ORIGINAL ARTICLE

Hypoglicemic and Antioxidant Activity of *Petiveria alliacea* in Diabetic Rat Models

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ABSTRACT

Introduction: Diabetes mellitus is a degenerative disease characterized by chronic hyperglycemia conditions in the body. Various complications of diabetes mellitus are caused by oxidative stress condition. Petiveria alliacea (*P. alliacea*) is a potential plant and easy to grow in hot regions. Leaf extracts of *P. alliacea* contain flavonoids and tannins which work as antidiabetic and antioxidant. In addition, other compounds found in P. alliacea leaf extracts like linoleic acid and allantoin show an increase in insulin secretion. Therefore, this study aimed to determine the antidiabetic activity of ethanolic extract of *P. alliacea*.
Methods: We investigated the hypoglycemic and antioxidant effect of *P. alliacea* on STZ-induced diabetic rats. Rats were randomly divided into six groups named normal control, diabetes control, metformin (150 mg/kg/d), low dose of P. alliacea (90 mg/kg/d), intermediate dose (180 mg/kg/d), and high dose (360 mg/kg/d). Rats were orally given the treatment daily in the morning for fourteen days. At the end of the study, blood glucose level was measured and rats were sacrificed to measure blood malondialdehyde level.malondialdehyde
Results: *P. alliacea* extract dose of 90 mg/kg and 360 mg/kg, and also metformin significantly decrease blood glucose levels. *P. alliacea* extract dose of 360 mg/kg was able to lower blood malondialdehyde level significantly which were not obtained on metformin.
Conclusion: This finding suggests that ethanolic extract of *P. alliacea* possess antidiabetic effect at least on rats.

Introduction

Diabetes mellitus (DM) is a degenerative disease that is experienced by most people in the world. According to WHO[1], 347 million people worldwide suffer from diabetes mellitus and is expected in 2030, diabetes mellitus will be the seventh leading cause of death in the world. The deaths allegedly due to complications of diabetes affect various organs of the body. In Indonesia, the estimated prevalence will increase to 21.3 million in 2030.[2]

DM is a condition of hyperglycemia in the body. DM can be caused due to the disruption of the synthesis and secretion of insulin, and also can be caused by the resistance of tissue sensitivity to insulin. Various complications of diabetes are caused by the imbalance condition of oxidants and antioxidants known as oxidative stress. Highly reactive free radicals can cause tissue damages.[3] This leads to complications in many organs. Oxidative stress in the body can be determined by measuring the levels of malondialdehyde (MDA) in the blood which is the result of lipid peroxidation. MDA levels of blood increase along with oxidative stress in the body.

Petiveria alliacea is a plant often used to treat various diseases. This plant is very useful in the health fields, such as cancer, diabetes, muscular pain, skin diseases, various central nervous system disorders, respiratory and pulmonary infections, and malaria.[4] In addition, *P. alliacea* also showed a protective effect against *L. monocytogenes* and immunomodulatory effect on hematopoiesis.[5]

The contents of *P. alliacea* leaf extract are flavonoids types astilbi, engeletin, and leridal-chalcone; flavanone types leridal, leridol, and leridol-5-methyl ether; flavonol types myricitrin; benzyl-2-hydroxyethylsulfide; isoarborinol; isoarborinol astetat; isoarborinol cinnamate; alkaloid types of allantoin; triterpene species ilexgenin; senfol, tannins, and polyphenols.[6,7] *P. alliacea* leaves are also known to contain lipids such as lignoceryl lignocerate, linoleic acid, nonadecanoic acid, palmitic acid, stearic acid, as well as inorganic compounds such as KNO_3.[7]

Tannins in *P. alliacea* could provide a hypoglycemic effect. Linoleic acid and allantoin can stimulate insulin secretion.[8,9]

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In addition, tannins and flavonoids also have antioxidant effects through capture free radicals, enhance the activity of endogenous antioxidants, and suppress oxidative stress. P. *alliacea* has an antioxidant capacity by 54%.[10] With its ability as an antioxidant, *P. alliacea* can suppress oxidative stress and reduce the complications of diabetes.

This study aims to determine the hypoglycemic and antioxidant activity of the ethanolic extract of *P. alliacea* in diabetic rats.

Methods

Plant materials

P. alliacea L. is an accepted Latin name, registered in The Plant List website. Leaves of *P. alliacea* were collected from Materia Medica, Batu, East Java, Indonesia. The identification made by Dr. Husin RM, Apt., M.Kes as chief of Materia Medica with certificate number 074/137/101.8/2015.

