

HYPOGLYCAEMIC EFFECTS OF JAVANESE GINSENG (*Talinum paniculatum* (Jacq.) Gaertn.) ROOT INFUSION ON ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

To examine whether Javanese ginseng root has an antihyperglycaemic effect, twenty-one male *Rattus norvegicus* rats were randomly divided into four groups: negative control (n= 3), positive control (n= 4), treatment (n= 7), and placebo (n= 7). Alloxan was injected intraperitoneally at a single dose of 80 mg/kg BW to rats to induce hyperglycaemia. Infusion of Javanese ginseng root or distilled water was given at a dose of 2% w/v solution with a volume of 1.8 mL/200 g BW per day for 14 days through an enteral feeding tube to either the treatment or placebo group, respectively. Blood glucose levels were measured using the colorimetric method. One-way analysis of variance (ANOVA) was used to examine differences in the mean of delta blood glucose (post intervention minus post alloxan blood glucose levels) among groups, followed by Tukey's post hoc analysis. A P value < 0.05 was considered statistically significant. There was a significant difference (P= 0.0001) in delta blood glucose among groups. Post hoc analysis revealed that delta blood glucose in the treatment group (-102.99±2.26 mg/dL) was significantly (P= 0.0001) greater than in the placebo (2.45±0.29 mg/dL), positive control (3.05±0.70 mg/dL) and negative control (1.60±0.17 mg/dL) group. In conclusion, Javanese ginseng root has potential as a hypoglycaemic agent in alloxan-induced diabetic animal models.

Key words: alloxan, animal models of diabetes, blood glucose, Javanese ginseng

ABSTRAK

Penelitian ini bertujuan mengetahui efek hipoglikemik akar ginseng jawa. Desain penelitian eksperimental pada 21 ekor tikus *Rattus norvegicus*, yang terbagi 4 kelompok, yaitu kontrol negatif (n= 3), kontrol positif (n= 4), perlakuan (n= 7) dan plasebo (n= 7). Aloksan diberikan secara injeksi intraperitoneal dengan dosis 80 mg/kg bobot badan untuk menginduksi hiperglikemia. Tujuh puluh dua jam kemudian, diberikan infusa akar ginseng jawa atau akuades dengan dosis 2% w/v dengan volume 1,8 mg/200 g bobot badan per hari selama 14 hari per sonde pada tikus coba kelompok perlakuan atau plasebo. Kadar glukosa darah diukur dengan metode kolorimetri. ANOVA satu arah dilakukan untuk menguji perbedaan delta kadar glukosa darah antar kelompok, yang dilanjutkan dengan uji post hoc Tukey. Nilai P<0,05 ditetapkan sebagai batas signifikansi. Terdapat perbedaan bermakna (P= 0,0001) delta kadar glukosa darah antar kelompok. Uji post hoc Tukey menunjukkan delta kadar glukosa darah kelompok perlakuan (-102,99±2,26 mg/dL) lebih rendah secara bermakna (P= 0,0001) daripada kelompok plasebo (2,45±0,29 mg/dL), kontrol positif (3,05±0,70 mg/dL), dan kontrol negatif (1,60±0,17 mg/dL). Disimpulkan bahwa akar ginseng jawa memiliki potensi sebagai agen hipoglikemia tikus model diabetes yang diinduksi dengan aloksan.

Kata kunci: aloksan, model hewan coba diabetes, glukosa darah, ginseng jawa

INTRODUCTION

Non-communicable diseases (NCD), also known as chronic diseases, are responsible for around 60% of human morbidity and mortality (Dans *et al.*, 2011). Diabetes is among the big four of NCD, besides cardiovascular diseases (including ischemic heart disease and stroke), cancer, and chronic pulmonary diseases (such as obstructive pulmonary disease and asthma) (WHO, 2018). Despite treatment with oral hypoglycaemic drugs (for example metformin and glimepiride) and insulin, the prevalence of diabetes still increases. The International Diabetes Federation (IDF) reported that from 1980 to 2014, worldwide age-standardized adult diabetes prevalence increased from 4.3% (95% CI 2.4-7.0) to 9.0% (7.2-11.1) in men and from 5.0% (2.9-7.9) to 7.9% (6.4-9.7) in women. Moreover, IDF suggested that diabetes affects approximately 300 million people around the world, and that number is predicted to reach almost 450 million by the year 2030 (NCD RiskC, 2016). Meanwhile, the number of adults with impaired glucose tolerance (IGT) will increase to almost 500 million by 2030 (Hu, 2011). The devastating effects of diabetes cause significant cost-of-illness (COI), in which direct cost is higher than indirect costs. The direct cost

portrays the cost of treatment, whereas the indirect costs represent the losses of productivity and earnings (Seuring *et al.*, 2015).

