# Antihyperglycemic Activity of *Piper crocatum* Leaves and *Cinnamomum burmannii* Bark Mixture Extract in Streptozotocin-induced Diabetic Rats

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**Abstract.** Indonesia presently has the fourth largest diabetic mellitus prevalence of all countries in the world. In a previous study, a mixture extract of *Piper crocatum* leaves and *Cinnamomum burmannii* bark showed in vitro antihyperglycemic activity. It acted as inhibitor of the α-glucosidase enzyme and had no toxic effect when it was administered orally to male and female rats for 28 days. In the present study, mixture extracts of *P. crocatum* leaves and *C. burmannii* bark were used to observe antihyperglycemic activity in diabetic rats induced with streptozotocin. Mixture extracts of *P. crocatum* leaves and *C. burmannii bark* were orally given to diabetic Sprague Dawley rats at various doses for 16 days. The results showed that the treatment led to a reduction of the blood glucose level, an increase in blood insulin level up to 170.75% at 1260 mg/kg body weight, maintaining the blood lipid level of the diabetic rats at a normal level, and an increase of pancreatic ß cells in the islets of Langerhans up to 2.2-fold at 1260 mg/kg body weight. The mixture extracts of *P. crocatum* and *C. burmannii* have antihyperglycemic activity, which enhances the number of pancreatic β cells.

**Keywords:** *antihyperglycemic agents; Cinnamomum burmannii; glucose; insulin; lipid profile; Piper crocatum.*

## 1 Introduction

Hyperglycemia, or a high blood glucose level, is an indication of diabetes mellitus. It is a metabolic syndrome that is caused by disruption of the insulin hormone. Chronic hyperglycemia can lead to imbalances in the body, such as damage to eyes, kidneys and other organs [1]. The increase of the number of people with diabetics based on statistical projections from the years 2000 to

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2010 will reach about 70 million in Indonesia in 2050. Indonesia has the highest diabetic prevalence after India, China and the USA [2,3].

Using traditional medicines that come from plants has been a priority for antihyperglicemic agents because they are more advantegeous than modern drugs [4]. Traditionally, in diabetic treatment, people used decoctions of *Piper crocatum* (Piperaceae). According to a previous study [5], a ten-day treatment with various doses of decoctions of *P. crocatum* extract reduced the blood glucose level up to 10-38%. A phytochemical assay of *P. crocatum* showed that the decoction contained alkaloids, flavonoids and tannins [5]. These are known to be bioactive antidiabetic and antioxidant compounds [6-8]. *P. crocatum* extract has a bitter taste. It can be combined with *Cinnamomum burmannii* in order to obtain a more acceptable flavor and help preserve the product, because *C. burmannii* has antibacterial and antibiotic activity.

*Cinnamomum burmannii* is inhibitory against pathogenic microorganisms with high activity. This can be seen from the diameter of the clear zones of each bacteria (mm). These are 15.4 mm (*B. cereus*), 11.5 mm (*L. monocytogenes*), 15.7 mm (*S. aureus*), 8.7 mm (*E. coli*) and 12.1 mm (*S. anatum*). Further, it also has antioxidant capacity (107.7 mmol trolox/100 g dry weight) [9]. Cinnamon has many benefits in food preparation with its unique flavor and taste. Besides that, it may play a role in the regulation of blood pressure and glucose metabolism, i.e. it can contribute to the uptake of glucose into cells [10]. It has been reported that giving cinnamon at different doses per day has beneficial effects for type 2 diabetic patients, such as reduction of the blood glucose level and cholesterol in the body [11]. The present study was conducted to observe the effects of mixture extracts of *P. crocatum* and *C. burmannii* as antihyperglycemic agents in diabetic rats induced by streptozotocin.

## 2 Methods

### 2.1 Functional Food Preparation of *P. crocatum* and *C. burmannii*

*P. crocatum* and *C. burmannii* were collected from the Indonesian Medicinal and Aromatic Crops Research Institute (IMACRI). The material was identified in the Herbarium Bogoriense, Indonesian Institute of Sciences (LIPI), Bogor. Dried leaves of *P. crocatum* (10 g) were boiled in water (200 mL); boiling was terminated when the volume reached 100 mL. Dried bark of *C. burmannii* (20 g) was boiled in water (200 mL); boiling was terminated when the volume reached 200 mL. The extract that was obtained from filtering with Whatman filter paper number 4 was used for further assays. The *P. crocatum* extract was mixed with the *C. burmannii* extract at a ratio of 1:0.6, after which it was added

with an amount of stevia (0.67 % v/v) as natural sweetener with a low glycemic index.

