The Potency of Antidiabetic of *Psidium guajava* Fruit Extract Against Streptozotocin-induced Type 2 Diabetic Rats

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ABSTRACT

Background: The chronic disease known as diabetes mellitus is brought on by either the pancreas's inability to make enough insulin or the body's inability to use it. Purpose: This plant finds applications for treating diarrhea, dysentery, gastroenteritis, hypertension, diabetes, caries and pain relief. **Purpose:** The current study aimed to determine how *Psidium guajava* fruit extract affected the blood glucose, body weights, and insulin levels of streptozotocin (STZ)-induced diabetic rats over 21 days. **Methods:** The extract's effectiveness was compared to that of glibenclamide, a common hypoglycemic medication. 30 male Wistar albino rats were divided into 5 groups with 6 animals in each group. V: Normal control (Group-I), diabetic control (Group-II), diabetic rats treated with glibenclamide 0.6mg/kg bw (Group-III), diabetic rats treated with *Psidium guajava* fruit extract 200 mg/kg bw (Group-IV) and *Psidium guajava* fruit extract 400 mg/kg bw (Group-V). All group of rats were subjected to evaluation of body weight, blood glucose and serum insulin levels on day 0, 7, 14 and 21 of the experiment. **Results:** There was significant ($P<0.05$) decrease in body weight and serum insulin and significant ($P<0.05$) increase in blood glucose level in Group-II compared to Group-I rats. In the Present study, daily oral administration of *Psidium guajava* fruit extract at dose rate of 200 and 400 mg/kg bw and glibenclamide at 0.6mg/kg bw in diabetic rats for 21 days showed a progressive improvement in body weight, blood glucose and serum insulin concentration. **Conclusion:** It can therefore be concluded the results of this study indicate that *Psidium guajava* fruit extract possesses anti-diabetic properties in Wistar albino rats.

Keywords: streptozotocin, *Psidium guajava*, glibenclamide, blood glucose, insulin

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition that is highly common in both humans and animals. All forms of diabetes are characterized by a decrease in the circulating concentration of insulin (insulin deficiency) and a decrease in the response of peripheral tissues to insulin (insulin resistance) which leads to disruptions in the metabolism of carbohydrates, fats, and proteins (Eurich et al., 2007; Amin, 2011). The disorder has reached epidemic levels and threatens a worldwide epidemic. According to World Health Organization (WHO), the disease incidence in 2010 was about 285 million people worldwide, and the number is projected to grow to 438 million by 2030 (Boyle et al., 2001).

There are two types of DM, type I and type II. Type I is caused due to insulin insufficiency, because of lack of functional beta cells. Patients suffering from type I DM are therefore totally dependent on exogenous source of insulin, while type II DM which is the most common type, is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (Chika and Bello, 2020;

Dineshkumar et al., 2010). The therapeutic management of DM with minimal side effects remains a clinical challenge. There is growing interest in the potential use of medicinal plant products as an alternative treatment for DM as these are commonly cheaper, less toxic, and with fewer side effects (Malviya et al., 2010).

Its fruit of *Psidium guajava* is rich in vitamins A, C, iron, phosphorus and calcium and minerals (Michael et al., 2002). It contains high content of organic and inorganic compounds like secondary metabolites e.g. antioxidants, polyphenols, antiviral compounds, anti-inflammatory compounds (Jimenez-Escrig et al., 2001; Abdel et al., 2004; Vieira et al., 2014). This plant finds applications for the treatment of diarrhea, dysentery, gastroenteritis, hypertension, diabetes, caries, and pain relief and for improvement in locomotors coordination. Its *Psidium guajava* extract is being used as a medicine in cough, diarrhea, and oral ulcers and in some swollen gums wound (Lozoya et al., 1994; Begum et al., 2002; Vikrant et al., 2012). Using streptozotocin (STZ)-induced diabetic rats, the current study aimed to ascertain the effects of *Psidium guajava* fruit extract on the rats' body weights, blood glucose levels, and insulin levels during a 21-day period.

METHODS

Experimental of Animal

Wistar rats weighing 250–300 g were purchased from the LPPT at the Universitas Gajah Mada in Indonesia. Before the trial began, the rodents were acclimated for one week in plastic enclosures under controlled conditions. The temperature was maintained at 26 ± 2^{o}C, and the rats were subjected to a 12-hour light/dark cycle. All rats had unrestricted access to drinking water and commercial pellets during this period.