Diabetes mellitus is classified into four types: 1) type 1 diabetes mellitus (T1DM), which is caused by β -cell destruction leading to absolute insulin deficiency; 2) type 2 diabetes mellitus (T2DM), which is due to a progressive loss of β -cell of the pancreatic islets; 3) gestational diabetes; 4) specific types of diabetes due to other causes: neonatal diabetes, maturity-onset diabetes of the young (MODY), diseases of exocrine pancreas, and drug- or chemical-induced diabetes (such as glucocorticoid use, HIV/AIDS treatment, post organ transplantation) (ADA, 2019). In epidemiological studies, study subjects with diabetes can be defined as those having either fasting plasma glucose (FPG) of 7.0 mmol/L or higher, history of diagnosis with diabetes, or insulin injection or oral hypoglycaemic drugs (NCD RiskC, 2016).

Ginseng has been recorded as one of the traditional medicinal herbs that have an anti-diabetic effect. The most common ginseng types that are most frequently used and studied are the Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius* L.). Both kinds of ginseng are reported to have hypoglycaemic effects in laboratory animals and humans. In fact, the

effect of lowering blood glucose can be found in all its parts, such as roots, berries, and leaves (Chang *et al.*, 2013; Chen *et al.*, 2019).

Ginseng contains triterpenoid saponins, which are designated as ginsenosides. Ginsenosides are the major compounds of ginseng; therefore, they are often used as markers for the identification of ginseng. The molecular structure of ginsenoside is similar to that of steroids, which contains four rings known as cyclopentanoperhydrophenanthrene ring. Therefore, ginsenosides are written sometimes as steroidal saponins (Xie *et al.*, 2005; Barret *et al.*, 2019; Chen *et al.*, 2019).

Based on the chemical structure, ginsenosides are classified into the dammarane family and oleanane family. The dammarane family is divided into: 1) the protopanaxadiol (PPD) group, such as ginsenosides Rb1, Rb2, Rb3, Rd, Rc, Rg3, and Rh2; 2) the protopanatriol (PPT) group, such as ginsenosides Rg1, Rg2, Re, Rf, and Rh1. The oleanane family is, however, the minority and contains only ginsenoside Ro (Chen *et al.*, 2019). One other classification divides ginsenosides into large and small molecule ginsenosides. Small molecule ginsenosides, such as ginsenosides Rg3 and component K, are suggested to be more active than the large molecule ginsenosides, such as ginsenosides Rb, Rc, and Re (Chen *et al.*, 2014).

In Indonesia, there is a succulent plant which slowly grows up to 100-120 cm tall and has pink flowers. The plant's Latin name is *Talinum paniculatum* (Jacq.) Gaertn, and its roots swell in a similar manner to *Panax* ginseng root. Therefore, *T. paniculatum* is called Javanese ginseng (Thanamool *et al.*, 2013; Manuhara *et al.*, 2015; Santoso *et al.*, 2016). *T. paniculatum* (Jacq.) Gertn has other names, such as *T. patens* (L) Wild, *T. Carssifolium* Wild, *Portulaca patens*, and *T. reflexum* Cav. As it belongs to the Portulacaceae family, *T. paniculatum* has a "sibling": *T. triangulare* (Jacq.) Wild, which is traditionally called krokot belanda, and both are known as kolesom or som Java. This plant grows not only in Indonesia, but also in other parts of the world, especially in South East Asia. In Muangthai, it is called *wan pak pang* (Kohda *et al.*, 1992; Santa and Prajogo, 1999; Thanamool *et al.*, 2013).

Javanese ginseng has been traditionally used to treat various health problems from inflammation of the skin, cough and inflammation of the lungs, gastrointestinal disorders such as diarrhea, reproductive problems such as irregular menstruation and vaginal discharge, and finally, to boost the production of breast milk. The anti-inflammatory and anti-oxidant activities of Javanese ginseng have been observed in both from the water extracts contained within it and the ethanolic extracts of its leaves (Lestario *et al.*, 2009) and roots (Estiasih and Kurniawan, 2006). An *in vitro* study reported that phytosterols in Javanese ginseng leaf extract has antimicrobial bioactivity. They also possess a low cytotoxic effect (Dos Reis *et al.*, 2015). Moreover, another study reported that the phytoestrols of