## 2.2 Experimental Animals

Sprague Dawley male and female rats (210-260 g) were obtained from the experimental animal facility of the National Agency of Drugs and Food Control (BPOM), Republic of Indonesia. The rats were fed with standard pellet diet during the experiment (CP Rodent, Thailand; 18% protein, 3% fat, 13% water, 10% ashes, 9% fiber, 9000 IU/kg vitamin A, 1800 IU/kg vitamin $D_3$, 80 IU/kg vitamin E, and 800 mg/kg vitamin C). Randomized rats were acclimatized for 7 days in 24 ± 1 ºC with a light:dark cycle of 12 hours and a relative humidity of 55-75%. The Institutional Animal Ethics Committee (PT. Bimana Indomedical R.02-11-IR) gave clearance for the use of the test animals in this study.

## 2.3 Experimental Design

There were 6 experimental groups, which were randomly obtained from 24 Sprague Dawley rats (210-260 g), i.e. group KN – normal control (administered intraperitoneally (i.p.) with NaCl 0.9% and orally (p.o.) with aquadest 2 mL per day for 16 days); group KP – diabetic control (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with aquadest 2 mL per day for 16 days); groups KDS 1, KDS 2, and KDS 3 were given the mixture extract treatment at different doses (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with extract 630 mg/kg body weight (KDS 1), 1260 mg/kg body weight (KDS 2), and 1890 mg/kg body weight (KDS 3)); group SM was administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with *P. crocatum* extract 1350 mg/kg body weight.

Observation of the rats' body weight and food consumption was conducted on days 1, 3 and 16. Rats that had been induced with streptozotocin for 48 hours were used for testing the mixture extract. Blood sampling from the tail vein of the rats was conducted on the rats that were left fasting for 18 hours. The instrument used for measuring the blood glucose level was an electronic glucometer (Miles Inc, USA). Measuring of the glucose level of the fasting rats was conducted on days 1, 3, and 16 after NaCl 0.9% injection or streptozotocin 50 mg/kg body weight and aquadest or mixture extract treatment at different doses. The blood lipid levels and blood insulin levels of the fasting rats were measured on day 16. All rats were observed for their body weight, food and water consumption on days 1, 3, and 16. Necropsy and pancreas immunohistopathological examinations were done on day 16 (3 rats per group). Before necropsy, rats were injected with euthal 200 mg/kg body weight, ketamine 80 mg/kg body weight, and xylazine 10 mg/kg body weight.

## 2.4 Measurement of Blood Glucose Level, Blood Insulin Level, and Blood Lipid Level

Blood samples from all groups, obtained from the tail vein of the rats, were used for measurenment of the blood glucose levels using a single touch glucometer (GlucoDr kit). Measurenment of the blood insulin levels was done using a kit (Mercodia, Sylveniusgatan, Uppsala) and a microplate reader (BioRad 3550) with the enzyme linked immunosorbant assay (ELISA), while the blood total cholesterol, HDL and triglyserida levels were analyzed using a commercial kit (Roche Diagnostics GmbH, Mannheim, Germany). The blood lipid levels were measured using a Photometer 5010 (Robert Riele GmbH & Co kG).

## 2.5 Eosin Haematoxylin Staining and Immunohistochemical Staining

The tissue preparation was immersed in xylol solvent followed by absolute alcohol 95% and alcohol 70%. The obtained product was washed with flowing water and then immersed in dye reagent of Mayer's haematoxylin for 5 minutes and then washed again. In the next step, the tissue preparation was immersed in acid alcohol solvent, a solution of ammonia and dye of eosin, alternately. Furthermore, the preparation was washed with alcohol 95%, absolute alcohol and xylol. The obtained preparation was observed under a light microscope to count the number of Langerhans islets. Photomicrographs of microscopical sections were taken with a Nikon Eclipse 80i DS Fi1.

Furthermore, immunohistochemical staining was conducted to observe the number of pancreatic β cells. Staining was started with making the histopathology preparation. After deparaffinization and rehydration, the preparation was immersed in a phosphate buffer solution (PBS) for 2 minutes. Then, the preparation was added with a few drops of blocking protein and mouse monoclonal anti-insulin antibody and then incubated for 60 minutes. The preparation was immersed in PBS and added with a solution of secondary antibody i.e. Trek Universal Link and then immersed again in PBS, and Trek-Avidin-HRP was added. Subsequently, the preparation was visualized using 1.3-deaminobenzidin (DAB) and immersed in distilled water for 5 minutes and then given a hematoxylin dye. The last step was dehydration, clearing, mounting and observation under the microscope.