Preparation of extract

The extract used is the result of maceration using 96% ethanol at the Laboratory of Pharmacology, Faculty of Medicine, Universitas Airlangga. Preparation of extract followed protocol implemented by Pharmacology Department of Universitas Airlangga. Dried powder of *P. alliacea* leaves (500 g) were soaked in 96% aquaeus etanol within 3x24 hours (2 l, 1.5 l, 1.5 l) at room temperature. The leaves extract was filtered and concentrated by heating in a waterbath at temperature of 45°C in order to obtain a thick leaf extract of *P. alliacea*.

The extract was suspended in 1% CMC-Na at concentration of 9 mg/ml, 18 mg/ml, and 36 mg/ml. To make this, sequentially, 900 mg, 1,800 mg, and 3,600 mg extracts were dissolved in 100 ml of 1% CMC-Na. Different volume is given depends on the bodyweight of rats. The new suspension made every 6 days.

Animals

Experimental animals used were healthy males Wistar strain albino rats (*Rattus norvegicus* L.) aged 2-3 months with bodyweight ± 200 grams. Rats were obtained from the Laboratory of Pharmacology, Faculty of Medicine, Universitas Airlangga. Selection of male sex aims to reduce hormonal influences. All animals were accustomed for approximately 1 week in clean cage with ad libitum water and food. The animals were treated in accordance with the standard guideline. The research work was approved by the Institutional Ethics Committee of Medical Research, Faculty of Medicine, Universitas Airlangga (174/EC/KEPK/FKUA/2015).

Induction of experimental diabetes and experimental design

Streptozotocin (STZ) used was purchased from Department of Physiology, Faculty of Medicine, Universitas Airlangga. Induction of diabetes followed induction protocol implemented by Purwanto and Liben.11. Rats were fasted for 4 hours and received single intraperitoneal injection of 50 mg/kg STZ which was freshly dissolved in 0.01 mol/L citrate buffer (pH 4.5). After injection, rats were given 10% w/v dextrose solution to prevent sudden hypoglycemic post-injection. Two days after injection, rats were fasted for 6 hours and blood glucose levels were measured. Rats with blood glucose levels ≥200 mg/dl were used.

On third day, rats were divided randomly into 6 groups of 6 rats each. The first group (N) was normal rats treated with 1% CMC-Na. Negative control group (NC), also treated with 1% CMC-Na was used as negative control. Positive control group (PC) were given metformin at doses 150 mg/kg. Metformin dose was determined based on Reagan-Shaw et al. Formula.12 Groups Pal90, Pal180, and Pal360, served as *P.alliacea*-treated groups and received extract at doses 90, 180, and 360 mg/kg respectively. Metformin and *P.alliacea* were dissolved in a 1% CMC-Na. Therapy was given intragastrically every morning for 14 days.

Blood glucose level measurement

After 14 days of treatments, rats were fasted for 6 hours and blood samples were taken from the lateral tail vein. Blood glucose level was measured using EasyTouch GCU (Bioptik Technology Inc.).

Blood malondialdehyde (MDA) level measurement

At the end of the study (day 15) levelof MDA was measured. The rats were sacrificed and blood was drawn from the heart. Measurement of MDA using Esterbauer and Cheeseman13 technique modified by the researcher and conducted at the Laboratory of Biochemistry, Faculty of Medicine, Universitas Airlangga. 500 µl sample taken and added 4.5 ml of cold PBS (phosphate buffered saline). 4 ml of the supernatant is then taken and added to 1 ml of 15% w/v Trichloroacetic acid (TCA). Furthermore, given 1 ml of 0.37% w/v Thiobarbituric acid (TBA) solution in 0.25 N HCl and heated in a waterbath at temperature 80°C for 15 minutes. Then cooled at room temperature for 60 minutes, and centrifuged at speed of 3,000 rpm for 15 minutes. Supernatant absorbance is then measured on a spectrophotometer at $\lambda = 532$ nm.

Statistical analysis

All raw datas were analyzed using one-way ANOVA with Welch Test F continued with Games-Howell Post Hoc test, Paired t-test, and Wilcoxon Signed Rank test. Statistical analysis is using SPSS 17.0 statistical package for Windows. All values were displayed as mean±SD. P values < 0.05 were considered as significant.

Results

There was no difference in blood glucose level between groups before induction of diabetes. After 48 hours of injection, blood glucose level significantly increased and persisted until the end of the study (Figure 1).

As shown in table 1, blood glucose level was decreased in *P. alliacea* leaf extracts and metformin treated-groups. Unlike metformin, a significant reduction in MDA level was found in the group treated with *P. alliacea* at dose 360mg/kg compared to negative control group (p=0.021).

P. alliacea shows hypoglicemic activity with chronic treatment of diabetic rats

After administration of *P. alliacea* leaf extracts for 14 days, blood glucose level was found to decrease significantly from 374.8 mg/dl to 222.5 mg/dl (90 mg/kg) and 420 mg/dl to 209.2 mg/dl (360 mg/kg). Metformin as an oral antidiabetic used in this experiment was also showed a significant reduction of blood glucose level (Figure 2).