Javanese ginseng have an estrogenic activity, in which acute consumption of Javanese ginseng extract increases cornification and keratinization of the vaginal epithelia of the ovariectomized rats in a dose-dependent manner (Thanamool *et al.*, 2013). Phytosterols of Javanese ginseng not only have estrogenic activities, but also show androgenic potencies (Sulistiono *et al.*, 2017). A study in male mice (*Mus musculus albinus*) demonstrated that the root extract of Javanese ginseng given at a dose of 3 mg/20 g BW increases the number of live spermatozoa and decreases the number of abnormal spermatozoa; in short, it showed that Javanese ginseng has positive effects on spermatogenesis (Rahmi *et al.*, 2011). Javanese ginseng also supports sperm viability (Sulistiono *et al.*, 2017). In line with its androgenic potency, Javanese ginseng has been used as an aphrodisiac for a long time (Heyne, 1987; Balitbangkes, 2000; Thanamool *et al.*, 2013). It was reported that mounting latency was significantly lower in Wistar rats that had been given ethanolic extracts of Javanese ginseng leaves per oral, when compared to the negative control group (Septiani *et al.*, 2021). Javanese ginseng also has an effect on the neural system. It was demonstrated that ethanolic extract of Javanese ginseng root given orally at a dose of 12 and 24 mg/rat for 18 days significantly increased the thickness of pyramidal lamina of the hippocampus of *Rattus norvegicus*. The hippocampus is known for its role in long-term memory (Sari *et al.*, 2006).

Javanese ginseng contains several compounds, such as phytosterols, steroids, flavonoids, tannins, polyphenols, and essential oils. The types of phytosterols detected in Javanese ginseng leaves are campesterol, β -sistosterol, stigmasterol, stigmastan-3-ol, stigmast-22-en-3-ol and stigmastanol. Similar to the Asian and American ginsengs, Javanese ginseng also contains triterpenoid saponins, which are designated as ginsenosides. It was reported that ginsenosides are detected in high concentrations in Javanese ginseng roots. Types and levels of ginsenosides in Javanese ginseng root have been determined using the liquid chromatography-mass spectrometry (LC-MS) method. The results showed that both hot water and ethanolic extract from Javanese ginseng root contains thirteen ginsenosides, i.e., ginsenoside K, Rh2, Tg2, Rg3, Rf, Rg1, Rd, Re, Ro, Rb2, Rb3, Rc, and Rb1. The order of ginsenosides from the highest to the lowest concentration in Javanese ginseng root is Rb3, Rb2, Rb1, Rc, and Rg1. Meanwhile, Javanese ginseng root cultures have been developed to augment the production of saponins (including ginsenosides) (Manuhara *et al.*, 2015; Santoso *et al.*, 2016; Faizal and Sari, 2019).

Previous studies have shown that ginsenosides have been linked to ginseng's hypoglycaemic effects (Xie *et al.*, 2005; Chang *et al.*, 2013; Bai *et al.*, 2018; Chen *et al.*, 2019). As Javanese ginseng contains ginsenosides, it is thus worthwhile to examine whether or not Javanese ginseng can decrease blood glucose levels.

MATERIALS AND METHODS

Drugs and Chemicals

The samples of Javanese ginseng (*Talinum paniculatum* (Jacq.) Gaertn.) used in this study were obtained from Boyolali, Central Java, Indonesia. The samples had been authenticated in the Integrated Research and Laboratory Testing, Universitas Gadjah Mada, Yogyakarta. Alloxan monohydrate ($C_4H_2N_2O_4 \cdot H_2O$) was purchased from Sigma-Aldrich.

Preparation of Samples

The roots of the Javanese ginseng (*Talinum paniculatum* (Jacq.) Gaertn.) were sorted in wet condition. Then they were washed with running water and chopped. The roots were then dried by aerating them under sunlight. A black fabric was used to shield the simplicia of the roots from excessive damage caused by the ultraviolet (UV) light. After that, the simplicia were ground into powder using a blender. The next step was weighing the powder of the Javanese ginseng root as much as 2 g and adds 100 mL of distilled water to obtain 2% w/v solution. Then after a temperature of 90° C was reached, the solution was then heated for another 15 minutes. The solution was continuously stirred during the heating. Then the solution was filtered with a flannel cloth, and through the dregs, hot distilled water was added to the solution to obtain the desired infusion volume of 100 mL.

Experiment Animals

A total of 21 adult male Wistar (*Rattus norvegicus*) rats, 10-12 weeks old, weighing 150-200 g, were obtained from the Inter-University Research Center, Universitas Gadjah Mada, Yogyakarta. The rats were housed in metabolic cages in groups of maximally four per cage in a temperature-controlled room (25° C) and maintained on a 12:12 hour light/dark cycle. They were fed a standard rat chow and water *ad libitum*, and acclimatized for a period of seven days prior to the experiment.