## 3 Results and Discussion

### 3.1 The Effect of *P. crocatum* and *C. burmannii* Mixture Extract Treatment on Rat Body Weight

The body weights of the rats in all groups that were given the mixture extracts of *P. crocatum* and *C. burmannii* on day 1 were statistically similar (see Table 1), but they decreased after streptozotocin induction on days 3 and 16, except group KN. The body weights of the rats in group KP showed the highest reduction compared to the others (5.26% on day 3 and 10.98% on day 16 compared to the body weight on day 1). This indicates that the *P. crocatum* and *C. burmannii* mixture extract treatment suppressed the reduction of the rats' body weights. The 16-day treatment with various doses of *P. crocatum* and *C. burmannii* mixture extract administered orally to streptozotocin-induced diabetic rats suppressed reduction of the rats' body weights at 2-8%. Giving hot water extract of mahogany bark for 13 days with doses of 250 mg/kg body weight also suppressed the body weight reduction of rats that were alloxan-induced up to 8.54 % in [12]. Diabetic rats that were induced by alloxan for 10 days showed suppression of body weight reduction up to 4.41% after being given 322 mg/kg body weight of *P. crocatum* extract in [5].

**Table 1** Effect of 16-day treatment with *P. crocatum* and *C. burmannii* mixture extract on rat body weight.

| Groups | Day 1 | Day 3 | Day 16 |
|---|---|---|---|
| KN | $244.08\pm7.37^{ab}$ | $259.45\pm7.32^{b}$ | $267.55\pm7.08^{b}$ |
| KP | $262.40\pm19.15^{a}$ | $248.63\pm20.32^{a}$ | $233.55\pm19.63^{a}$ |
| KDS 1 | $261.30\pm23.16^{a}$ | $253.43\pm27.74^{a}$ | $244.88\pm35.31^{a}$ |
| KDS 2 | $263.28\pm30.19^{a}$ | $259.30\pm18.54^{a}$ | $255.40\pm18.30^{a}$ |
| KDS 3 | $268.98\pm14.50^{a}$ | $256.30\pm25.02^{a}$ | $245,35\pm12.79^{a}$ |
| SM | $269.55\pm30.14^{a}$ | $266.18\pm28.95^{a}$ | $253,15\pm36.89^{a}$ |

Amounts that share the same superscript characters (a,b) are statistically similar at $p < 0.05$ ($n = 4$). Group KN is normal control (administered i.p. with NaCl 0.9% and p.o. with aquadest 2 mL per day for 16 days); group KP is diabetic control (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with aquadest 2 mL per day for 16 days); groups KDS 1, KDS 2, and KDS 3 are mixture extract treatment at different doses (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with extract 630 mg/kg body weight (KDS 1), 1260 mg/kg body weight (KDS 2), and 1890 mg/kg body weight (KDS 3) per day for 16 days); group SM was administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with *P. crocatum* extract 1350 mg/kg body weight per day for 16 days.

The effect of giving mixture extract of *P. crocatum* and *C. burmannii* on the rats' food consumption on day 1 in all groups was statistically similar (see Table 2), but it was decreased after streptozotocin induction on days 3 and 16, except in group KN. The highest reduction occurred in group KP (diabetic control – 5.10% on day 3 and 10.83% on day 16 compared to food consumption on day 1). Diabetic rats that were induced by alloxan for 10 days also showed reduced food consumption up to 34.88% after being given 800 mg/kg of methanol-water *Coccinia indica* leaves extract [13]. The lowest reduction occurred in group KDS 2 at a dosage of 1260 mg/kg body weight (1.27% on day 3 and 3.16% on day 16 compared to food consumption on day 1). This indicates that treatment with *P. crocatum* and *C. burmannii* mixture extract for 16 days can maintain relatively stability of the amount of food consumption by the rats. This shows that *P. crocatum* and *C. burmanni* mixture extract can improve the condition of diabetic rats.

**Table 2** Rat food consumption for 16-day treatment with P. crocatum and C. burmannii mixture extract.

| Groups | Day 1 | Day 3 | Day 16 |
|---|---|---|---|
| KN | $14.65\pm1.3^{ab}$ | $15.7\pm1.2^{b}$ | $16.1\pm1.2^{b}$ |
| KP | $15.7\pm3.2^{a}$ | $14.9\pm3.4^{a}$ | $14.0\pm3.3^{a}$ |
| KDS 1 | $15.7\pm3.9^{a}$ | $15.2\pm4.6^{a}$ | $14.7\pm5.9^{a}$ |
| KDS 2 | $15.8\pm5.0^{a}$ | $15.6\pm3.1^{a}$ | $15.3\pm3.1^{a}$ |
| KDS 3 | $16.1\pm2.4^{a}$ | $15.4\pm4.2^{a}$ | $14.7\pm2.1^{a}$ |
| SM | $16.1\pm5.0^{a}$ | $15.9\pm4.8^{a}$ | $15.2\pm6.2^{a}$ |

Amounts that share the same superscript characters (a,b) are statistically similar at $p < 0.05$ ($n = 4$). Group KN is normal control (administered i.p. with NaCl 0.9% and p.o. with aquadest 2 mL per day for 16 days); group KP is diabetic control (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with aquadest 2 mL per day for 16 days); groups KDS 1, KDS 2, and KDS 3 are mixture extract treatment with different doses (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with extract 630 mg/kg body weight (KDS 1), 1260 mg/kg body weight (KDS 2), and 1890 mg/kg body weight (KDS 3) per day for 16 days); group SM was administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with *P. crocatum* extract 1350 mg/kg body weight per day for 16 days.

