

Antihyperglycemic and Antihyperlipidemic Effects of Methanolic Seeds Extract of *Pandanus odoratissimus* in Alloxan-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is one of the most serious diseases affecting a larger global population, accompanied with other long-term complications. Also, the management of this disease comes with various side effects, hence, the need for substances with little or no side effect. Therefore, this study aimed at evaluating the antidiabetic and antihyperlipidemic effects of methanolic extract of *Pandanus odoratissimus* seeds in alloxan-induced diabetic rats. 30 adult male rats (Sprague Dawley) weighing 180-220 g were randomly selected and placed into 6 groups of 5 animals each. The alloxan-induced diabetic rats were orally supplemented with three doses of *P. odoratissimus* methanolic seed extracts, 300, 600 and 900 mg/kg bw, or 0.5 mg/kg of glibenclamide for 2 weeks. The glucose, HbA1c, and lipid profile were measured from the blood sample taken from all studied groups. The results were expressed as mean \pm SEMs and compared with repeated measurement using SPSS Version 19.0. The methanolic seed extract of *P. odoratissimus* significantly reduced ($p < 0.05$) the blood glucose level, with a high dose producing 79.15% reduction after 14 days, compared with the control and glibenclamide-treated groups. Furthermore, the seeds extract significantly reduced the concentration of HbA1c, TC, TG and LDL while increasing HDL in alloxan-induced diabetic rats. The *P. odoratissimus* could be used to effectively manage diabetes and other related complications.

Keywords: *Pandanus odoratissimus*, seed, methanolic extract, alloxan, glibenclamide.

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INTRODUCTION

Diabetes is a long-term disease affecting the lives and well-being of individuals, families, and societies at large. This chronic condition is among the top 10 causes of death in adults, and estimated to have caused over four million deaths globally in 2017(1). Diabetes mellitus is a complex disorder characterized by hyperglycemia due to malfunction in insulin secretion, caused by impaired metabolism of glucose, lipids and protein (2). Its complication is linked with oxidative stress induced by hyperglycemia suppressing the body's natural anti-oxidant system (3). In the later stages of this condition, lipid metabolism is affected, in the form of hyperlipidemia and hypercholesterolemia, which are risk factors in atherosclerosis (4). Also, the frequency of hyperlipidemia in diabetic patients is usually very high, depending on the type of diabetes and its degree of control (5).

The disease is managed through insulin injection and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides (6). However, most antidiabetic medications usually come with various side effects, such as liver problem, lactic acidosis, diarrhea and other secondary issues (7). Managing the disease without any side effects is still a challenge in the medical system. This has led to an increase in demand for natural products with anti-hyperglycemic property having little or no side effects. Hence, natural drugs are receiving more attention due to their safety. Thus, plants with antidiabetic activities are vital in the development of diabetes mellitus drugs (8). Also, there are many medicinal herbs which have been recommended for the treatment of diabetes (9).

One of these medicinal plants is *Pandanus odoratissimus* which is member of Pandanaceae family. It is a coarsely branched tree, with open crown and aerial roots close to its base. This plant is popularly known as "screw pine" and locally called "pandan samak". It is widely distributed in Indonesia and commonly found on sandy beaches, littoral thickets, on the edges of brackish marshes and mangrove, and inland along watercourses with low altitudes (10) (11). The leaves of *P. odoratissimus* have been reported to be useful for preventing epilepsy (12)]; infertility (13); inflammation (14) and as CNS-depressant (15). Also, its roots have been reported to have anticancer (16); and antidiabetic effects in patients (17); the flowers are antidiabetic (18), while its fruits are useful as anti-inflammatory and analgesic substances (19). The active component of the leaves extract include alkaloid, flavonoid and saponin (13) while alkaloid, glycosides, steroid, tannin and phenol are the active component of the fruit extract (19).