Experimental Design

Rats were randomly divided into four groups: healthy rats, designated as the negative control group (n= 3), that would be given neither injection of alloxan, infusion of Javanese ginseng root, nor infusion of distilled water; diabetic rats, designated as the positive control group (n= 4), that would be given an injection of alloxan without an infusion of Javanese ginseng root nor infusion of distilled water; diabetic rats, designed as the treatment group (n= 7), that would be given an injection of alloxan with an infusion of Javanese ginseng root; and finally, diabetic rats which were designated as the placebo group (n= 7) that would be given an injection of alloxan with an infusion of distilled water.

Induction of Diabetes

All rats, except those in the negative control group, were rendered diabetic by intraperitoneal

administration of alloxan monohydrate (Sigma Chemical Co., St Louis, USA) at a single dose of 80 mg/kg BW in a solution of 0.90% w/v of NaCl. Rats showing blood glucose levels > 200 mg/dL after 72 hours following an injection of alloxan were considered diabetic.

Intervention

After the diabetic condition was established, infusion of either Javanese ginseng root or distilled water (as placebo) was administered to rats in the treatment group or placebo group, respectively, at a dose of 2% w/v solution with a volume of 1.8 mL/200 g BW per day for 14 days through an enteral feeding tube.

Measurement of Body Weight

Body weight of rats was measured five times: before acclimatization, after acclimatization, after alloxan injection, on the 7th day of intervention, and at the end of the study, before the rats were euthanized.

Measurement of Blood Glucose Level

Blood glucose levels were measured three times: once after acclimatization, again after the alloxan injection, and lastly at the end of study. Blood plasma was obtained from the rats' tails and blood glucose levels were measured using the colorimetric glucose oxidase-peroxidase (GOD-POD) method.

Ethical Clearance

All experimental procedures used in this study had been approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Muhammadiyah Surakarta, with ethical clearance letter no. 3232/A.2/KEPK-FKUMS/I/2021.

Statistical Analyses

A paired t-test was employed to analyze differences between pre-test and post-test data. One-way ANOVA and Tukey's post hoc analysis were performed to analyze differences among groups. The significance level was set at $P < 0.05$ and all calculations were performed using SPSS version 16.0.

RESULTS AND DISCUSSION

Body Weight

The results of body weight measurements in Wistar rats during this study are presented in Table 1. Body weight of the rats was measured before acclimatization, after acclimatization, after alloxan injection, on the 7th day of intervention, and at the end of the study. Measured rats' body weights obtained after acclimatization were used to calculate the dose of alloxan injection, while body weights collected after alloxan injection and on the 7th day of intervention were used to calculate the dose of infusion of Javanese ginseng root or distilled water and to adjust the calculated dose of infusion of Javanese ginseng root or distilled water, respectively.

Using one-way ANOVA, there were no significant differences in the rats' body weights among groups, namely the negative control, positive control, treatment, and placebo, either before ($P=0.127$) or after ($P=0.185$) acclimatization. All rats had increased body weight from before acclimatization (194.29 ± 2.99 g) to after acclimatization (203.86 ± 3.04 g) (Table 1), thus gaining 9.57 ± 0.75 g BW.

The body weight measured after acclimatization was used as the baseline level to calculate the total dose of alloxan injection. Not only body weight but also blood glucose was measured after acclimatization (see below). The dose of alloxan injection was 80 mg/kg of the rat's body weight, which was intraperitoneally injected in 0.90% w/v of NaCl solution to the positive control, treatment, and placebo group, but not to the negative control group. The rats' body weights as well as blood glucose levels (see below) were then measured 72 hours after alloxan injection.

Using one-way ANOVA, it was revealed that rats' body weights among groups significantly differed ($P=0.0001$) after the alloxan injection. Using Tukey' test for post hoc analysis, it was shown that rats' body weights in groups that were given an alloxan injection, i.e., the positive control (198.25 ± 5.25 g), treatment (197.00 ± 2.58 g), and placebo (198.43 ± 3.10 g) group, were significantly lower than the negative control group (212.00 ± 3.00 g) ($P=0.0001$) (Table 1). All groups given alloxan injection, i.e., the positive control, treatment, and placebo (-6.14 ± 3.24 g) group, consistently experienced decreased body weights of -4.00 ± 0.82 g, -5.86 ± 1.35 g, and -6.14 ± 3.24 g, respectively; meanwhile, the negative control group that was not given alloxan injection had experienced an increased body weight of 5.33 ± 1.15 g. Among those groups that were given alloxan injection (the positive control, treatment, and placebo group), rats' body weights did not significantly differ ($P=0.86-1.00$) (Table 1).