## 3.2 Effect of *P. crocatum* and *C. burmannii* Mixture Extract Treatment on Rat Blood Glucose Levels

Blood glucose levels were measured on days 0, 4, 9 and 15 in order to observe the effect of the aquadest or *P. crocatum* and *C. burmannii* mixture extract or *P. crocatum* extract administered orally to diabetic rats induced by streptozotocin.

Table 3 shows that on day 1, when the treatment had not yet started, the blood glucose levels of all the rats were significantly different ($p < 0.05$), while the range was normal (90-100 milligram/deciliter). On the third day, after induction with streptozotocin, the blood glucose levels of the rats had increased. The blood glucose levels for the other groups, i.e. groups KP, KDS 1, KDS 2, KDS 3 and SM, had increased up to 2- to 3-fold. The increase in blood glucose levels of the rats on the third day, after induction with streptozotocin, was significantly different ($p < 0.05$) compared with the KN group (normal control).

**Table 3** Rat blood glucose levels for 16 days of treatment with P. crocatum and C. burmannii mixture extract.

| Groups | Day 1 | Day 3 | Day 16 |
|---|---|---|---|
| KN | $100.00 \pm 7.44^{a}$ | $100.00 \pm 5.03^{a}$ | $92.00 \pm 8.76^{a}$ |
| KP | $100.50 \pm 6.56^{a}$ | $342.75 \pm 46.61^{b}$ | $383.75 \pm 73.49^{b}$ |
| KDS 1 | $104.75 \pm 16.50^{a}$ | $274.75 \pm 47.03^{b}$ | $141.00 \pm 67.19^{ab}$ |
| KDS 2 | $97.50 \pm 3.87^{a}$ | $308.50 \pm 19.43^{b}$ | $151.25 \pm 83.51^{a}$ |
| KDS 3 | $97.00 \pm 18.60^{a}$ | $306.25 \pm 48.49^{b}$ | $212.25 \pm 70.32^{ab}$ |
| SM | $100.00 \pm 5.48^{a}$ | $237.50 \pm 44.47^{b}$ | $145.00 \pm 57.00^{ab}$ |

Amounts that share the same superscript characters (a,b) are statistically similar at $p < 0.05$ ($n = 4$). Group KN is normal control (administered i.p. with NaCl 0.9% and p.o. with aquadest 2 mL per day for 16 days); group KP is diabetic control (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with aquadest 2 mL per day for 16 days); groups KDS 1, KDS 2, and KDS 3 are mixture extract treatment with different doses (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with extract 630 mg/kg body weight (KDS 1), 1260 mg/kg body weight (KDS 2), and 1890 mg/kg body weight (KDS 3) per day for 16 days); group SM was administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with *P. crocatum* extract 1350 mg/kg body weight per day for 16 days.

The blood glucose levels of the rats were reduced up to 30 to 51% after 16 days of treatment with various dosages of *P. crocatum* and *C. burmannii* mixture extract (groups KDS 1, KDS 2 and KDS 3) compared with the KP group. Then, the rats in groups KDS 1 and KDS 3 were able to reduce their blood glucose levels up to 48.69% and 30.68%, respectively, which were statistically similar ($p < 0.05$) to the blood glucose levels of the rats on day 3. However, after 16 days of treatment, group KDS 2 was able to reduce their blood glucose levels up to 50.98%, which was statistically different compared with group KDS 2 after 3 days. Nevertheless, treatment with *P. crocatum* extract at a dosage of 1350 mg/kg body weight for 16 days (group SM) was able to reduce the blood glucose levels of the rats up to 38.95%, which was statistically similar at $p < 0.05$ compared with the blood glucose levels of the rats on day 3 (group

SM). This indicates that *P. crocatum* and *C. burmannii* mixture extract at a dosage of 1260 mg/kg body weight could reduce the blood glucose levels of the rats towards a normal level.