According to Tiwari and Rao (20) and Hakim et al (21), plants with flavonoid, alkaloid and glycosides possess some antioxidant and antidiabetic properties. Flavonoid reproduce the damaged cell of pancreas and saponin while polyphenol compounds reduce the transportation of glucose transport by suppressing sodium glucose co-transporter 1 (S-GLUT) in the intestine. Similarly, Yang et al (22) reported that flavonoid and polyphenol have lipid lowering effects by stimulating glucose utilization in peripheral tissues. There are limited research on the seed of *P. odoratissimus*, as previous studies on focused on the leaves, roots, flowers and fruits. Therefore, this study aimed at evaluating the antidiabetic and antihyperlipidemic effect of methanolic extract of *P. odoratissimus* seeds and the

biochemical parameters for the management of diabetes in alloxan-induced rats.

MATERIALS AND METHODS

Plant Material

The *P. odoratissimus* seeds were collected from Calang village, Aceh Jaya district, Aceh Province. The plant fruits from which the seeds were collected were mature, shown by the orange color of its exoderm. These seeds were shade dried, and mechanically grinded to a powder form.

Preparation of Methanolic Extract of Pandanus odoratissimus Seed

About 1000 g of the seed powder were subjected to cold maceration using 98% methanol for 15 days, while changing the solvent every 5 days. The collected solvent were filtered with whatman paper and then evaporated at 40°C under low pressure till a semisolid residue was obtained.

Preliminary Phytochemical Screening

The qualitative phytochemical evaluation of the methanolic extracts was conducted to determine the presence or absence of flavonoids through Shinoda test, sterols through Libermann Buchard test, phenols through ferric chloride test, alkaloids through Dragendorff test, and saponins through saponification test, as described by (26).

Antioxidant Activity

DPPH Radical Scavenging Activity

The free radical scavenging capacity of the extracts was determined using DPPH (27). DPPH solution (0.004% w/v) was prepared in 95% methanol, which was then mixed with the seeds extracts to prepare the stock solution of 5 mg/ml. Freshly prepared DPPH solution (0.004% w/v) was dispensed into test tubes after which the seed extract was added. This was followed by the serial dilution (1 to 500 µg) of each test tube to a final volume of 3 ml, then the absorbance read at 515 nm using a spectrophotometer after 10 minutes. Ascorbic acid was used as a reference standard and dissolve in distilled water to make a stock solution of 5 mg/ml. The control sample prepared has same volume but with no extract and ascorbic acid, as the 95% methanol was served as blank. Then, the percentage scavenging of the DPPH free radical was measured using the equation:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

The inhibition curve was plotted for duplicate experiments and represented as percentage of mean inhibition \pm standard deviation IC₅₀ values were obtained by probit analysis (28).

Experimental Animals

Adult male sprague Dawley weighing 180-220 g were used in this study. The animals were kept in cages with standard laboratory condition (temperature 22-23°C with a 12/12 hr light-dark cycle), in animal house of Faculty of Biology, Universitas Nasional Jakarta, Indonesia. Food and water were the available ad libitum. The experimental procedure was approved by the Ethic Committee of Health research from Universitas Pembangunan Nasional "Veteran Jakarta, Indonesia No. B/1719/3/2019/KEPK.

Induction of Diabetes Mellitus

The animals were allowed to acclimatize for one week and diabetic models were made by chemically inducing the rats with freshly prepared solution of alloxan monohydrate dissolved in NaCl 0.9%, at a dose of 125 mg/kg body weight. This was injected by intraperitoneally, then the blood glucose level of the rats with hyperglycemia were measured through the tail vein using glucometer. The concentration of glucose level above 200 mg/dl was considered in the experiment.

Experimental Design

All animals were randomly divided into six group (n=5) and treated daily for 2 weeks as follows:

Group I (CN) : made up of normal rats, orally given water and food.