During intervention, rats' body weights were measured two times; once in the middle (on 7th day) and at the end of the study. In the course of the intervention, the treatment group that was given an infusion of Javanese ginseng root experienced

increased body weight from 197.00 ± 2.58 g after the alloxan injection to 200.14 ± 2.67 g in the middle of intervention and 205.43 ± 2.51 g at the end of the study, meaning that the body weight of rats in the treatment group had reached that level before the alloxan injection (Table 1). A similar pattern of increased body weight was also experienced by rats in the negative control group, with weights of 212.00 ± 3.00 g after alloxan injection, 222.00 ± 2.65 g in the middle of intervention, and 231.00 ± 2.00 g at the end of the study. Both the positive control and placebo group had, however, consistently decreased body weight during the course of the study (Table 1).

Blood Glucose Level

The results of blood glucose measurements in Wistar rats during this study can be observed in Table 2. Using one-way ANOVA, baseline blood glucose levels after acclimatization (baseline) did not significantly differ ($P=0.57$) among groups, i.e., the negative control (78.98 ± 1.0 mg/dL), positive control (80.64 ± 2.0 mg/dL), treatment (79.83 ± 2.1 mg/dL), and placebo (80.58 ± 1.7 mg/dL) group. These results showed that these groups were comparable in terms of a baseline blood glucose level.

Blood glucose levels were measured 72 hours after the alloxan injection in the positive control, treatment, and placebo group as well as in the negative control group, which was not given alloxan. Using a paired t-test, this post alloxan blood glucose level was significantly higher than the baseline blood glucose level within the positive control (217.88 ± 1.55 vs. 80.64 ± 2.00 mg/dL; $P=0.0001$), treatment (219.74 ± 1.33 vs. 79.83 ± 2.10 mg/dL; $P=0.0001$) and placebo (218.54 ± 1.70 vs. 80.58 ± 1.7 mg/dL, $P=0.0001$) group. Within the negative control group, the second measurement of blood glucose level was not significantly different from the baseline measurement (78.85 ± 0.82 vs. 78.98 ± 1.00 mg/dL; $P=0.58$). Using one-way ANOVA, post alloxan blood glucose levels significantly differed among groups ($P=0.0001$). Using Tukey's test for post hoc analysis, post alloxan blood glucose levels in the positive control (217.88 ± 1.55 mg/dL), treatment (219.74 ± 1.33 mg/dL), and placebo

Table 1. Body weight measurements in experiment animals

Groups	Definition	Before acclimatization	At the end of acclimatization	After alloxan injection	On the 7 th day of intervention	At the end of the study
Negative control	Healthy rats (n=3)	197.33±2.08	206.67±2.08	212.00±3.00	222.00±2.65	231.00±2.00
Positive control	Diabetic rats given injection of alloxan (n=4)	193.00±4.32	202.25±4.57	198.25±5.25	193.25±5.19	190.50±5.07
Treatment	Diabetic rats given injection of alloxan and infusion of Javanese ginseng root (n=7)	193.00±2.16	202.86±2.19	197.00±2.58	200.14±2.67	205.43±2.51
Placebo	Diabetic rats given injection of alloxan and infusion of distilled water (n=7)	195.00±2.44	204.57±2.57	198.43±3.10	195.43±3.48	192.57±3.82
	P value	0.127	0.185	0.0001**	0.0001**	0.0001**

**= Significantly difference ($P<0.001$) using one-way ANOVA. Unit measurement of rat's body weight was gram (g). Data was presented as mean ± standard deviation

(218.54±1.70 mg/dL) group were significantly higher ($P=0.0001$) than in negative control group (78.85±0.82 mg/dL). There was no significant difference ($P=0.15$) in post alloxan blood glucose levels among the positive control, treatment, and placebo group (Table 2). These results showed that a single dose of alloxan could significantly increase blood glucose levels in Wistar rats.

Blood glucose levels of post infusion of Javanese ginseng root (116.75±1.96 mg/dL) measured at the end of the study were significantly lower ($P=0.0001$) than those of post injection of alloxan (219.74±1.33 mg/dL), but were still significantly higher ($P=0.0001$) than that of the baseline level (79.83±2.09 mg/dL). Within the placebo group, blood glucose levels of post infusion of distilled water (220.99±1.70 mg/dL) were significantly higher than those of the post alloxan injection (218.54±1.70 mg/dL) ($P=0.0001$) and of the baseline level (79.83±2.09 mg/dL) ($P=0.0001$). Within either the negative or positive control group, there were no significant differences ($P>0.05$) among the three measures of blood glucose (Table 2).

