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Paederia foetida Twig Extract: A Dual-Purpose Antidiabetic and Antioxidant Agent in High-Fat Diet-Low Dose Streptozotocin Rats

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Abstract: This study was conducted to determine the antidiabetic and antioxidant effect of *Paederia foetida* twigs in high-fat diet-low dose streptozotocin-induced Sprague Dawley rats to provide essential information for safe use in humans. Thirty-six male Sprague Dawley rats were acclimatized for a week, 6 were assigned to normal control, and 30 were induced into obesity using a high-fat diet for 4 weeks. After the obesity induction, 24 of 30 obese rats were induced diabetes by 40mg/kg streptozotocin and further divided into diabetic control and three diabetic treated groups (50mg/kg, 100mg/kg *P. foetida*, and 300mg/kg metformin). In the sub-chronic study, *P. foetida* twigs extract and metformin were orally administered to the rats for 28 days. Repeated single oral administration of 50mg/kg *P. foetida* twigs extract on diabetic rats for 28 days revealed this dosage as the most effective in lowering blood glucose (27.2%), comparable to metformin (23.1%). Treatment with 50mg/kg *P. foetida* twigs extracts displayed significant antioxidant properties that reduce oxidative stress based on the protein carbonyl levels and exhibited clinical signs of hyperglycemia and oxidative stress marker. *P. foetida* supplementation and metformin both reversed the clinical manifestation of type 2 diabetes

Keywords: *Paederia foetida*; antioxidant; antidiabetic; high-fat diet; diabetes; oxidative stress.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by high blood glucose from insufficient insulin secretion and insulin resistance [1]. Type 2 diabetes results from the failure of pancreatic β-cell to secrete insulin sufficiently in response to elevated blood glucose. Type 2 diabetes has been related to many chronic comorbidities that can undermine patients' quality of life. People living with diabetes have a higher risk of morbidity and mortality than the general population [2]. The global prevalence of diabetes in adults has been increasing over recent decades. The prevalence of diabetes in adults aged 20-79 years was about 10.5% in 2021

and was predicted to be an alarming 12% in 2045 [2]. The high prevalence of diabetes in adults has significant social, financial, and developmental implications.

Some major microvascular complications of diabetes mellitus, including diabetic retinopathy, neuropathy, and nephropathy, cause dialysis, blindness, and amputation [3]. Most of these secondary complications were caused by reactive oxygen species (ROS) induced by diabetes. The ROS were produced in the cell during normal cellular metabolism. Excessive ROS were neutralized by the antioxidant system in the body [4]. There were several species or molecules, endogenous or exogenous, that play a role in antioxidant defense and may be considered as biomarkers of oxidative stress, such as involving catalase (CAT), glutathione peroxidase (GPx), protein carbonyl content (PCO), and receptor for advanced glycation end-products (RAGEs) [5]. However, this system's failure may create an imbalance between generating and eliminating OS in favor of the formation of excessive oxidants called oxidative stress [5].

Animal or rodent models are effective methods for exploring and understanding the complex mechanisms of type 2 diabetes [6]. Recently, a type 2 diabetes model has become increasingly prevalent. This model is established by a combination of a high-fat diet (HFD) and low-dose streptozotocin (STZ) to cause mild dysfunction in β-cells without ultimately compromising insulin secretion [7]. This experimental model is effective for evaluating the pathogenesis of diabetes complications related to most humans, such as diabetic cardiomyopathy, nephropathy, neuropathy, and retinopathy [6,8].

Antioxidant (natural or synthetic) therapies may help reduce the risk of developing T2DM in healthy people and reduce the secondary complications of T2DM in patients. *Paederia foetida*, belonging to the Rubiaceae family, is a climbing plant widely distributed in Asian countries, including Malaysia. The plant has traditional properties, such as treatments for toothaches, dysentery, sores, gastrointestinal issues like enterosis and enteromegaly, rhinos (possibly referring to nasal conditions), rheumatism, edema, night blindness, gastritis, diarrhea, and ulceration [9]. Scientific research shows that *P. foetida* exhibits good anti-inflammatory [10], antioxidant [11,12], antimicrobial [12] activities, etc. Our previous studies reported *P. foetida* twigs chloroform extract has good antioxidant and antidiabetic activity *in silico* and *in vitro* [9,13]. *P. foetida* twigs showed strong 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and β-carotene bleaching effects. Besides, *P. foetida* twigs showed good inhibition against α-amylase and α-glucosidase enzymes. A coumarin, a high content of scopoletin isolated from *P. foetida* twigs, showed high antioxidant and antidiabetic activities [9,14]. Several antidiabetic metabolites of *P. foetida* twigs were identified in Gas Chromatography-Mass Spectrometry metabolomics, including tocopherol, fatty acid, steroids, ketone, amino acid, and glycerides [13]. In addition, the existence of alkaloids, tannins, saponin, terpenoids, flavonoids, and phenol in *P. foetida* was also reported to show the antioxidant properties based on DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays [12]. The acute toxicity evaluation of *P. foetida* confirmed that no mortality or sign of toxicity was observed in the animals up to a dose of 2000mg/kg [15].