Experimental Induction of Diabetes

After an overnight fast, a single intraperitoneal injection of a freshly made solution of streptozotocin (50 mg/kg b w) in 0.1 M cold citrate buffer with a pH of 4.5 was given to groups II, III,IV and V of rats to induce diabetes (Junod et al., 1967; Pushparaj and Tan, 2007). Animals in Group I (control) were given citrate buffer only. To verify that the animals were diabetic, the blood glucose levels were measured 72 hours after the STZ injection using an Accu-Check Glucometer (Rheney and Kirk, 2000). Diabetic animals were defined as those whose blood glucose levels were more than 200 mg/dL. Following the determination that each group had diabetes, each group underwent daily treatment for 21 days.

Experimental Design

The study utilized rats randomly divided into five groups with eight rats in each group. Group I: Normal control; Group II: Diabetic control: Streptozotocin i.p.50mg/kg BW; Group III: Standard: Streptozotocin i.p. + 0.6mg/kg BW Glibenclamide (oral); Group IV: Streptozotocin i.p.+200mg/kg BW *Psidium guajava* fruit extract (oral); Group V: Streptozotocin i.p.+400mg/kg BW *Psidium guajava* fruit extract (oral). *Psidium guajava* fruit extract nanoparticles were given orally once a day for 21 days. On day 0, 1,7,14 21, all groups of rats were blood was taken to measure levels of blood glucose, and insulin. And also body weight was recorded using an electronic weighing balance.

Collection of Blood Samples

Blood samples were taken from all rats on days 0, 7, 14, and 21 of the experiment by puncturing the retro-orbital plexus while the animals were under light ether anesthesia using a capillary tube (Babu and Prince, 2004).

One milliliter of blood was collected in centrifuge tubes without anticoagulant, and the samples were left to clot at room temperature (26 ± 2°C). To obtain serum for biochemical examination, it was centrifuged at 1500 x g for 10 minutes at 15°C. The serum was then refrigerated at -80°C.

Recording of Body Weights

Every rats body weight was recorded using an electronic weighing balance, first on Day 0 of the trial and then on Days 7, 14, and 21 of the therapy.

Determination of Blood Glucose and Insulin Levels

Blood samples were taken from the rats' orbital sinuses. The glucose-oxidase principle was used to determine the blood glucose levels (Sellamuthu, et al., 2009; Graham et al., 2011). The animals' blood glucose levels were measured using Accu-Check (Roche Diagnostics, Manheim, Germany), with values reported as mg/dl [11]. The ELISA approach (Insulin ELISA test kits, Wuhan Fine Biotech Co., Ltd. China) was used to determine insulin levels while fasting.

Statistical Analysis

The collected data (body weights, blood sugar levels, and insulin hormone) were statistically analyzed using the statistical package for social sciences (SPSS) version 25.0 and Two-way ANOVA. Duncan's multiple comparison tests were used to compare the means, and a significance level of $P<0.05$ was established.

RESULT AND DISCUSSION

The effect of Psidium guajava fruit extract on Body weight of diabetic rats

Table 1 shows the body weights (g) of groups I, II, III, IV, and V ranged from 241±6.80 to 240.8±1.64 on the first day of the experiment, 250.1±5.84 to 242.6 ± 2.23 on the seventh day, 261±7.64 to 244.8 ±2.21 on the fourteenth day, and 276.5±8.89 to 248.2 ± 2.24 on the twenty-first day. On the 14th and 21st day of the trial, group II rats' mean body weights were significantly ($P<0.05$) lower than those of groups I, III, IV, and V.

Table 1. Body weights (g) in different experimental groups of rats

Groups	Days post-treatment			
	0	7	14	21
Normal Control (Group I)	241±6.80 ^{a,p}	250.1±5.84 ^{a,p}	261±7.64 ^{a,p}	276.5±8.89 ^{a,p}
Diabetic Control (Group II)	241±4.32 ^{a,p}	235.3± 3.90 ^{b,p}	225.8±6.05 ^{b,q}	223.8±6.43 ^{b,q}
DM+ Glibenclamide (Group III)	240.5±3.69 ^{a,p}	241.1± 2.85 ^{b,p}	247.3±3.92 ^{c,p}	252.6±4.57 ^{c,p}
DM+ *Psidium guajava* 200 (Group IV)	241.5±6.85 ^{a,p}	244.2 ± 7.11 ^{b,p}	246 ± 6.74 ^{c,p}	250.6 ± 6.94 ^{c,p}
DM+ *Psidium guajava* 400 (Group V)	240.8±1.64 ^{a,p}	242.6 ± 2.23 ^{b,p}	244.8 ±2.21 ^{c,p}	248.2 ± 2.24 ^{c,p}

Values are mean ± standard error (n=6); Two way ANOVA (SPSS)
^{a,b,c} Means sharing different superscripts in a column differ significantly ($P\leq0.05$)
^{p,q,r} Means sharing different superscripts in a row differ significantly ($P\leq0.05$)