Using one-way ANOVA, there was significant difference ($P=0.0001$) in post intervention blood glucose levels among groups. Using Tukey's test for post hoc analysis, it was revealed that blood glucose levels in the treatment group after infusion of Javanese ginseng root (116.75±1.96 mg/dL) were significantly lower from blood glucose levels in the positive control (220.93±1.87 mg/dL) ($P=0.0001$) and placebo (220.99±1.70 mg/dL) ($P=0.0001$) group, but was significantly higher than in the negative control group (80.46±0.65 mg/dL) ($P=0.0001$) (Table 2).

At the end of study, the mean blood glucose level in the treatment group was 104.23 mg/dL (95% confidence interval (CI) 106.88 to 101.59 mg/dL) lower than in the placebo group, 104.17 mg/dL (95% CI 107.28 to 101.07 mg/dL) lower than in the positive control group, but 36.29 mg/dL (95% CI 32.88 to 39.71 mg/dL) higher than in the negative control group.

Delta blood glucose was the gap between post intervention and post alloxan blood glucose levels (Table 3). Using one-way ANOVA, there was significant difference ($P=0.0001$) in delta blood glucose

among groups. Using Tukey's test for post hoc analysis, delta blood glucose in the treatment group (-102.99±2.26 mg/dL) was significantly less ($P=0.0001$) than in the placebo (2.45±0.29 mg/dL), positive control (3.05±0.70 mg/dL), and negative control (1.60±0.17 mg/dL) group.

At the end of study, blood glucose level reduction in the treatment group was 105.43 mg/dL (95% CI 103.57 to 108.51 mg/dL) greater than in the control group, 106.04 mg/dL (95% CI 103.33 to 107.54 mg/dL) greater than in the positive control group, and 104.59 mg/dL (95% CI 101.87 to 107.31 mg/dL) greater than in the negative control group.

Plasma glucose levels are pivotal in diagnosing diabetes. The American Diabetes Association (ADA) has recommended random plasma glucose (RPG), fasting plasma glucose (FPG), and oral glucose tolerance test (OGTT) to diagnose diabetes. The threshold for plasma glucose levels for the diagnosis of diabetes is ≥ 200 mg/dL (11.1 mmol/L) for RPG in a person with classic symptoms of hyperglycemia, or either ≥ 126 mg/dL (7.0 mmol/L) for FPG or ≥ 200 mg/dL (11.1 mmol/L) for OGTT in a person without classic symptoms of hyperglycemia (ADA, 2019).

Laboratory animals are often used to study diabetes experimentally. Rat models of diabetes mellitus can be divided into genetic models and chemically induced models. In genetic models of diabetic rats, there are type 1 diabetes (T1D) models and type 2 diabetes (T2D) models. Genetically modified T1D models are autoimmune Bio Breeding (BB) rats, which are derived from Wistar rats, and LEW 1AR1/-iddm rats, which are colonized from Lewis rats. Genetically modified T2D models are Zucker Diabetic Fatty (ZDF) rats, which developed a mutation in the Leptin receptors, and Goto-kakizaki rats, which, like BB rats, are developed from Wistar rats. So far, chemically induced diabetic rats have been used most frequently in diabetes studies. In these models, either streptozotocin (STZ) or alloxan is applied to damage the pancreatic β cells (Al-awar *et al.*, 2016).

In this study, alloxan monohydrate was used, which is the aqueous solution of alloxan, to induce diabetes in Wistar rats. The molecular structure of alloxan is

Table 2. Blood glucose levels in experiment animals

Groups	Baseline blood glucose level (mg/dL)	Post alloxan blood glucose level (mg/dL)	Post intervention blood glucose level (mg/dL)
Negative control (n= 3)	78.98±1.00	78.85±0.82	80.46±0.65
Positive control (n= 4)	80.64±2.02	217.88±1.55	220.93±1.87
Treatment (n= 7)	79.83±2.09	219.74±1.33	116.75±1.96
Placebo (n= 7)	80.58±1.68	218.54±1.70	220.99±1.70

Data was presented as mean±standard deviation

Table 3. Delta blood glucose in experiment animals

Groups	Delta blood glucose (mg/dL)
Negative control (n=3)	1.60±0.17
Positive control (n=4)	3.05±0.70
Treatment (n=7)	-102.99±2.26
Placebo (n=7)	2.45±0.29