Group II (DM): made up of diabetic rats which received alloxan 125 mg/kg b.w through intraperitoneal injection

Group III (DM+Gliben): made up of diabetic rats were received glibenclamide 5 mg/kg b.w through intraperitoneal injection

Group IV (DM+ MEPOS 300) : made up of diabetic rats orally given *P. odoratissimus* methanolic seed extract 300 mg/kg bw once daily for 14 days

Group V (DM+ MEPOS 600) : made up of diabetic rats orally given *P. odoratissimus* methanolic seed extract 600 mg/kg bw once daily for 14 days

Group VI (DM+ MEPOS 900) : made up of diabetic rats orally given *P. odoratissimus* methanolic seed extract 900 mg/kg bw once daily for 14 days

Determination of Blood Glucose Levels

Blood samples were collected through the rats' retro orbital plexus to know the blood glucose levels at intervals of 0, 7 and 14 days. The actual glucose level was measured through Achu-check glucometer and reported as mg/dl.

Serum Biochemical Analysis

Glycosylated hemoglobin (HbA_{1c}) levels were determined through the anion exchange HPLC methods using Biored D10, Thiruvananthapuram, India, in accordance with standard protocols described by the manufacturer. The serum total cholesterol (TC) levels were estimated using colorimetric enzymatic methods using CHOD-PAP, Dialab GmbH, Viena, Austria, using the standard protocols described by manufacturer. Serum triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) levels were all estimated using random access chemistry analyzer BT 3500, Rome, Italy, based on the standard protocols described by manufacturer.

Statistical Analysis

All the relevant data were analyzed with One-Way ANOVA from SPSS 19.0, USA, followed by Tukey's multiple comparison test. Then, *p* values less than 0.05 (*p* < 0.05) were considered significant.

RESULT

Phytochemical Screening

Phytochemical screening of the methanolic extract of *P. odoratissimus* seed tested positive for flavonoids, tannins,

saponins, and quinons, but tested negative for alkaloid, steroid and triterpenoid, as shown in Table 1.

Table 1. Phytochemical screening of *Pandanus odoratissimus* seed extract

Phytochemicals	<i>P. odoratissimus</i>
Flavonoids	+
Alkaloids	-
Tannins	+
Saponins	+
Quinons	+
Steroid	-
Triterpenoid	-

Presence of phytochemicals is denoted by (+) sign, while the absence is denoted by (-) sign.

Antioxidant Activity

The antioxidant activity of the seed extract was evaluated using *in vitro* assays namely DPPH. The concentration up to 50% inhibition (IC50) is given in Table 2. The antioxidant activities were compared with ascorbic acid which were found

in (29) and the better activity was reflected by lower IC50 values. The *P. odoratissimus* seed extract exhibited a significant antioxidant activity, IC= 31.25 µg/ml, which was found to be more potent than those of the reference antioxidant, ascorbic acid, with IC50 = 73.61 µg/ml.

Table 2. Antioxidant properties of crude extract of *Pandanus odoratissimus* seed

Sample	Radical scavenging activity IC50 (µg/ml)
<i>Pandanus odoratissimus</i>	31.25
Ascorbic Acid	73.61 [29]

Antidiabetic Effect of Methanolic Extract from *Pandanus odoratissimus* Seed

The effect of *P. odoratissimus* seed methanolic extract on blood glucose level in alloxan-treated rats is presented in Table 3. The injection of alloxan induced a significant increase ($p<0.05$) in blood glucose level in this group at 418.50 ± 113.65 mg/dl, compared with normal control group at 97.75 ± 5.44 mg/dl. However, the methanolic extract showed a continuous significant reduction in the glucose level ($p<0.05$), particularly 14 days after treatment in the diabetic rats. Also, low dosage of the extract significantly reduced ($p<0.05$) the glucose level from 496.000 ± 76.95 mg/dl to 338.50 ± 67.36 mg/dl at day 7 and to 145.75 ± 36.67 mg/dl at day 14, in

comparison with untreated rat at 418.50 ± 113.65 mg/dl. In addition, this effect obtained with the lower dose is consistent with glibenclamide, which was initially at 946.250 ± 112.82 mg/dl, reduced to 241.25 ± 29.74 mg/dl at day 7, and then to 130.00 ± 14.72 mg/dl at day 14. Similarly, treatment with a high dose of the seed extract (900mg/kg bw) caused a maximum reduction in blood glucose level, from 507.75 ± 72.62 mg/dl to 131.50 ± 13.50 mg/dl at day 7 and then to 104.75 ± 4.50 mg/dl at day 14, compared with the normal control of 96.00 ± 5.66 mg/dl at day 7 and 101.50 ± 8.35 mg/dl at day 14. Furthermore, this effect was more potent than those of the reference drug.