The mixture extract of *P. crocatum* and *C. burmannii* at a dosage of 1260 mg/kg body weight in streptozotocin-induced diabetic rats reduced the level of blood glucose to 50.98%. This is a sign of antihyperglycemic activity. This reduction indicates higher antihyperglycemic activity than from treatment with *P. crocatum* extract at a dosage of 1350 mg/kg bw (38.95%). Diabetic rats that were induced by alloxan for 10 days had decreased blood glucose levels up to 10.46% after being given 322 mg/kg of *P. crocatum* extract [5]. This indicates that *C. burmannii* extract can increase antihyperglicemic activity. Diabetic rats that were induced by alloxan for 7 days had decreased blood glucose levels up to 73.50% after being given 0.240 mg/kg of *Morinda lucida* steam bark extract [14]. This indicates that the different species have different activity affecting blood glucose level reduction. This depends on the content of bioactive compounds in each species. The bioactive compounds in *P. crocatum* consist of alkaloid, flavonoid and tannin [5], while those in *C. burmannii* consist of cinnamaldehyde, cinnamic benzyl and eugenol [15-16]. The presence of bioactive compounds in the extract mixtures was able to reduce the blood glucose levels of the rats. This indicates that bioactive compounds can stimulate pancreatic β cells by releasing a large amount of insulin hormone, which brings glucose to the cells [17].

### 3.3 Effect of *P. crocatum* and *C. burmannii* Mixture Extract Treatment on Rat Insulin Levels

The rat blood insulin levels increased up to 13 to 170% (see Table 4) after 16 days of treatment with various doses of *P. crocatum* and *C. burmannii* mixture extract (groups KDS 1, KDS 2, and KDS 3). The rats in groups KDS 1, KDS 2, and KDS 3 were able to increase their blood insulin levels up to 50.34%, 170.75% and 13.61%, respectively, which is statistically similar at $p < 0.05$ compared with group KN (normal control) and group KP (diabetic control). The same pattern was observed in group SM, which was treated with *P. crocatum* extract at 1350 mg/kg body weight for 16 days. In this group, the blood insulin levels were increased up to 41.50%, which is statistically similar at $p < 0.05$ compared with groups KN and KP. This result indicates that *P. crocatum* and *C. burmannii* mixture extract at a dosage of 1260 mg/kg body weight affects the rats blood insulin towards a normal level.

In the present study, the mixture extract of *P. crocatum* and *C. burmannii* at 1260 mg/kg body weight administered to streptozotocin-induced diabetic rats increased blood insulin levels towards a normal level. This finding is similar to

the results of treatment with ginseng root and green tea extract at 400 mg/kg body weight, which increased the blood insulin levels in streptozotocin-induced diabetes rats towards a normal level too. This shows that increased rat blood insulin levels can reduce rat blood glucose levels. The increase of the blood insulin levels in the test animals was caused by the capability of the bioactive compounds in the mixture extracts of producing regeneration of pancreatic islets [18]. Alkaloid compounds are known to be responsible for stimulating the multiplication of pre-existing islet cells [19].

**Table 4** Effect of 16-Day treatment with P. crocatum and C. burmannii mixture extract on rat blood insulin levels.

| Groups | Insulin (µg/L) |
|---|---|
| KN | $6.85\pm5.46^{a}$ |
| KP | $1.47\pm0.31^{b}$ |
| KDS 1 | $2.21\pm0.41^{ab}$ |
| KDS 2 | $3.98\pm1.54^{ab}$ |
| KDS 3 | $1.67\pm0.23^{ab}$ |
| SM | $2.08\pm0.49^{ab}$ |

Amounts that share the same superscript characters (a,b) are statistically similar at $p < 0.05$ ($n = 4$). Group KN is normal control (administered i.p. with NaCl 0.9% and p.o. with aquadest 2 mL per day for 16 days); group KP is diabetic control (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with aquadest 2 mL per day for 16 days); groups KDS 1, KDS 2, and KDS 3 are mixture extract treatment at different doses (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with extract 630 mg/kg body weight (KDS 1), 1260 mg/kg body weight (KDS 2), and 1890 mg/kg body weight (KDS 3) per day for 16 days); group SM was administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with *P. crocatum* extract 1350 mg/kg body weight per day for 16 days.

## 3.4 Effect of *P. crocatum* and *C. burmannii* Mixture Extract Treatment on Rat Blood Lipid Levels

Measurement of the blood lipid levels was carried out after 16 days of oral treatment of streptozotocin-induced diabetic rats with aquadest or *P. crocatum* and *C. burmannii* mixture extract or *P. crocatum* extract. Table 5 shows that the rat blood lipid levels in all groups were statistically similar at $p < 0.05$. However, the blood triglyceride level in group KP tended to increase up to approximately 92.46% compared to the blood triglyceride level of group KN (normal control). This indicates that *P. crocatum* and *C. burmannii* mixture extracts and *P. crocatum* extract could maintain the rat blood lipid levels at a normal level.