However, these data may not represent the real circumstances around a living organism. *In vivo* studies are essential for drug or herbal-drug development because they provide the ability to study the physiological and biochemical processes, including the adverse effects, which cannot be obtained from *in vitro* studies. *In vitro* studies are crucial for research because they represent most of human diseases' structural, functional, and biochemical characteristic [16]. The antidiabetic and antioxidant assessments of *P. foetida* twigs on the alternative rat

model have not been available to date. Therefore, the present study aimed to identify the antidiabetic and antioxidant effects of *P. foetida* twigs chloroform extract in high-fat diet-low-dose streptozotocin-induced Sprague Dawley rats. We expect that the joint approaches of *in silico*, *in vitro* with *in vivo* results could reflect human and animal physiological responses towards *P. foetida* twigs extract. The data generated can be used to minimize the late-stage failures during human clinical trials.

2. Materials and Methods

All experiments complied with the guidelines for the welfare of experimental animals by the Care and Use of Animals for Scientific Purposes. This experimental procedure was approved by Universiti Sains Malaysia (USM) Institutional Animal Care and Use Committee (IACUC) on 7th October 2019 with animal ethics approval number of USM/IACUC/2019/(119) (1009). The study was conducted at the Animal Research and Service Centre (ARASC), USM Health Campus.

2.1. Preparation of plant extract.

The *Paederia foetida* twigs were collected from Ledang, Johor, and submitted to the Institute of Bioscience, Universiti Putra Malaysia (UPM) Serdang for plant identification by Dr. Mohd. Firdaus Ismail (Biodiversity Unit) who gave a specimen voucher number of SK3177/17. No permission is required for the plant collection. The plant twigs were dried at ambient temperature and subsequently crushed into powder form. The powdered twigs were extracted in the chloroform by cold maceration method for 72 hours in a conical flask [9]. The extraction was filtered with Whatman No.1 filter paper and then concentrated under reduced pressure using the rotary vacuum evaporator to get the chloroform crude extract. The extract was subjected to *in vivo* study.

2.2. Experimental animals.

Thirty-six of 12 weeks old male Sprague Dawley rats were purchased from ARASC, USM Health Campus, individually housed in polypropylene cages with stainless steel covers and maintained under controlled room temperature of 25 ± 2°C, the humidity of 60 ± 5% and 12/12 h light/darkness condition with the light on at 07:00 AM and off at 7:00 PM. Male rats were chosen to eliminate variations in food intake due to ovarian hormones and their faster growth rate than female rats, enabling easier detection of body weight changes [17]. A week of acclimatization was applied to all rats with a normal pellet diet (Altromin 1324, Brogaarden, Denmark) and water ad libitum. Power analysis for one-way ANOVA with six groups was conducted in G*Power version 3.1 to determine a sufficient sample size using an alpha of 0.05, a power of 0.80, and an effect size of 0.60. A total of 6 rats per group were considered necessary. All the rats were observed daily for health status based on the criteria of reduced food and water consumption, weakness, abnormal behavior, or any sign indicating severe stress, such as severe dyspnea, aggression, and reduced body weight. No adverse events were observed.

2.3. Preparation of a high-fat diet.

The high-fat diet (HFD) was formulated based on our group [1] and Lim and his team [17] with modifications, containing a mixture of 50% of normal chow pellets, 38% of ghee

(Crispo brand), 8% of full-cream milk powder (Fernleaf), and 4% sugar. According to Manna and colleagues [18], ghee is produced by milusing traditional methods and contains about 65% saturated fat and 33% monounsaturated fatty acids (MUFAs). The HFD mixture was shaped into a hand-ball size. The HFD was then placed in an oven at 65°C for 24h before storing it in a fridge at 4°C. The HFD was prepared weekly to protect lipids from the oxidation process.