***P. alliacea* shows antioxidant activity with chronic treatment of diabetic rats**

MDA level in the diabetic group (7.048 nmol/ml) was increased compared with non-diabetic (6.747 nmol/ml) although statistically not found a significant increase ($p = 0.098$). A significant reduction in MDA level was found in the group treated with P. alliacea at dose 360mg/kg compared to negative control group ($p = 0.021$). . As shown in Figure 3, the higher dose of the extract, the lower the blood level of MDA. This showed that higher antioxidant level was achieved while increasing the dose.

Table 1. Blood glucose level and blood MDA level of the experimental rats

Group	Average of blood glucose level ± SD (mg/dl)			Average of blood MDA level ± SD (nmol/ml)
	Pre-STZ	Pre-test	Post-test	
Normal rats (N)	114.6 ± 13.3	109.0 ± 10.3	107.4 ± 9.4	6.747 ± 0.820
Negative control (NC)	118.4 ± 13.0	400.1 ± 130.6	309.0 ± 147.3	7.048 ± 1.419
Metformin treated-rats (PC)	120.7 ± 9.4	427.0 ± 66.4	215.3 ± 92.8	4.751 ± 0.756
90 mg/kg Pal-treated rats (Pal90)	121.5 ± 12.4	374.8 ± 63.4	222.5 ± 104.3	6.312 ± 4.486
180 mg/kg Pal-treated rats (Pal180)	115.1 ± 12.2	401.3 ± 64.4	365.4 ± 111.7	4.040 ± 2.457
360 mg/kg Pal-treated rats (Pal360)	128.2 ± 7.9	420.0 ± 47.5	209.2 ± 66.7	2.101 ± 0.423

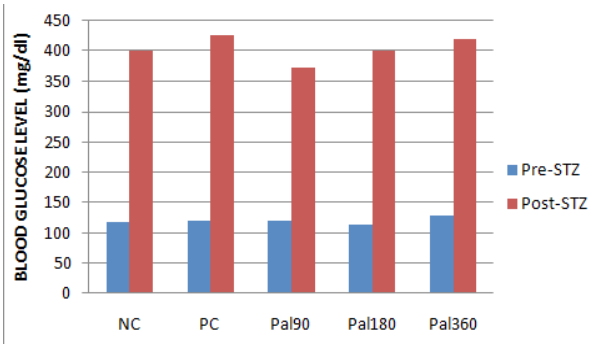

Figure 1 Comparison blood glucose level before and after STZ induction.

*NC: diabetic control rats
PC: metformin-treated diabetic rats
Pal90: diabetic rats treated with *P.alliacea* extract of 90 mg/kg
Pal180: diabetic rats treated with *P.alliacea* extract of 180 mg/kg
Pal360: diabetic rats treated with *P.alliacea* extract of 360 mg/kg.
Blood glucose level was measured after 6 hours fasting.

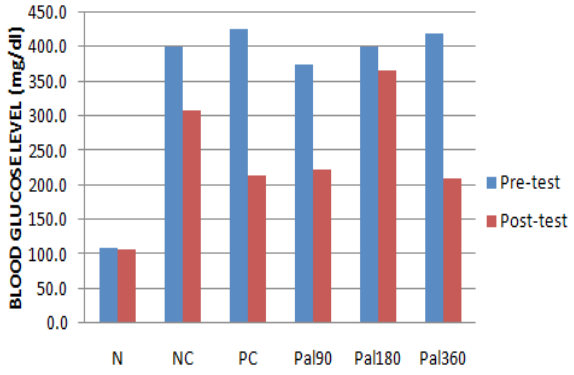

Figure 2. Effect of P. alliacea extract on pre-test and post-test blood glucose level of the experimental rats.

*N: normal rats
NC: diabetic control rats
PC: metformin-treated diabetic rats
Pal90: diabetic rats treated with *P.alliacea* extract of 90 mg/kg
Pal180: diabetic rats treated with *P.alliacea* extract of 180 mg/kg
Pal360: diabetic rats treated with *P.alliacea* extract of 360 mg/kg.
Blood glucose level was measured after 6 hours fasting

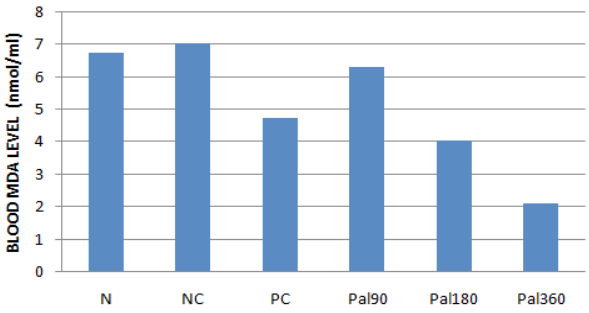

Figure 3. Effect of P. alliacea extract on blood MDA level of the experimental rats

*N: normal control rats
NC: diabetic control rats
PC: metformin-treated diabetic rats
Pal90: diabetic rats treated with *P.alliacea* extract of 90 mg/kg
Pal180: diabetic rats treated with *P.alliacea* extract of 180 mg/kg
Pal360: diabetic rats treated with *P.alliacea* extract of 360 mg/kg.
Blood glucose level was measured after 6 hours fasting.