**Table 5** Effect of 16-Day treatment with P. crocatum and C. burmannii mixture extract on rat blood lipid levels.

| Groups | Total Cholesterol (mg/dl) | HDL (mg/dl) | Triglyceride (mg/dl) |
|---|---|---|---|
| KN | 63.00±15.38[a] | 61.75±9.46[a] | 49.75±17.76[a] |
| KP | 55.75±7.68[a] | 46.00±8.08[a] | 95.75±37.88[a] |
| KDS 1 | 70.00±5.48[a] | 69.50±8.74[a] | 40.00±7.39[a] |
| KDS 2 | 66.75±11.95[a] | 61.75±12.97[a] | 52.00±11.79[a] |
| KDS 3 | 48.00±6.73[a] | 49.00±8.64[a] | 53.50±37.19[a] |
| SM | 63.75±8.66[a] | 58.50±12.40[a] | 69.50±45.27[a] |

Amounts that share the same superscript characters (a,b) are statistically similar at $p < 0.05$ ($n = 4$). Group KN is normal control (administered i.p. with NaCl 0.9% and p.o. with aquadest 2 mL per day for 16 days); group KP is diabetic control (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with aquadest 2 mL per day for 16 days); groups KDS 1, KDS 2, and KDS 3 are mixture extract treatment at different doses (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with extract 630 mg/kg body weight (KDS 1), 1260 mg/kg body weight (KDS 2), and 1890 mg/kg body weight (KDS 3) per day for 16 days); group SM was administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with *P. crocatum* extract 1350 mg/kg body weight per day for 16 days.

## 3.5 Effect of *P. crocatum* and *C. burmannii* Mixture Extract Treatment on Number of Rat Pancreatic Islets

To understand more about the molecular mechanism of *P. crocatum* and *C. burmannii* mixture extract and *P. crocatum* extract in reducing hyperglycemia via insulin production from pancreatic islets (see Figure 1), we evaluated the quantities of Langerhans islets and pancreatic β cells (see Table 6).

**Table 6** Effect of 16-day treatment with P. crocatum and C. burmannii mixture extract on rat Langerhans Islet and number of β cells.

| Groups | Langerhans islets | β cells |
|---|---|---|
| KN | 29.50±0.71[a] | 166.00±9.90[a] |
| KP | 4.50±0.71[c] | 66.50±7.78[c] |
| KDS 1 | 9.00±1.41[bc] | 112.00±7.07[b] |
| KDS 2 | 15.00±2.83[b] | 143.50±10.61[ab] |
| KDS 3 | 7.00±4.24[bc] | 103.00±4.24[b] |
| SM | 10.00±1.41[bc] | 105.50±9.19[b] |

Amounts that share the same superscript characters (a,b) are statistically similar at $p < 0.05$ ($n = 4$). Group KN is normal control (administered i.p. with NaCl 0.9% and p.o. with aquadest 2 mL per day for 16 days); group KP is diabetic

control (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with aquadest 2 mL per day for 16 days); groups KDS 1, KDS 2, and KDS 3 are mixture extract treatment at different doses (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with extract 630 mg/kg body weight (KDS 1), 1260 mg/kg body weight (KDS 2), and 1890 mg/kg body weight (KDS 3) per day for 16 days); group SM was administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with *P. crocatum* extract 1350 mg/kg body weight per day for 16 days.

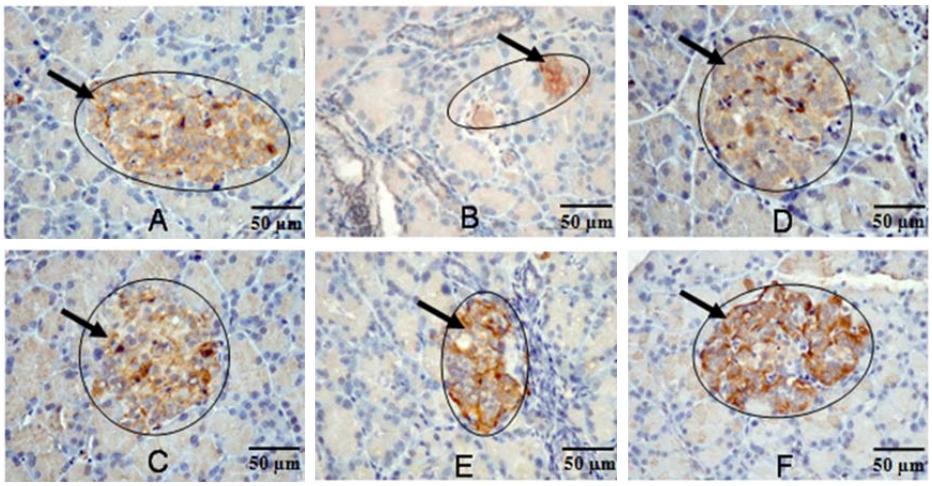


**Figure 1** Insulin immunostaining on β-cells (brown) in islets of Langerhans. The scale bars are 50 µm. (A) Group KN is normal control (administered i.p. with NaCl 0.9% and p.o. with aquadest 2 mL per day for 16 days); (B) group KP is diabetic control (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with aquadest 2 mL per day for 16 days); (C) groups KDS 1, (D) KDS 2 and (E) KDS 3 are mixture extract treatment at different doses (administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with extract 630 mg/kg body weight (KDS 1), 1260 mg/kg body weight (KDS 2), and 1890 mg/kg body weight (KDS 3) per day for 16 days); (F) group SM was administered i.p. with streptozotocin 50 mg/kg body weight and p.o. with *P. crocatum* extract 1350 mg/kg body weight per day for 16 days.