Table 3. Effect of MEPOS on blood glucose and HbA1c in alloxan-induced diabetic rat

Group	Fasting blood glucose (mg/dL)			HbA1c (%)
	Day 0	Day 7	Day 14	
Normal control	97.75 ± 5.44 [#]	96.00 ± 5.66 [#]	101.50 ± 8.35 [#]	6.50 ± 0.12 [#]
Diabetic control	418.50 ± 113.65	462.50 ± 97.43	499.75 ± 146.04	11.75 ± 0.87
Glibenclamide	462.50 ± 112.82	241.25 ± 29.74*	130.00 ± 14.72*	6.50 ± 0.34*
MEPOS (300 mg/kg)	496.00 ± 76.95	338.50 ± 67.36*	145.75 ± 36.67*	9.38 ± 0.12*
MEPOS (600 mg/kg)	505.50 ± 109.00	222.50 ± 35.09*	110.75 ± 9.50*	7.68 ± 0.48*
MEPOS (900 mg/kg)	507.75 ± 72.62	131.50 ± 13.50*	104.75 ± 4.50*	6.32 ± 0.15*

All values are Mean±SE. [#] $p<0.05$ as compare with normal control * <0.05 as compare to diabetic control. SE : Stabdard Error : MEPOS : Methanolic extract of *Pandanus odoratissimus* seed

Table 4. Effect of MEPOS on lipid profile in alloxan-induced diabetic rat

Serum lipid profile (mg/dL)				
Groups	TCH	TG	LDL	HDL
Normal control	68.25 ± 5.67 [#]	74.25 ± 6.23 [#]	10.25 ± 0.95 [#]	45.25 ± 1.50 [#]
Diabetic control	88.75 ± 5.25	191.25 ± 3.86	22.25 ± 2.98	32.25 ± 2.06
Glibenclamide	75.50 ± 2.64	85.50 ± 8.81	18.25 ± 1.89	36.50 ± 2.38
MEPOS (300 mg/kg)	67.50 ± 1.29*	110.75 ± 2.50*	13.75 ± 0.50*	41.50 ± 1.73*
MEPOS (600 mg/kg)	61.50 ± 1.73*	93.50 ± 3.10*	11.25 ± 0.95*	46.25 ± 1.25*
MEPOS (900 mg/kg)	52.75 ± 1.70*	74.75 ± 2.75*	10.50 ± 0.57*	56.75 ± 5.31*

All values are Mean±SE. [#]p<0.05 as compare with normal control *<0.05as compare to diabetic control. SE : Stabdard Error : MEPOS : Methanolic extract of *Pandanus odoratissimus* seed

Effect of *Pandanus odoratissimus* Seed Extract on Glycosylated Hemoglobin in Alloxan-Induced Diabetic Rats

The data in Table 3 show that the HbA1c% levels of untreated diabetic rats highly increased to 180.8% of normal control, while treatment with *P. odoratissimus* seed extract 300, 600, 900 mg/kg or glibenclamide reduced this elevation, where the HbA1c% levels were 144.3; 118.1; 97% and 100% respectively.

Effect of *Pandanus odoratissimus* Seed Extract on Lipid Profile in Alloxan-Induced Diabetic Rats

Table 4 shows the summary of the serum lipid profile, serum total cholesterol (TC), total triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL). There was a significant increase ($p<0.05$) in the level of serum TC, TG and LDL and significant decrease ($p<0.05$) in the level serum HDL of diabetic untreated rats, compared with the control. However, diabetic rats treated with *P. odoratissimus* seed extract had a reversal in the serum lipid profile to almost normal levels with the highest dose (900 mg/kg body weight) being the most effective.

