered. Similarly, the LME group also showed the islets of Langerhans with normal morphology. Histological observations of the rat pancreas indicated that the pancreatic cells had recovered from the damage induced by alloxan following 15 d of treatment with LME and LI in diabetic rats. Histological study was not carried out on the SME group, as all rats in the group were dead before the end of treatment.

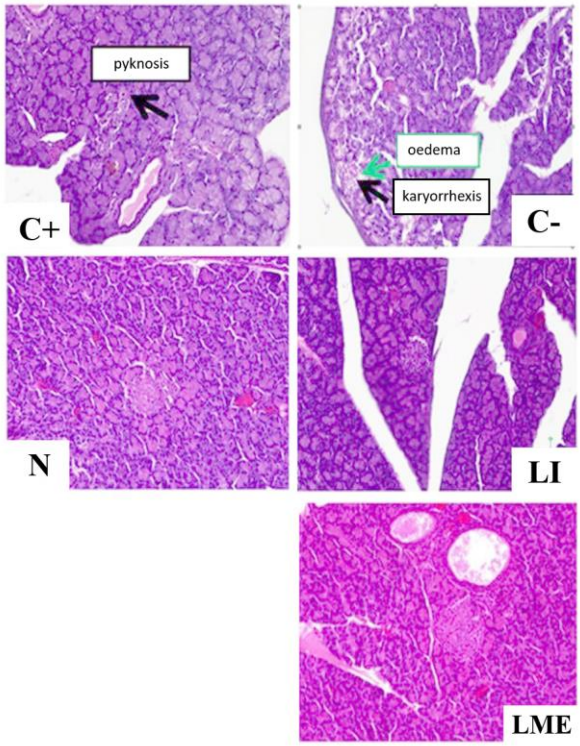

Figure 2. Histological observations of rat pancreas after treatments with *Brucea javanica* leaf methanol extract and leaf infusion for 15 d against the positive, negative and normal controls. Positive control (C+) showed slight cell damage in the form of pyknosis in tissue, while negative control (C-) showed more severe tissue damage as oedema and karyorrhexis. Otherwise, the normal group (N), leaf infusion (LI) group and leaf methanol extract (LME) group all showed morphology of normal pancreatic tissue. Samples were observed under 100× objective magnification.

IV. DISCUSSION

Phytochemical analyses on plant extracts are commonly conducted as the first step in bioactivity screening to qualitatively and/or quantitatively determine the chemical compounds responsible for their biological properties (Ablat *et al.*, 2014). The different hypoglycaemic effect of different preparations of *B. javanica* seeds and leaves fed to the alloxan-induced diabetic rats, and thus the effectivity of each preparation as an antidiabetic agent, was due to the different compounds extracted in each preparation. The extreme reduction of FBG in the rats

treated with SME might be attributed to the rather complete range of phytochemical compounds including alkaloids, flavonoids, steroids, tannins and saponins, in the extract. Methanol extracts of *B. javanica* seeds and leaves were shown to have different phytochemical components, with LME found to contain only alkaloids, terpenoids and tannins. Interestingly, LI derived from steeping the leaves in hot water at 95°C contained more phytochemical compounds.

The hypoglycaemic effect of different preparations of *B. javanica* seeds and leaves was manifested only as significant changes in FBG concentration, but not the body weight of diabetic rats throughout the treatment. Based on our results, the dose of SME applied at 50 mg/kg BW might be too high and resulted in the death of diabetic animals on day 11 of treatment, likely caused by the uncontrolled hypoglycaemic effect of SME. This is evident by the extreme reduction of FBG up to more than 75% from the baseline throughout the treatment as shown in Figure 1. Meanwhile, further investigation is needed to establish the effective dose of SME. Bruceines E and D are two compounds in *B. javanica* seeds that were found to significantly reduce blood glucose concentration in diabetic rats (NoorShahida *et al.*, 2009), besides some toxic components such as bruceosides A and B and yadanzioside F identified in methanol extract of *B. javanica* seeds (Okuyama *et al.*, 1990). Methanol extract of *B. javanica* leaves which contained the least groups of phytochemicals produced the least prominent antidiabetic effect as shown in Figure 1 and Table 3. The FBG of LME group was not significantly reduced from the baseline measured at day 1. Interestingly, *B. javanica* leaf extract in the form of leaf infusion managed to reduce FBG concentration in a safe and gradual manner in the LI group, making it desirable for use as a treatment for DM. The body weight of all control and treatment groups that did not change significantly throughout the treatment was unexpected, especially that of the negative control (Table 2). This suggests that the diabetic condition induced with alloxan might need more than 15 d to result in a significant change in body weight.