2.4. Treatment protocol.

The rats were randomly divided into the normal diet control (NC) group (n=6) and the obese group (n = 30). The NC group was fed continually on a normal pellet diet (NPD), while the obese group was fed with HFD for 4 weeks to induce obesity. Obesity was determined using the Lee obesity index [19]. It is an index to confirm obesity whereby its value of more than 315 was considered obese. It was calculated based on the formulae of the cube root of body weight (g) divided by the rat's nose-to-anus length (cm).

After 4 weeks of obesity induction, six of thirty obese rats were further subdivided into the obese control (OC) group (n=6). Twenty-four obese rats were fasted overnight and injected intraperitoneally with 40mg/kg of STZ (AdipoGen Life Sciences, USA) dissolved in 0.1M cold disodium citrate buffer (pH 4.5) to induce Type 2 diabetes. In contrast, NC and OC were injected intraperitoneally with normal saline. A glucometer confirmed hyperglycemia 96 hours after streptozotocin injection by measuring glucose levels in the blood obtained from the tail vein. Animals with fasting blood glucose levels above 11mmol/L were considered diabetic [20]. Diabetic rats were randomly separated into 4 groups, each consisting of six individuals (n=6). Below are the lists of the rat groups for the study.

Group 1: Normal control rats (NPD, untreated);
Group 2: Obese control rats (HFD, obese, untreated);
Group 3: Diabetic control rats (HFD, diabetic, untreated);
Group 4: Diabetic rats (HFD, diabetic) treated with 50mg/kg of *P. foetida* twigs extract;
Group 5: Diabetic rats (HFD, diabetic) treated with 100mg/kg of *P. foetida* twigs extract;
Group 6: Diabetic rats (HFD, diabetic) treated with 300mg/kg of metformin;

The *P. foetida* twigs extract was diluted to 50mg/mL in 0.5% (w/v) of carboxymethylcellulose (CMC) and administrated every day for 4 weeks in doses of 50mg/kg for Group 4 rats and 100mg/kg for Group 5 rats by oral gavage. The diabetic rats in Group 6 served as a positive control, in which metformin (300mg/kg) suspended in 0.5% (w/v) of CMC was given every day for 4 weeks. Group 1, 2, and 3 were as negative control, treated with only 0.5% CMC. The body weight of each rat was recorded before and after 4 weeks of treatment. The food intake of each rat was recorded weekly throughout the experiment.

2.5. Fasting blood glucose.

Tail venous blood was chosen to determine fasting blood glucose [21]. A Lancing device with disposable lancets was used to express venous blood. Weekly fasting blood glucose measurement with a glucometer (Accu-Chek ® Perfoma 2, Roche, USA) was performed.

2.6. Blood and tissue collection.

After 28 days of intervention, all rats fasted overnight for 16h. On 29th day at 10:30 AM, all rats were euthanized using a single intraperitoneal injection of sodium pentobarbital (100mg/kg) anesthesia to lose consciousness and minimize the animals' suffering [22]. The

blood samples were collected by cardiac puncture and placed in the proper blood collection tubes (BD Vacutainer®). The collected blood was centrifuged for 10 min at 4000 rpm at room temperature, and the supernatants were collected and stored at -80°C prior to use. The blood serum was obtained from the Plain tubes with gel. The liver and heart were surgically removed and rinsed with phosphate buffer saline solution.

2.7. Biochemical and antioxidant tests.

The blood serum was analyzed for lipid profile, renal, and liver function tests. Lipid profiles included total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). The renal function included creatinine, urea, and uric acid. The liver function included aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). The biochemical tests were characterized using Architect ci8200 (Abbott, Illinois, United States). The liver and heart tissues were homogenized with cold phosphate buffer saline using a homogenizer. The samples were centrifuged for 10 min at 4000 rpm using a refrigerated centrifuge, and the supernatants were collected and stored at -80°C. Two antioxidant enzymes (glutathione peroxidase (GPx) and catalase activity (CAT)) and two oxidative stress markers (protein carbonyl content (PCO) and rat RAGE levels) were measured using commercial reagent assay kits (Abcam, Cambridge, United Kingdom).

2.8. Statistical analysis.

The findings were presented as the mean ± standard deviation (SD). Statistical analysis were conducted using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to compare multiple groups. The values were considered statistically significant when $p < 0.05$.

3. Results and Discussion

3.1. Body weight of experimental animals.

Table 1 shows the body weight from week 0 to week 4 of NC, OC, and treated diabetic rats increased significantly ($p<0.05$), while untreated diabetic rats decreased significantly ($p<0.05$). In NC rats (Group 1), body weight increased by 7.40%, followed by 17.80% in OC rats (Group 2). Meanwhile, the body weight of diabetic control rats (Group 3) decreased by 18.40%.