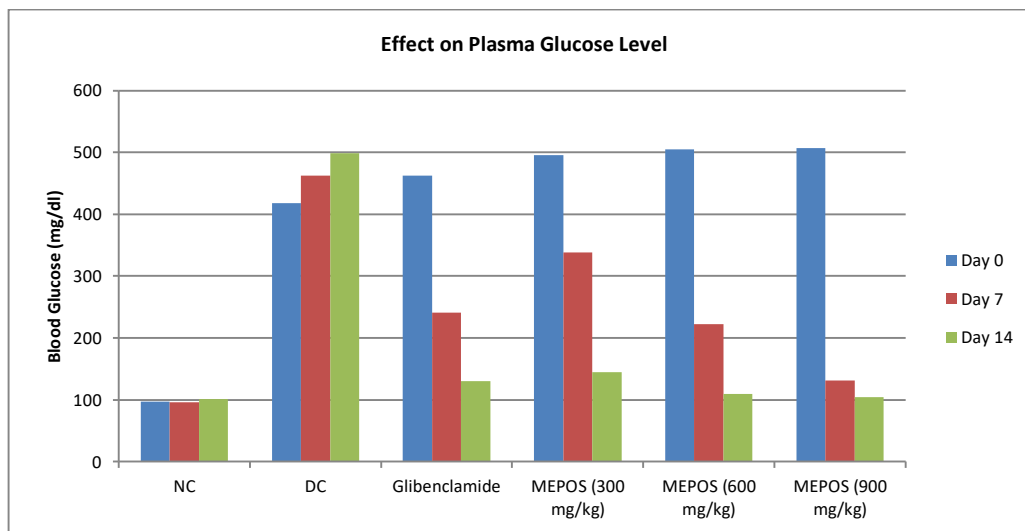


Figure 1. Effect of different dose of *Pandanus odoratissimus*, libenclamide and their combination on fasting plasma glucose on alloxan induce diabetes rat after two weeks treatment. Values are represented as mean ± SEM; n=5, [#]p<0.05 as compare with normal control *<0.05as compare to diabetic control. Here, NC (Normal Control). DC (diabetic control), MEPOS (Methanolic extract of *Pandanus odoratissimus* seed 300 mg/kg bw), MEPOS (Methanolic extract of *Pandanus odoratissimus* seed 600 mg/kg bw), MEPOS (Methanolic extract of *Pandanus odoratissimus* seed 900 mg/kg bw).