Table 2. Blood glucose concentration (mg/dL) in different experimental groups of rats

Groups	Days post-treatment			
	0	7	14	21
Normal Control (Group I)	99.4 ± 1.12 ^{a,p}	100.6 ± 2.18 ^{a,p}	99.1 ± 1.11 ^{a,p}	99.9 ± 1.31 ^{a,p}
Diabetic Control (Group II)	337.4±7.17 ^{b,p}	347.1 ±5.11 ^{b,p}	352 ± 3.87 ^{b,p}	360.9 ± 2.57 ^{b,p}
DM+ Glibenclamide (Group III)	341.6±5.53 ^{b,p}	323.6 ± 4.45 ^{b,p}	302.4 ± 3.58 ^{b,q}	282.51 ± 3.29 ^{b,r}
DM+ *Psidium guajava* 200 (Group IV)	338.7±6.62 ^{b,p}	330.7 ±5.34 ^{b,p}	313.9 ± 7.72 ^{b,q}	297.18 ± 7.04 ^{b,r}
DM+ *Psidium guajava* 400 (Group V)	339.9±5.62 ^{b,p}	327.7 ± 5.16 ^{b,p}	311.2 ± 5.71 ^{b,q}	293.1 ± 3.75 ^{b,r}

Values are mean ± standard error (n=6); Two way ANOVA (SPSS)
^{a,b,c} Means sharing different superscripts in a column differ significantly (P≤0.05)
^{p,q,r} Means sharing different superscripts in a row differ significantly (P≤0.05)

Table 3. Insulin concentration (µIU/mL) in different groups of rats

Groups	Days post-treatment			
	0	7	14	21
Normal Control (Group I)	19.27±1.20 ^{a,p}	19.66 ± 1.16 ^{a,p}	18.82. ± 1.10 ^{a,p}	19.73 ± 1.07 ^{a,p}
Diabetic Control (Group II)	9.13 ± 0.15 ^{b,p}	8.81 ± 0.17 ^{b,p}	7.975 ± 0.22 ^{b,q}	7.14 ± 0.07 ^{b,q}
DM+ Glibenclamide (Group III)	9.41 ± 0.12 ^{b,p}	10.89 ± 0.15 ^{b,p}	12.86 ± 0.15 ^{c,q}	14.35 ± 0.17 ^{b,r}
DM+ *Psidium guajava* 200 (Group IV)	9.16 ± 0.11 ^{b,p}	9.86 ± 0.14 ^{b,p}	11.22 ± 0.11 ^{c,q}	12.45 ± 0.10 ^{c,q}
DM+ *Psidium guajava* 400 (Group V)	9.26 ± 0.13 ^{b,p}	10.25 ± 0.15 ^{b,p}	12.09 ± 0.79 ^{c,q}	13.88 ±0.37 ^{c,q}

Values are mean ± standard error (n=6); Two way ANOVA (SPSS)
^{a,b,c} Means sharing different superscripts in a column differ significantly (P≤0.05)
^{p,q,r} Means sharing different superscripts in a row differ significantly (P≤0.05)

The effect of Psidium guajava fruit extract on Blood glucose of diabetic rats

Table 2 shows The mean blood glucose levels (mg/dL) in the I, II, III, IV, and V groups varied over the course of the experiment: on day 0, they were 99.4 ± 1.12 to 339.9±5.62; on day 7, they were 100.6 ± 2.18 to 327.7 ± 5.16; on day 14, they were 99.1 ± 1.11 to 311.2 ± 5.71; and on day 21, they were 99.9 ± 1.31 to 293.1 ± 3.75. On days 7, 14, and 21 of the trial, group II rats had significantly (P<0.05) higher mean blood glucose readings than groups I, III, IV, and V

The effect of Psidium guajava fruit extract on Insulin hormone of diabetic rats

Table 3 shows the mean serum insulin levels (µIU/mL) in the I, II, III, IV, and V groups varied over the course of the experiment: on day 0, they were 19.27±1.20 to 9.26 ± 0.13; on day 7, they were 19.66 ± 1.16 to 10.25 ± 0.15; on day 14, they were 18.82. ± 1.10 to 12.09 ±

0.79; and on day 21, they were 19.73 ± 1.07 to 13.88 ±0.37. On the seventh, fourteenth, and twenty-first days of the trial, group II rats showed significantly ($P<0.05$) lower mean values of serum insulin levels than groups I, III, IV, and V.