Discussion

DM is a degenerative disease characterized by chronic hyperglycemia in the body affecting millions of people worldwide. The deaths allegedly due to complications of diabetes which affect various organs of the body. New treatment using plants with fewer side effects began to be utilized and studied for many years. In this study, antidiabetic effect of *P. alliacea* leaves in STZ-induced type 2 DM rats was studied. STZ has selective toxic effects on pancreatic β cells that reach 90% success rate.[11] STZ causes the death of pancreatic β-cells through a process of alkylation on the DNA and the formation of ROS that lead into DNA fragmentation.[14,15] As a result, a significant increase in blood glucose level was obtained 48 hours post-injection (Figure 1).

When *P. alliacea* ethanolic leaves extract was administered for 14 days, it showed valuable hypoglycemic effect and significantly decreased blood glucose level almost same level as metformin-treated group especially at dose 90mg/kg and 360mg/kg (Figure 2). Lores and Cires (1990) found that extract from leaf and stem powder

of *P. alliacea* was able to lower blood glucose level more than 60% after one-hour oral administration.[16] This effect may due to *P. alliacea* containing flavonoids that increase the activity of endogenous antioxidant enzymes and regenerate damaged pancreatic β cells.[17] It has been found that antioxidants can improve insulin sensitivity. One of the oldest oral antidiabetic and commonly used as first line treatment in DM is metformin, which also works as insulin sensitizer. Few studies showed that metformin can decrease free radicals and restore antioxidant status which explained its protective role in preventing cardiovascular complications.[18,19]

Besides its antioxidant activity, *P. alliacea* leaves is thought to have a stimulating effect on insulin secretion. *P. alliacea* contain an unsaturated free fatty acids named linoleic acid which can stimulate insulin secretion and increase glucose-induced insulin secretion in rats in a dose-dependent manner.[8] In addition, in a dose-dependent manner, allantoin compounds are also known can increase insulin secretion and decrease basal blood glucose level.[9] Tannins have also been observed to increase insulin activity and enhance the glucose uptake through mediators of the insulin-signalling pathways, especially in adipocyte. These effects are proven by similarity of dose-response curve of tannin-induced glucose transport with insulin.[20,21,22] Phenolic compounds have been attributed in induction of β cell regeneration and direct action in adipose tissue that enhance the insulin activity.[22]

Interestingly, as seen in Figure 2, it was found the dose-response curves of *P. alliacea* was non-monotonic. Non-monotonic dose-response (NMDR) may presents as a bell-shaped or U-shaped profile with the highest responses at low and high dose as seen in our study.[23] Several studies have described the effects of chemical compounds that can generate NMDR curves such as endocrine disruptor chemicals (EDC).[24] These compounds affect the hormone system in the synthesis, secretion, transport, binding, and metabolism of hormones. These compounds can also mimic or block the effects of hormones and cause effect on sensitive tissues, including endocrine tissues, through several mode of actions.[23,25] This finding may suggests that *P. alliacea* leaf extracts contain chemical compounds that can work as hormones or affect the endogenous hormone.

Hyperglycemic conditions trigger oxidative stress that can be measured by MDA level. Many experimental studies have shown the potential of plant derived antioxidant as treatment of DM. In this study, *P. alliacea* leaves extract decreased blood MDA levels. These results is in line with previous study showed *P. alliacea* had antioxidant effect by 54%.[10] *P. alliacea* leaves extract contains flavonoids and tannins that can work as an antioxidant and reduce oxidative stress condition. Flavonoids can work directly as an antioxidant which protect the body from free radicals and also increase the activity of antioxidant enzymes.[17] Tannins are not only enhanced the activity of endogenous antioxidant, but also increased the antioxidant levels. Tannins also decreased the MDA concentrations in liver, heart, and kidney.[26] Figure 3 shows that *P. alliacea* leaves extract decreased blood MDA levels and its relationship was dose-dependent. This means that the higher dose of the extract, the lower the blood level of MDA.

Conclusion

P. alliacea has a direct hypoglycemic effect and is thought to have a stimulating effect on insulin secretion and enhancing insulin activity. Moreover, *P. alliacea* can also work as an antioxidant. This antioxidant effect is not obtained on metformin.

Conflict of Interest

The author stated there is no conflict of interest

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