Data was presented as mean±standard deviation

similar to glucose and both are hydrophilic. Alloxan as well as glucose is transported by a protein carrier, i.e., a glucose transporter 2 (GLUT2), to across the lipid bilayer of the β cell membrane of the pancreatic islets. As alloxan competes with glucose to occupy GLUT2, fasted animals are more sensitive to the effects of alloxan as compared to fed animals. Alloxan can be administered intraperitoneal at a single dose, over a dose range of 170-200 mg/kg BW, to induce diabetes (Ighodaro *et al.*, 2017). However, it has been proposed also to give alloxan at repeated doses to develop diabetic animals (Purwanto and Liben, 2002). Alloxan builds up reactive oxygen species (ROS), which in turn cause partial degradation of the β cells of pancreatic islets, therefore causing diminished insulin release. The hyperglycemic effect of alloxan can be observed as early as 2-4 hours, and it persists 24-48 hours, after its administration (Ighodaro *et al.*, 2017). In our study, blood glucose levels were measured and the hyperglycemic condition was established 72 hours post alloxan injection. A hypoglycemic event could occur in experiment animals as early as 30 minutes after alloxan exposure. It has been suggested that alloxan can induce a massive influx of Ca^{2+} ions, which stimulates an abrupt release of insulin from the β cells of pancreatic islets (Ighodaro *et al.*, 2017). In this study, there was no hypoglycemic casualty observed in the experiment animals after alloxan injection.

Diabetic hyperglycemia in rats is established when the blood glucose level reaches ≥ 200 mg/dL (11.1 mmol/L) either by chemically induced by STZ or alloxan. Moreover, STZ induced diabetic rats can be divided into two stages; stage 1 with the blood glucose levels 200-450 mg/dL and stage 2 with the blood glucose levels 451-750 mg/dL (Qinna and Badwan, 2015; Ighodaro *et al.*, 2017). In this study, rats given a single dose injection of alloxan showed blood glucose levels of ≥ 200 mg/dL, in the positive control (217.88 ± 1.55 mg/dL), treatment (219.74 ± 1.33 mg/dL) and placebo (218.54 ± 1.70 mg/dL) group (Table 2). This means that in this study the intraperitoneally injection of alloxan effectively induced hyperglycemic diabetes in Wistar rats.

Regarding the changes in the rats' body weights, this study revealed that: 1) rats' body weights were significantly decreased once hyperglycemic diabetes was established; 2) infusion of Javanese ginseng root resulted in increased rats' body weights in the treatment group, which reached the level before alloxan injection. At the end of study, rats' body weights in the treatment group were comparable to the negative control group; 3) infusion of distilled water did not change the rats' body weights in the control group, which was comparable to the positive control group. Other studies also reported a decrease of body mass-as much as 6-8.17% in STZ-induced Wistar and Sprague-Dawley diabetic rats (Nayak *et al.*, 2011, Rias and Sutikno, 2017). In these studies, however, body weight of normal rats showed increased body weight over the study period (Nayak *et al.*, 2011; Rias and Sutikno, 2017). Furthermore, diabetic rats treated with

glibenclamid, an oral antidiabetic drug, also demonstrated increased body weight but the magnitude of weight gain in these groups was still below the control negative group (Nayak *et al.*, 2011). In a clinical setting, weight loss is recognized as one of the classic symptoms of type 2 diabetes mellitus (Yang *et al.*, 2016). Loss of body weight in diabetes mellitus can be caused by increased protein catabolism in the skeletal muscles and lipolysis in the adipose tissues in order to supply amino acids and free fatty acid, respectively, to the liver to proceed the gluconeogenesis (Rias and Sutikno, 2017). Furthermore, loss of body water caused by osmotic diuresis, which is triggered by hyperglycemia, can also contribute to a decreased body weight in diabetic patients (Yang *et al.*, 2016). Osmotic diuresis can increase the osmolarity of the extracellular fluid, which in turn stimulates thirst and then polydipsia behavior, another classic symptom of diabetes mellitus (Shin *et al.*, 2012). Regarding the body weight in the treatment group, it had a trend to reach the level before alloxan injection and was just below the body weight of the negative control group at the end of the study. This suggests that insulin resistance has been reduced and proteolysis in the skeletal muscles and lipolysis in the adipose tissues have been prevented in those rats of the treatment group given the infusion of Javanese ginseng root (Mathews *et al.*, 2002; Rias and Sutikno, 2017).