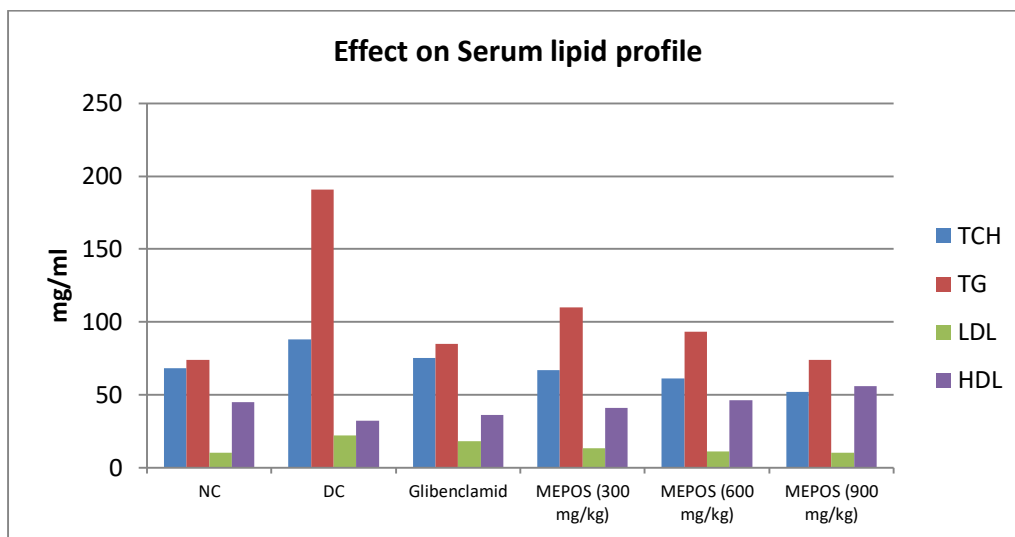


Figure 2. Effect of different dose of *Pandanus odoratissimus*, Glibenclamide and their combination on fasting plasma glucose on alloxan induce diabetes rat after two weeks treatment. Values are represented as mean \pm SEM; n=5, #p<0.05 as compare with normal control *<0.05as compare to diabetic control. Here, NC (Normal Control). DC (diabetic control), MEPOS (Methanolic extract of *Pandanus odoratissimus* seed 300 mg/kg bw), MEPOS (Methanolic extract of *Pandanus odoratissimus* seed 600 mg/kg bw), MEPOS (Methanolic extract of *Pandanus odoratissimus* seed 900 mg/kg bw).

DISCUSSION

The management of diabetes mellitus with a substance with no side effect is still a major challenge in the medical system. Consequently, there is an increase in the demand for natural products with anti-hyperglycemic activity but with fewer or no side effect. It is generally known that medicinal plants are used for treating diabetes due to their effectiveness, safety and acceptability. Most of these medicinal plants have antidiabetic in nature and contain different substances reported for various activities (30). Some reports showed that phytochemicals are responsible for some anti-hyperglycemic and antihyperlipidemic activities (31). The phytochemical test showed the presence of flavonoid, tannins, saponin and quinons in the seed extract of *P. odoratissimus*. Also, it has been shown that flavonoid play a vital role in controlling glucose level and lipid concentration. These compounds are also known to regenerate damaged β cells in alloxan induce animal and act as insulin secretagogues, an important medicine for type 2 diabetes, as well as help in the secretion of insulin, thereby maintaining the blood glucose level (32). Other components such as tannins and saponin have been found to hinder glucose transport by inhibiting sodium glucose cotransporter 1 (S-GLUT – 1) in intestinal brush border cells (33). In addition, saponins are known to lower cholesterol in the intestinal lumen, preventing its absorption and/or binding with bile acids, causing a reduction in the entero hepatic circulation of bile acids, and increasing fecal excretion (34). Increased bile acid excretion is offset by its synthesis from cholesterol in the liver, thereby lowering the plasma cholesterol (34, 35). It has also been reported that the administration of polyphenolic compound to alloxan-induced diabetic rats reduced hyperlipidemia and this was attributed to a reduction in the activity of hepatic HMG-CoA reductase, which is the first committed enzymatic step in cholesterol synthesis (36). This lowers elevated LDL cholesterol levels, hence, reducing the common coronary events and death from coronary heart disease (CHD) which occurs in diabetic

patients (37). Therefore, the observed hypolipidemic effect of *P. odoratissimus* seed could be linked to the synergistic action of phytochemicals like saponin and polyphenolic compound in it. Additionally, fiber of plants interfere with carbohydrate absorption, thereby maintaining the blood glucose level.