Alloxan was used to induce diabetes by destroying the β cells in the islets of Langerhans of pancreas and reducing the pancreatic β-cell populations via the formation of reactive oxygen species like nitric oxide (Chackrewarthy & Thabrew, 2012). In addition to monitoring changes in the body weight and FBG level, further histological observation of the pancreas morphology would provide additional clue to understanding the effect of the treatments with different preparations of *B. javanica* in recovering the diabetic condition. The blood glucose level is directly correlated with the conditions and functions of pancreas. Our results found that treatments with LME and LI resulted in normal morphology in pancreas histology, which means the treatments were effective in recovering the alloxan-induced damage of pancreas. However, even though treatment with LME managed to recover damage in the pancreatic islets of Langerhans, FBG reduction from the baseline following the treatment was not statistically significant. This suggested that optimisation for treatment of DM with LME by establishing a safe and effective dose that can reduce FBG to normal level is needed. Nevertheless, LME showed potential antidiabetic effect as long as it can recover the damage in pancreas. The 5% leaf infusion used in this study fed at the dose volume of 1% or 10 mL/kg BW seemed to be effective in reducing both FBG level and damage in pancreatic tissue.

Previous study reported several mechanisms of action for *B. javanica* extracts to decrease the blood glucose levels, including inhibition of glycogen phosphorylase (GP-α) and α-glucosidase to slow down the digestion of carbohydrates and enhance glycogen synthesis (Ablat *et al.*, 2017). Luteolin was among the compounds identified in the ethyl acetate fraction of *B. javanica* seeds with the most potent GP-α and α-glucosidase inhibitory activities. The compound was 10 times more potent than the standard GP-α inhibitor caffeine and showed 5.5-fold higher α-glucosidase inhibitory activity than the standard drug acarbose (Ablat *et al.*, 2017). This could explain the strong to moderate hypoglycaemic effect of SME and LI observed in our study, as the two preparations of *B. javanica* contained flavonoids which might include luteolin that had been identified as a potent GP-α and α-glucosidase inhibitor.

In vivo studies in rats with type 2 DM suggested that extracts of *B. javanica* reduced FBG level through increased glycogenesis mediated by the insulin-activated GP-α. The ethyl acetate fraction of ethanol extract prepared from *B. javanica* seeds was reported to increase

hepatic glycogen deposits and serum insulin levels. The insulin increment following treatment with the ethyl acetate fraction at both doses of 50 and 25 mg/kg BW was higher than that in the positive control treated with glibenclamide at a dose of 10 mg/kg BW (Ablat *et al.*, 2017). NoorShahida *et al.* (2009) suggested that bruceines isolated from the *B. javanica* seeds might act as insulin secretagogues that reduced the blood glucose level through increased insulin secretion by improving survival of the damaged pancreas cells through a mechanism similar to that of conventional sulfonylurea drugs such as glibenclamide. Similar mechanism of action might be responsible for the hypoglycaemic effect of *B. javanica* leaf infusion tested in this study. Histological observation on the pancreas indicated that leaf infusion aided in the recovery of β cells and thus improving the morphology of the islets of Langerhans as well as the secretion of insulin; therefore, the blood glucose level decreased after treatment with leaf infusion.

The findings suggested that *B. javanica* has the potential to be developed into an antidiabetic agent. Different preparations of *B. javanica* seeds and leaves at the same animal-equivalent dose converted from human dose exhibited a spectrum of hypoglycaemic effect on the alloxan-induced diabetic rats. An extreme hypoglycaemic effect that even resulted in the death of animal was observed in the SME group; while FBG in the LME group was not significantly affected, a potent hypoglycaemic effect was found in the LI group. Although the potential of 5% leaf infusion as an antidiabetic agent with a feed volume at 1% of BW had been demonstrated, further analyses for establishing the effective dose of SME and LME is necessary. However, limitations of alloxan-induced experimental diabetes have been documented, including antidiabetic effect of test compounds reported on alloxan-induced diabetes was not translated to success in human diabetes (Ighodato *et al.*, 2017). The hypoglycaemic effectivity of various *B. javanica* preparations would need to be further evaluated if the plant extracts or infusion were to be developed into antidiabetic agents.

V. CONCLUSION

This study showed that the same empirical human dose of *B. javanica* seeds and leaves converted to animal-equivalent dose in the forms of methanol extracts and leaf infusion produced different hypoglycaemic effects on the alloxan-induced diabetic rats without altering the body weight. Treatment with SME at a dose of 50 mg/kg BW brought about the death of animal probably from extreme hypoglycaemia. LME given at a dose of 50 mg/kg BW did not reduce the FBG level significantly but the treatment helped with the recovery of pancreatic tissue. The 5% LI treatment fed at a volume of 10 mL/kg BW was effective not only in reducing the FBG in a gradual manner, but also in the recovery of damaged pancreatic tissue.

VI. ACKNOWLEDGEMENTS

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