Table 1. The mean body weight and body weight gain during the treatment period.

Groups	Body weight (g) and body weight gain		
	Week 0	Week 4	Body weight gain
Group 1	281.83 ± 25.06^{a}	302.67 ± 20.40^{a}	20.84 ± 5.20^{a}
Group 2	281.17 ± 16.58^{b}	331.17 ± 12.12^{a}	50.00 ± 4.46^{a}
Group 3	285.00 ± 23.12^{b}	232.60 ± 5.86^{b}	-52.40 ± 17.26^{b}
Group 4	290.00 ± 24.44^{b}	312.20 ± 16.26^{a}	22.20 ± 8.18^{a}
Group 5	287.29 ± 15.67^{b}	309.60 ± 22.58^{a}	22.31 ± 6.91^{a}
Group 6	292.93 ± 24.52^{b}	312.67 ± 14.75^{a}	19.74 ± 9.77^{a}

Data are shown as mean ± SD. Data with different superscripts (a, b) in the same column were considered significantly ($p < 0.05$) different. Group 1: Normal control; Group 2: Obesity control; Group 3: Diabetic control; Group 4: Diabetic rats treated with 50mg/kg *P. foetida* twigs extract; Group 5: Diabetic rats treated with 100mg/kg *P. foetida* twigs extract; Group 6: Diabetic rats treated with 300mg/kg metformin.

However, the body weight of diabetic rats treated with *P. foetida* twigs extracts increased by 7.70% (Group 4) and 7.80% (Group 5) after 4 weeks of treatment. The body weight of diabetic rats in group 6, treated with metformin, increased by 6.70%.

3.2. Plasma blood glucose.

In the treatment period, the diabetic rats were treated with 50mg/kg *P. foetida* twigs extract (n=6), 100mg/kg *P. foetida* twigs extract, and 300mg/kg metformin, respectively, for 4 weeks (Table 2). Glucose levels in Group 1 and Group 2 have remained within the normal range throughout the experiment. Group 1 had a 7.03% increase in blood glucose, while Group 2 increased by 11.01%. The increase in blood glucose in Group 3 (10.29%) was significantly (p<0.05) different when compared to Groups 4, 5, and 6. However, treated diabetic rats showed decreased blood glucose levels compared to the diabetic control group (Group 3). Interestingly, the treatment of diabetic rats with 50 mg/kg body weight *P. foetida* twigs extract (Group 4) potentially decreased blood glucose levels compared to Groups 5 and 6. There was a 27.19% reduction in blood glucose levels in Group 4, followed by 23.14% in group 6 and 16.79% in Group 5. *P. foetida* twig extract's ability to reduce glucose levels was complemented by previous findings in an *in vitro* study. Thus, this study's lowest extract dose seemed to be the most effective.

Table 2. The plasma glucose level and changes during the treatment period.

Group	Plasma glucose level (mmol/L)		
	Week 0	Week 4	Changes
Group 1	4.55 ± 0.35^{a}	4.87 ± 0.26^{a}	0.32 ± 0.09^{a} (7.03%)
Group 2	5.72 ± 0.49^{a}	6.35 ± 1.55^{a}	0.63 ± 1.06^{a} (11.01%)
Group 3	26.64 ± 5.82^{b}	29.38 ± 2.43^{b}	2.74 ± 3.39^{a} (10.29%)
Group 4	13.68 ±6.70^{c}	9.96 ± 4.15^{a}	-3.72 ± 2.55^{b} (-27.19%)
Group 5	16.14 ± 4.07^{d}	13.43 ± 9.99^{b}	-2.71 ± 5.29^{c} (-16.79%)
Group 6	21.48 ± 1.99^{b}	16.51 ± 11.38^{b}	-4.97 ± 9.39^{c} (-23.14%)

Data are shown as mean ± SD. Data with different superscripts (a, b, c, d) in the same column were considered significantly (p < 0 .05) different. Group 1: Normal control; Group 2: Obese control; Group 3: Diabetic control; Group 4: Diabetic rats treated with 50mg/kg *P. foetida* twigs extract; Group 5: Diabetic rats treated with 100mg/kg *P. foetida* twigs extract; Group 6: Diabetic rats treated with 300mg/kg metformin.