The numbers of Langerhans islets decreased up to 6.6-fold in 16 days after treatment with aquadest and streptozotocin induction. Nevertheless, the numbers of Langerhans islets increased up to 1.6- to 3.3-fold after 16 days of treatment with various doses of *P. crocatum* and *C. burmannii* mixture extract (KDS 1, KDS 2 and KDS 3). The rats in groups KDS 1 and KDS 3 were able to increase their numbers of Langerhans islets up to 2-fold and 1.6-fold, respectively, which is both statistically similar at $p < 0.05$ compared with group KP. The same pattern was observed in group SM, which was treated with *P.*

*crocatum* extract at 1350 mg/kg body weight for 16 days. This group was able to increase the number of Langerhans islets up to 2.2-fold. This increased number was statistically similar at $p < 0.05$ compared with group KP (diabetic control). However, group KDS 2 was able to increase their numbers of Langerhans islets up to 3.3-fold, which is statistically different compared with groups KN and KP. This indicates that the *P. crocatum* and *C. burmannii* mixture extract at a dosage of 1260 mg/kg body weight led the number of Langerhans islets towards a normal level.

Streptozotocin can be used to induce diabetic in rats by destroying pancreatic β cells [20]. In the present study it was shown that *P. crocatum* and *C. burmannii* mixture extract at a dosage of 1260 mg/kg body weight increased the number of pancreatic β cells towards a normal level. This finding is similar to the results from Karaca, *et al.* [21], where diabetic rats were given an extract of ginseng at a dosage of 400 mg/kg body weight for 42 days and the treatment aimed at improving insulin-immunoreactivity showed a significant positive effect on the number of β cells in the Langerhans islets. The present study indicates that *P. crocatum* and *C. burmanni* mixture extract can reduce blood glucose levels in rats by enhancing the number of pancreatic β cells in order to increase the rat blood insulin level.

## 4 Conclusion

The present study showed that antihyperglicemic activity of *P. crocatum* and *C. burmannii* mixture extract enhances the number of pancreatic β cells in rats, which can increase the rats' blood insulin levels, which in turn can reduce the rats' blood glucose levels. In addition, *P. crocatum* and *C. burmannii* mixture extract can maintain blood lipid levels in rats at a normal level. Further research is needed to investigate the antihyperglicemic activity of the bioactive compounds in *P. crocatum* and *C. burmannii* mixture extract. Also, more information is needed about the molecular mechanism of the antihyperglicemic activity of *P. crocatum* and *C. burmannii* mixture extract.

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## References


[1] American Diabetes Association, *Standards of Medical Care in Diabetes-2011*, Diabetes Care, **35**(1), pp. 564-571, 2012.

[2] King, H., Aubert, R.E. & Herman, W.H., *Global Burden of Diabetes 1995-2025: Prevalence, Numerical Estimates and Projections*, Diabetes Care, **21**(9), pp. 1414-1431, 1998.

[3] Boyle, J.P., Honeycutt, A.A., Narayan, K.M., Hoergoer, T.J., Geiss, L.S., Chen, H. & Thompson, T.J., *Projection of Diabetes Burden through 2050: Impact of Changing Demography and Disease Prevalence in the U.S.*, Diabetes Care, **24**(11), pp. 1936-1940, 2001.

[4] Rates, S.M., *Plants as A Source of Drugs*, Toxicon, **39**(5), pp. 603-613, 2001.

[5] Safithri, M. & Fahma, F., *Potency of Piper crocatum Decoction as an Antihiperglycemia in Rat Strain Sprague Dawley*, Hayati Journal of Bioscience, **15**(1), pp. 45-48, 2008.

[6] Satyanarayana, T., Katyayani, B.M., Hema, L.E., Anjana, A.M. & Chinna, E. M., *Hypoglycemic and Anti-hyperglycemic Effect of Alcoholic Extract of Euphorbia Leucophylla in Normal and in Alloxan Induced Diabetic Rats*, Pharmacoognosy Magazine, **2**(8), pp. 244-255, 2006.

[7] Battu, G.R., Mamidipalli, S.N., Parimi, R., Viriyala, R.K., Patchula, R.P. & Mood, L.R., *Hypoglycemic and Anti-Hyperglycemic Effect of Alcoholic Extract of Benincasa Hispida in Normal and in Alloxan Induced Diabetic Rats*, Pharmacoognosy Magazine, **3**(10), pp. 101-105, 2007.