At present, diabetes is a common pancreas disorder that afects at least 100 million people globally. This number will double by the year 2030. In most of the lowest-income countries, including Indonesia, India, and Pakistan, there has been an alarming increase in the number of diabetics over the past decade. Several pharmaceutical drugs, such as thiazolidinediones, biguanides, and insulin, are used in modern medicine to control blood glucose. These drugs have hypoglycemic activities but can produce several health problems, such as neural disorders, diarrhea, heart diseases, and many others. All these health problems have been managed by using alternative herbal drugs. They have lesser side effects and, however, improved therapeutic values. The present study has evaluated the antidiabetic properties of *Psidium guajava* fruit extract. Beta-cells in the pancreatic islets of Langerhans die after being injected with streptozotocin (Suba et al., 2004). The loss of cells in the pancreas causes a considerable reduction in serum insulin levels. The drug's modest impact on STZ induced diabetic rats can be related to the fact that there are practically any cells in the pancreas to create any secretagogue activity. It has already been documented that this animal took glibenclamide. Administration of *Psidium guajava* fruit extract only displayed a moderately potent antihyperglycaemic effect in rats with STZ-induced diabetes. The extracts' potential mechanism could be an increase in peripheral glucose uptake or a reduction in liver endogenous glucose synthesis. At this point, it is impossible to rule out the potential of one or more of these pathways, with each extract contributing to the activity. The process involving intestinal delay or glucose inhibition can be disregarded because the study's subjects' animals fasted the night before the experiment began. In a chronic STZ-induced diabetes investigation, an aqueous extract of every plant was employed at a concentration of 500 mg/kg based on the findings in the acute models. The lack of serum insulin in STZ-diabetic rats have been extensively established (Graham et al., 2011). In the current investigation, diabetic control rats' blood insulin levels were significantly lower than those of normal rats. In STZ-induced diabetes rats, endogenous insulin has a very small function. The results demonstrate that *Psidium guajava* fruit extract therapy has a signifcant antihyperglycaemic efect in diabetic rats caused by STZ. The form of diabetes that STZ produces in rats is IDDM. *Psidium guajava* fruit extract extract can increase insulin secretion from the pancreas which is supported by research results which show that the extract is able to reduce blood glucose levels in mice when compared to healthy mice that were not treated.

In STZ-induced diabetic rats, blood glucose levels increased significantly ($P<0.05$). Conversely, when oral *Psidium guajava* fruit extract (300 mg/kg & 400 mg/kg) and standard medication glibenclamide (0.6 mg/kg) were given to diabetic rats, blood glucose levels decreased. According to reports from (Suba at al., 2004), glibenclamide treatment seems to be more successful in controlling blood glucose when compared to *Psidium guajava* fruit groups. Furthermore, there doesn't seem to be much of a benefit to using *Psidium guajava* fruit at higher concentrations compared to lesser doses. blood insulin levels in STZ-

induced diabetic rats showed a significant (P<0.05) decline, while oral administration of *Psidium guajava* fruit extract (200 mg/kg & 400 mg/kg) and conventional medication glibenclamide (0.6 mg/kg) resulted in a progressive increase in blood insulin levels in diabetic rats.

The inclusion of phytochemical elements such as flavonoids, phenol, terpenoids, glycosides, alkaloids, and kaempferol (Michael et al., 2002; Abdel et al., 2004), along with flavonoids and phenol (Vikrant et al., 2012), may have potentiated glucose-induced insulin production from existing β cells or prevented damage to these cells with *Psidium guajava* fruit extract.

The duration of the current study, which lasted 21 days, could be the reason that the insulin and glucose levels in the group given *Psidium guajava* fruit extract did not return to values that were close to normal. Therefore, to find out the antidiabetic benefits of *Psidium guajava* fruit extract in the treatment of type II diabetes, research must be carried out over a long period of time.

CONCLUSION

When the administration of *Psidium guajava* fruit extract was increased from 200 mg/kg bw to 400 mg/kg bw, there was only a slight increase in the hypoglycemic effect compared to the standard drug glibenclamide. *Psidium guajava* fruit extract can be considered as an alternative medicine for the treatment of Type II DM.

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Author Contribution

S.C.R and S.S.C conceptualization. S.S.C and S.C.R contributed to the animal study S.S.C analyzed data and draft preparation. S.S.C and S.C.R writing review and editing. All author have read and agreed to the published version of the manuscript.

Competing Interest

The authors declare that there is no conflict of interest.

Ethical Approval

All trials were examined and approved by the Faculty of Veterinary Medicine at Airlangga University's Ethical Approval Committee assessed this work, and it was given ethical approval under No. 1.KEH.081.06.2023.

Data Availability

The article includes data that was used to support the study's conclusions.

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