3.3. Serum lipid profile.

Table 3 shows the mean of the serum lipid profile of all groups at week 4. The lipid profile levels of NC rats (Group 1) were in the normal range. However, the serum total cholesterol, TG, and LDL of OC rats (Group 2) were significantly (p<0.05) higher than Group 1, while there was no significant difference in HDL and VLDL levels. The TC, TG, LDL, and VLDL levels of Group 3 were significantly (p<0.05) higher than those of Group 1 and 2 while significantly (p<0.05) lower HDL levels than those of Group 1 and 2. Besides that, The TC, TG, and LDL levels of Group 4 were significantly (p<0.05) lower than those of Group 3, 5, and 6. Group 4 showed a non-significant (p>0.05) difference in HDL levels compared to Groups 5 and 6. The hypocholesterolemia effect is higher in Group 4, followed by Groups 5 and 6.

Table 3. Serum lipid profile of rats' groups.

Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Group 1	66.3 ± 0.82^{a}	77.62 ± 1.00^{a}	57.10 ± 1.05^{a}	6.68 ± 1.08^{a}	15.52 ± 0.20^{a}
Group 2	74.73 ± 2.99^{b}	82.67 ± 0.52^{b}	52.50 ± 0.55^{a}	20.67 ± 2.48^{a}	16.53 ± 0.10^{a}

Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Group 3	112.65 ± 5.14^{c}	113.55 ± 4.46^{c}	31.00 ± 0.82^{b}	88.25 ± 2.87^{b}	22.71 ± 0.89^{b}
Group 4	72.92 ± 2.47^{b}	80.20 ± 0.45^{ab}	54.04 ± 1.71^{a}	16.36 ± 2.59^{a}	16.04 ± 0.09^{a}
Group 5	83.42 ± 3.90^{d}	96.75 ± 0.96^{d}	50.95 ± 0.62^{ab}	36.71 ± 2.81^{c}	19.35 ± 0.19^{a}
Group 6	88.67 ± 3.21^{d}	103.00 ± 2.65^{d}	51.57 ± 0.38^{ab}	43.84 ± 4.44^{c}	20.60 ± 0.53^{a}

Data are shown as mean ± SD. Data with different superscripts (a, b, c, d) in the same column were considered significantly ($p < 0.05$) different. Group 1: Normal control; Group 2: Obese control; Group 3: Diabetic control; Group 4: Diabetic rats treated with 50 mg/kg *P. foetida* twigs extract; Group 5: Diabetic rats treated with 100mg/kg *P. foetida* twigs extract; Group 6: Diabetic rats treated with 300mg/kg metformin.

3.4. Serum renal function.

As shown in Table 4, Group 1 has no significant ($p>0.05$) difference in creatinine compared to other groups. Besides that, Group 4 showed a significant ($p<0.05$) decrease in uric acid level compared to Groups 3, 5, and 6. Among the treatment groups, Group 4 showed the lowest levels of creatinine, uric acid, and urea (53.31 ± 2.42, 121.58 ± 37.37, and 4.00 ± 1.85µmol/L, respectively) as compared to Group 5 (53.64 ± 4.85, 131.59 ± 49.57, and 4.17 ±1.95µmol/L, respectively) and Group 6 (58.56 ± 11.89, 121.59 ± 29.71, and 5.09 ± 2.35µmol/L, respectively).

Table 4. Creatinine, uric acid, and urea levels of rats' groups.

Group	Creatinine (umol/L)	Uric acid (umol/L)	Urea (umol/L)
Group 1	52.15 ± 5.81^{a}	116.00 ± 30.56^{a}	6.01 ± 0.46^{a}
Group 2	55.25 ± 6.98^{a}	138.81± 32.87^{a}	3.87 ± 0.39^{a}
Group 3	73.15 ± 11.78^{a}	181.98 ± 46.65^{b}	9.69 ± 8.37^{a}
Group 4	53.31 ± 2.42^{a}	121.58 ± 37.37^{a}	4.00 ± 1.85^{a}
Group 5	53.64 ± 4.85^{a}	131.59 ± 49.57^{b}	4.17 ± 1.95^{a}
Group 6	58.56 ± 11.89^{a}	121.59 ± 29.71^{b}	5.09 ± 2.35^{a}

Data are shown as mean ± SD. Data with different superscripts (a, b) in the same column were considered significantly ($p < 0.05$) different. Group 1: Normal control; Group 2: Obese control; Group 3: Diabetic control; Group 4: Diabetic rats treated with 50mg/kg *P. foetida* twigs extract; Group 5: Diabetic rats treated with 100mg/kg *P. foetida* twigs extract; Group 6: Diabetic rats treated with 300mg/kg metformin.