[8] Tapas, A.R., Sakarkar, D.M. & Kakde, R.B., *Flavonoids as Nutraceuticals: A Review*, Tropical Journal of Pharmaceutical Research, **7**(3), pp. 1089-1099, 2008.

[9] Shan, B., Cai, Y.Z., Brooks, J.D. & Corke, H., *The In-vitro Antibacterial Activity of Dietary Spice and Medicinal Herb Extracts*, International Journal of Food Microbiology, **117**(1), pp. 112-119, 2007.

[10] Preuss, H.G., Echard, B., Polansky, M.M. & Anderson, R., *Whole Cinnamon and Aqueous Extracts Ameliorate Sucrose-induced Blood Pressure Elevations in Spontaneously Hypertensive Rats*, Journal of the American College of Nutrition, **25**(2), pp. 144-150, 2006.

[11] Khan, A., Safdar, M., Khan, M.M.A., Khattak, K.N. & Anderson, R.A., *Cinnamon Improves Glucose and Lipids of People with Type 2 Diabetes*, Diabetes Care, **26**(12), pp. 3215-3218, 2003.

[12] Falah, S., Safithri, M., Katayama, T. & Suzuki, T., *Hypoglycemic Effect of Mahagony (Swietenia macrophylla king) Bark Extracts in Alloxan-induced Diabetic Rats*, Wood Research Journal, **2**(1), pp. 89-94, 2010.

[13] Mallick, C., Chatterjee, K., Guha Biswas, M. & Ghosh, D., *Antihyperglycemic Effects of Separate and Composite Extract of Root of*

*Musa Paradisiaca and Leaf of Coccinia Indica in Streptozotocin-induced Diabetic Male Albino Rat*, Afr. J. Trad. CAM, **4**, pp. 362-371, 2007.

[14] Odutuga, A.A., Dairo, J.O., Minari, J.B. & Bamisaye, F.A., *Antidiabetic Effect of Morinda lucida Steam Bark Extracts on Alloxan-induced Diabetic Rats*, Research Journal of Pharmacology, **4**(3), pp. 78-82, 2010.

[15] Paranagama, P.A., Wimalasena, S., Jayatilake, G.S., Jayawardena, A.L., Senanayake, U.M. & Mubarak, A.M., *A Comparison of Essential Oil Constituents of Bark, Leaf, Root and Fruit of Cinnamon (Cinnamomum zeylanicum Blum) Grown in Sri Lanka*, J. Natn. Sci. Foundation Sri Lanka, **29**, pp. 147-153, 2001.

[16] Schmidt, E., Jirovetz, L., Buchbauer, G., Eller, G.A., Stoilova, I., Krastanov, A.I., Stoyanova, A. & Geissler, M., *Composition and Antioxidant Activities of the Essential Oil of Cinnamon (Cinnamomun zeylanicum Blume) leaves from Sri Lanka*, Journal of Essential Oil Bearing Plants, **9**(2), pp. 170-182, 2006.

[17] Jadhav, R. & Puchchakayala G., Hypoglycemic *and Antidiabetic Activity of Flavonoids: Boswellic Acid, Ellagic Acid, Quercetin, Rutin on Streptozotocin-Nicotinamide Induced Type 2 Diabetic Rats*, International Journal of Pharmacy and Pharmaceutical Sciences, **4**(2), 2012.

[18] Wisetmuen, E., Pannangpetch, P., Kongyingyoes, B., Kukongviriyapan., U., Yutanawiboonchai, W. & Itharat, A., *Insulin Secretion Enhancing Activity of Roselle Calyx Extract in Normal and Streptozotocin-induced Diabetic Rats,* Pharmacognosy Research, **5**(2), pp. 65-71, 2013.

[19] Singh, N., & Gupta, M., *Regeneration of β Cells in Islets of Langerhans of Pancreas of Alloxan Diabetic Rats by Acetone Extract of Momordica Charantia (Linn.) (Bitter Gourd) Fruits*, Indian Journal of Experimental Biology, **45**, pp. 1055-1062, 2007.

[20] Szkdelski, T., *The Mechanism of Alloxan and Streptozotocin Action in β Cells of the Rat Pancreas, Physiological Research*., **50**(1), pp. 536-546, 2001.

[21] Karaca, T., Yoruk, M., Yoruk, I.H. & Uslu, S., *Effect of Extract Green Tea and Gingseng on Pancreatic Beta Cells and Levels of Serum Glucose, Insulin, Cholesterol, Triglycerides In Rats with Experimentally Streptozotocin Induced Diabetes: A Histochemical And Immunohistochemical Study*, Journal of Animal Veterinary Advances, **9**(1), pp. 102-107, 2010.