Alloxan is a selective β cytotoxic agent widely used to mimic diabetic pathology rodent (38). It is uric acid derivative which causes significant reduction in serum insulin, thus, destroying the β cells of the islets of Langerhans (39) and causing hyperglycemia in rats (40). In addition, it is selectively toxic for pancreatic β cells of the islets of langerhans thereby inducing necrosis (41). Alloxan is cytotoxic glucose analog with a molecular shape similar with glucose (42) and has two pathological effects, namely, selective reduction of glucose-induced insulin secretion through specific inhibition of glucokinase and generation of free radicals. The mechanism through which alloxan induce diabetes is the generation of free radicals which damage and cause the death of β cells; it also infiltrate to the pancreatic β cells through the GLUT 2 transporter (43). Also, it is reduced in cytosol, in the presence of different cellular reducing agents to dialuric acid. This leads to a redox cycle and production of superoxide radicals (O_2^-), and the dismutation of these radicals generates hydrogen peroxide (H_2O_2) which reacts with ferrous (Fe^{2+}) to form hydroxyl radical (OH^-), a high oxidative agent (40). These oxidative agents cause necrosis in pancreas β cells, thereby leading to diabetes mellitus type 1 (38). Insulin is secreted by pancreas, which is the primary organ involved in determining the organism's dietary and energetic status through the amount of glucose present in the blood (44). This study shows that the *P. odoratissimus* seed extract reduced the glucose level in animal made diabetic with alloxan. One possible explanation for this could be the presence of antioxidant in the plant part, as reported in a previous study (45). Antioxidants are protective agents which inactivate the ROS causing cell damage (46). Interestingly, plants with flavonoids possess

both antioxidant and anti-hyperglycemic activities (47). Additionally, it has been reported that the antioxidant activity of herbal hypoglycemic plant extract could form a protective mechanism against the ROS associated with chronic hyperglycemia and diabetic complications such as microvascular and macrovascular conditions (46). Also, albino rats have surviving β cells, capable of regenerating due to the use of lower dose alloxan (125 mg/kg body weight), which produce partial destruction of pancreatic β cells (48). Other possible mechanism include the stimulation of cells and subsequent release of insulin and activation of the insulin receptor. Estimation of insulin level and receptor might give more insight into the mechanism of antidiabetic activity exhibited by the plant extract (49).

Another important clinical marker in diabetes is the glycosylate haemoglobin. It helps to regulate the degree of protein glycation during diabetes mellitus. In insistent hyperglycemic, formation of HbA1c takes place through the non-enzymatic reaction between free amino groups of haemoglobin and glucose. In diabetic patients, HbA1c levels help to assess both the long-term glycemic control and the progression of damage or development of complication associated with the condition. Hence, marked increase in HbA1c levels was noticed in the alloxan-induced diabetic animals, compared with the control group. However, treatment with the seed extract showed a reduction in HbA1c levels. This could be as a result of the reduction in the blood glucose caused through increasing insulin secretion by rejuvenated pancreatic β cells (50).

One of the complications as due to diabetic hyperglycemia is dyslipidemia. Generally, lipid play a major role in maintaining the integrity, structure, as well as the function of the biomembrane. However, due to insulin deficiency, alteration in lipid and lipoprotein associated with diabetes have a higher chance of developing into atherosclerosis. This dyslipidemic profile during diabetes, include increase in the level of total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and decrease in plasma high density lipoprotein (HDL) (51). The serum lipid profile of rats was evaluated in this study and as expected, untreated diabetic animals showed a significant increase in TC, TG and LDL concentration but with low level of HDL. This increase in serum lipid is mainly due to the increase in fatty acid mobilization from adipose tissue, since insulin inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is the key enzyme in cholesterol biosynthesis (52). Hence, insulin deficiency or resistance could be responsible for hyperlipidemia. Also, treatment of type 2 diabetic rats with the methanolic seed extract (900 mg/kg body weight), completely reversed dyslipidemia as shown by the significant decrease ($p < 0.05$) in TC, TG and LDL, coupled with the increase in HDL ($p < 0.05$). These alleviating effects, therefore, show the antihyperlipidemic potential of methanolic extract of *P. odoratissimus* seeds.

CONCLUSION

The beneficial effects on the regulation of serum glucose levels and hyperlipidemia in alloxan-induced diabetic rats is evident in treatment with the dose of 900 mg/kg body weight of the *P. odoratissimus* seed extract. There is need for further research on it which could possibly be used in the development of diabetic drugs. Based on the results, the seed extract was

more potent and effective than the standard drug. Therefore, this study give scientific support to the use of *P. odoratissimus* in traditional beliefs for the treatment of diabetes and could be further explored for producing effective drugs for this condition due to its antidiabetic activity.

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Conflict of interest

The authors reported no conflict of interest

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