Many studies have examined the hypoglycemic and antidiabetic effects of Asian ginseng and American ginseng (Xie *et al.*, 2005; Chang *et al.*, 2013; Gui *et al.*, 2016; Bai *et al.*, 2018; Chen *et al.*, 2019). For example, a randomized controlled trial (RCT) for the supplementation of Korean red ginseng on 42 subjects with prediabetes and newly diagnosed T2DM was reported. Prediabetes was defined as subjects who had impaired fasting glucose (IFG) (more than 100 mg/dL but still below 126 mg/dL) or impaired glucose tolerance (IGT) 2 hours following oral glucose tolerance test (OGTT) (more than 126 mg/dL but still below 200 mg/dL). In that study, supplementation of capsules containing 16.58 mg/g total ginsenosides of Korean red ginseng as compared to supplementation of placebo capsules for 3 times a day in 12 weeks effectively decreased fasting serum glucose levels in prediabetes and T2DM subjects (Bang *et al.*, 2014). Another RCT study on American ginseng showed similar results. A gelatinized capsule containing 3 g of American ginseng or corn flour as placebo was randomly allocated to T2DM group or healthy group in one shot. Post prandial capillary glucose levels were measured following OGTT 4 hours after the treatment. In that study, supplementation of American ginseng was shown to significantly reduce post prandial glucose levels as compared to the placebo in 10 healthy subjects and 9 subjects with T2DM (Vuksan *et al.*, 2000). Moreover, fermentation in ginseng is getting more attention because it increases the amount of small ginsenosides, the active form of ginsenosides (Chen *et al.*, 2019), which enhances the efficacy of ginseng, such as the anti-inflammatory effects (Oh *et al.*, 2014).

Regarding fermented ginseng, a RCT was done to 42 subjects with either prediabetes or type 2 diabetes mellitus. These subjects were allocated to either red ginseng root extract that had undergone the fermentation process using *Lactobacillus plantarum* or placebos containing dried yeast, which was given with dose 2.7 g/day for consecutive 28 days. The study showed that the fermented red ginseng group had an improvement in post prandial insulin and glucose but not fasting insulin and glucose levels as compared to the placebo group (Oh *et al.*, 2014).

In this study, the infusion of Javanese ginseng root significantly decreased hyperglycemia from 219.74±1.33 mg/dL to 116.75±1.96 mg/dL in diabetic rat models. On the other hand, hyperglycemia persisted in both the placebo group and the positive control group (Tabel 2). At the end of study, infusion of Javanese ginseng root decreased blood glucose levels by 102.99±2.26 mg/dL. In contrast, infusion of distilled water just increased the rats' blood glucose levels by 2.45±0.29 mg/dL (Table 3). To date, this is this first study to show that Javanese ginseng root has a hypoglycemic effect on diabetic rat models.

Based on this study, it is advisable that ginsenosides, which are detected in the Javanese ginseng root (Santoso *et al.*, 2016; Faizal and Sari, 2019), are used to determine the hypoglycemic effects of Javanese ginseng. Ginsenosides, a member of the triterpenoid saponins, are mainly responsible for the hypoglycemic and antidiabetic effects of Asian ginseng and American ginseng (Chang *et al.*, 2013; Santoso *et al.*, 2016; Chen *et al.*, 2019). Javanese ginseng roots contain ginsenosides Rb3, Rb2, Rb1, Rc, and Rg1 based on the LC-MS method (Santoso *et al.*, 2016).

The mechanisms of the antidiabetic effects of ginsenosides in the Asian ginseng and the American ginseng, as revealed by numerous studies, are exerted through peripheral and central action. Peripherally, ginsenosides improve insulin resistance. The effects of ginsenosides on the liver, such as maintaining normal fasting blood glucose levels, are a part of the peripheral antidiabetic action of ginsenosides. Centrally, ginsenosides protect the β -cells of pancreatic islets from apoptosis, thus preserving the function of mitochondria in the pancreatic β -cells (Chang *et al.*, 2013; Bai *et al.*, 2018; Li *et al.*, 2019). As of yet, there are no studies that can be found in the literature that examine the mechanisms behind the hypoglycemic and antidiabetic effects of specific ginsenosides of the Javanese ginseng root.

In this study, the infudation method was used as the method of extraction of Javanese ginseng root with water, rather than ethanol or oil used as a solvent. Triterpenoid saponins such as ginsenosides are actually water-soluble (Brain and Turner, 1975). It has been shown that the types of ginsenosides detected in Javanese ginseng root which have been extracted with hot water are similar to those extracted with ethanol (Faizal and Sari, 2019). Moreover, the infudation method is in concordance with the way people on the island of Java, Indonesia, have traditionally prepared

Javanese ginseng root as a medicine, which is by washing it and then brewing it in hot water (Santoso *et al.*, 2016).

CONCLUSION

In conclusion, Javanese ginseng root has potential as a hypoglycemic agent in alloxan-induced diabetic animal models.

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