3.5. Serum liver function.

Table 5 shows the aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphate (ALP) levels of the rats. There are no significant ($p>0.05$) differences in the level of ALT among all the groups. Besides that, Group 4 showed significantly ($p<0.05$) lower AST levels than other groups. Serum activities of ALP of diabetic treated groups were significantly decreased ($p<0.05$) after 4 weeks of treatment of *P. foetida* twigs extract and metformin, as compared to the diabetic control rats (Group 3). 50mg/kg *P. foetida* twigs extract (Group 4) treatment was the effective dosage to improve the AST and ALP activities compared to 100mg/kg *P. foetida* twigs extract and 300mg/kg metformin.

Table 5. AST, ALT, and ALP levels of rats' groups.

Group	AST/ SGOT (U/L)	ALT/ SGPT (U/L)	ALP (U/L)
Group 1	139.70 ± 24.13^{a}	72.00 ± 14.52^{a}	321.83 ± 37.74^{a}
Group 2	169.00 ± 11.88^{a}	85.33 ± 15.58^{a}	726.00 ± 51.39^{b}
Group 3	152.75 ± 19.50^{a}	93.75 ± 9.84^{a}	957.25 ± 24.69^{c}
Group 4	125.00 ± 19.33^{b}	70.20 ± 14.48^{a}	391.00 ± 43.79^{d}
Group 5	126.25 ± 12.18^{a}	74.00 ± 7.83^{a}	420.25 ± 34.16^{e}
Group 6	129.20 ± 18.17^{a}	71.40 ± 6.66^{a}	484.00 ± 82.82^{f}

Data are shown as mean ± SD. Data with different superscripts (a, b, c, d, e, f) in the same column were considered significantly ($p < 0.05$) different. Group 1: Normal control; Group 2: Obese control; Group 3:

Diabetic control; Group 4: Diabetic rats treated with 50mg/kg *P. foetida* twigs extract; Group 5: Diabetic rats treated with 100mg/kg *P. foetida* twigs extract; Group 6: Diabetic rats treated with 300mg/kg metformin.

3.6. Antioxidant enzymes and oxidative stress markers.

Antioxidant enzyme activities such as catalase (CAT) and glutathione peroxidase (GPx) were carried out using the liver and heart tissues. Also, oxidative stress markers were measured, such as protein carbonyl content (PCO) and receptor for advanced glycation end-products (RAGEs) (Table 6). In the liver tissue, NC rats (Group 1) had higher CAT and GPx activities than OC rats (Group 2). Diabetic control rats (Group 3) followed the trend of Group 2, which had a significantly ($p<0.05$) decreased CAT activity but non-significant ($p>0.05$) GPx activity decrement, compared to Groups 1 and 2. Diabetic-treated rats had significantly ($p<0.05$) increased CAT activity and non-significantly ($p>0.05$) increased GPx activity compared to rats of Group 3. Group 4 had higher CAT activities compared to Group 5 and Group 6. Group 4 had a significant ($p<0.05$) increased GPx activity compared to Group 6 but non-significantly ($p>0.05$) to Group 5. Group 3 had a significantly ($p<0.05$) increased PCO and RAGE activities compared to Groups 1 and 2. Diabetic-treated groups had lower PCO and RAGE activities compared to Group 3. Regarding PCO marker, the level was ranked as follows: Group 4<Group 6<Group 5<Group 3. The RAGE marker was ranked as Group 4<Group 5<Group 6<Group 3.

In the heart tissue evaluation of Groups 1, 2, and 3, Group 3 had significantly ($p<0.05$) the lowest CAT activity while non-significantly ($p>0.05$) the lowest GPx activity. Among the Groups 3, 4, 5, and 6, Group 3 had the lowest CAT and GPx activities. Group 5 had the highest CAT activity, followed by Group 6 and Group 4, while Group 4 had the highest GPx activity compared to Groups 5 and 6. Group 3 had a non-significant ($p>0.05$) increase in PCO and RAGE markers compared to Groups 1 and 2. Regarding PCO markers, the level was ranked as follows: Group 4<Group 5<Group 6<Group 3. On the other hand, Group 4 had a decreased RAGE marker followed by Group 5; however, Group 6 had increased RAGE compared to Group 3.

Table 6. Antioxidant activities and oxidative stress of rats' groups.

Groups	CAT (U/mg protein)		GPx (nmol/mg protein)		PCO (nmol/mg protein)		RAGE (ng/ml)	
	Liver	Heart	Liver	Heart	Liver	Heart	Liver	Heart
Group 1	16.60 ± 0.02^{a}	29.47 ± 0.05^{a}	19.96 ± 0.47^{a}	11.59 ± 0.05^{a}	20.69 ± 0.03^{a}	24.42 ± 0.01^{a}	1.38 ± 0.02^{a}	1.29 ± 0.00^{a}
Group 2	5.52 ± 0.03^{ab}	26.39 ± 0.10^{a}	18.84 ± 0.10^{a}	12.26 ± 0.06^{a}	23.06 ± 0.06^{a}	25.67 ± 0.06^{b}	1.72 ± 0.02^{b}	1.64 ± 0.02^{a}
Group 3	4.34 ± 0.03^{b}	10.39 ± 0.02^{b}	10.90 ± 0.26^{a}	8.37 ± 0.04^{a}	23.95 ± 0.06^{b}	34.76 ± 0.02^{a}	2.22 ± 0.06^{c}	2.31 ± 0.02^{a}
Group 4	25.96 ± 0.07^{bc}	28.29 ± 0.14^{a}	17.62 ± 0.06^{b}	9.01 ± 0.04^{a}	20.33 ± 0.06^{b}	27.48 ± 0.06^{a}	1.47 ± 0.00^{a}	2.03 ± 0.02^{b}
Group 5	11.78 ± 0.04^{a}	37.26 ± 0.12^{a}	15.18 ± 0.09^{a}	8.87 ± 0.01^{a}	20.89 ± 0.12^{a}	27.66 ± 0.03^{a}	1.55 ± 0.01^{b}	2.06 ± 0.02^{c}
Group 6	14.27 ± 0.09^{a}	32.11 ± 0.14^{b}	15.17 ± 0.02^{a}	8.75 ± 0.02^{b}	22.65 ± 0.02^{b}	27.81 ± 0.01^{c}	1.70 ± 0.01^{b}	2.33 ± 0.01^{a}

Data are shown as mean ± SD. Data with different superscripts (a, b, c, d) in the same column were considered significant ($p < 0.05$). Group 1: Normal control; Group 2: Obese control; Group 3: Diabetic control; Group 4: Diabetic rats treated with 50mg/kg *P. foetida* twigs extract; Group 5: Diabetic rats treated with 100mg/kg *P. foetida* twigs extract; Group 6: Diabetic rats treated with 300mg/kg metformin.

3.7. Discussion.

In this study, we tested *Paederia feotida* twig extract for antidiabetic and antioxidant activities using an animal model. Animals have been used in several research studies to determine toxicity, mechanism of action of drugs or plant extracts, and other related works. In studying the progression of type 2 diabetes in humans, researchers commonly induce diabetes by administering a high-fat diet (HFD) along with a low-dose streptozotocin (STZ). Feeding the rats with HFD increases their energy expenditure and leads to insulin resistance in the organs. Besides, low-dose STZ induced hyperglycemia in rats, disrupting insulin secretion by destroying β-cell. This progressive development of type 2 diabetes is parallel to humans.

In the present study, plasma glucose levels among rats of all groups were measured after the acclimatization period, at the end of diet manipulation, and after a week of STZ injection. Meanwhile, plasma glucose levels among the rats in the treatment period were measured before and after four treatment weeks. The glucose levels increased significantly after STZ injection compared to those after diet intervention. The impact of HFD on insulin secretion and glucose tolerance is recognized to be time-dependent. Another study showed the increment in phenotype and metabolic of obesity after 4 weeks of administration of HFD [23]. HFD-based fatty acids may modulate the function of β-cells and impair insulin production in the presence of other factors, such as diabetes and obesity, due to lifestyle or genetic reasons [23,24].

Injection of STZ induced rapid destruction of pancreatic β-cells in rats, led to impaired glucose-stimulated insulin release, and insulin resistance marked features of type 2 diabetes [25]. After 4 weeks of treatment, treated diabetic rats had decreased blood glucose levels compared to non-treated diabetic rats. 50mg/kg *P. foetida* twigs extract decreased blood glucose levels more significantly compared to 100mg/kg *P. foetida* twigs extract and 300mg/kg metformin. *P. foetida* twigs ”” ’extract’s ability to reduce glucose level was complimented by the previous findings in an *in vitro* study [9,13] *P. foetida* twigs extract inhibited α-amylase and α-glucosidase *in vitro*, leading to a reduced rate of carbohydrate digestion, delayed intestinal absorption and a reduced rise in postprandial blood sugar levels [9,26]. *P. foetida* leaf extract also improved glucose tolerance in diabetic rats on oral glucose tolerance tests, suggesting the ability to reduce glucose absorption. A further histopathological study suggested that *P. foetida* leaf extract also regenerated and reactivated the β-cells, resulting in increased synthesis and secretion of insulin in STZ-induced diabetic rats [27]. A lower *P. foetida* twigs extract dose seemed to be more effective.

It is already known that the saturated fats containing HFD biosynthesized fatty acids in the rat’s liver inhibited the synthesis of apoproteins and lipoproteins, disrupted the active transport of fatty acids to tissue cells, and increased the accumulation in the liver and blood plasma [24]. The HFD rats in the present study had higher levels of TC and TG than the normal control. The association of HFD with saturated fatty acids, their quality and quantity, and duration of treatment, as well as the development of hyperlipidemia, still controversial [28,29]. However, studies showed that consumption of saturated fats contributes to the development of hypercholesterolemia [30,31]. The increase in different lipid contents is also time-dependent in HFD-fed rats. TC, TG, and LDL levels increased, while HDL concentration levels changed after 12 weeks of treatment [32].

Diabetic dyslipidemia is defined as several lipid and lipoprotein interrelated defects associated with type 2 diabetes or any defects in the lipid levels [33]. Type 2 diabetes is

generally associated with high cholesterol, high triglycerides (TG) (hypertriglyceridemia), low high-density lipoprotein cholesterol (HDL), and small dense low-density lipoprotein (LDL) particles. Diabetic rats had higher levels of TG among all the groups. Rat treated with HFD had dyslipidemia and other diabetic syndromes. Besides that, the lipogenic activity deficiency was also caused by a defect in insulin secretion due to STZ. Insulin plays a vital role in stimulating lipogenesis in mammals, and decreased secretion of insulin implicates high levels of lipids in plasma. The most striking result from this study was that lipids in serum were ameliorated when treated with 50mg/kg of *P. foetida* twigs extract. These results may be associated with *P. foetida* twig extract's possible protective effect on pancreatic β-cells. Another reason could be the low activity of cholesterol biosynthetic enzymes or the insignificant level of lipolysis under insulin control [34]. STZ-induced diabetic rats treated with different doses of *P. foetida* leaf extract also significantly decreased the level of glucose-6-phosphatase and fructose 1-6 bisphosphates, the enzymes responsible for converting carbohydrates into fats [27]. Therefore, glycol and lipid metabolism may improve the renovation of β-cells and increase insulin secretion.

Diabetic nephropathy, also called diabetic kidney disease, is one of the most severe and common microvascular complications [35]. It was characterized by glomerular vascular injury, glomerulosclerosis, nodular lesion formation, and worsening renal function, leading to renal disease at the end of the stage [35]. The kidney contains nephrons that filter waste products from the blood, thereby maintaining an overall fluid balance in the body. The diabetic human and rats with nephropathy exhibited higher levels of inflammatory cytokines (TNF-α, IL-6, and IL-1β) in serum. TNF-α may induce renal cell injury [36]. Hence, the loss of cells along any part of the nephron can compromise or alter its functions. IL-6 and IL-1β induce glomerular membrane thickening, alterations in endothelial permeability, and mesangial cell proliferation [37]. Further, these cytokines (TNF-α and IL-1β) also activate transcriptional factor NF-kB of genes responsible for nephropathy in a positive feedback manner [5,37]. Diabetic rats exhibited the highest levels of creatinine, uric acid, and urea compared to other groups, which is in agreement with the study of Goji and colleagues [38] that stated increased serum urea and creatinine levels in diabetic rats decreased renal function and caused progressive damage. Treatment of diabetic rats with 50mg/kg *P. foetida* twigs extracts significantly reduced serum creatinine, uric acid, and urea compared to metformin, suggesting compared to metformin, suggesting a non-toxic effect on the kidney. The methanolic extract of *P. foetida* leaves may contain scopoletin-reduced serum TNF-α levels in diabetic rats [36,39]. The *P. foetida* leaf extract also lowered levels of IL-6 and IL-1β in blood serum and inhibited transcription factor NF-kB activation in diabetic rats [27,36]. Overall, the biomarkers of renal damage in the serum were significantly disturbed in diabetic rats and obese rats compared to normal rats. *P. foetida* twig extract was found to be non-toxic to the kidney.

As the primary target organ of insulin, the liver plays an essential role in developing insulin resistance and type 2 diabetes [40]. Liver disease is the primary cause of death among patients with diabetes mellitus, including non-alcoholic fatty liver disease (NAFLD). Fat accumulation in hepatocytes constitutes the spectrum of liver disease in diabetes mellitus. Conditions like hypertriglyceridemia and hypercholesterolemia have been associated with NAFLD [1,41]. Besides, high blood sugar levels in individuals with diabetes are associated with altered glucose and lipid metabolism, as well as modification in liver enzyme levels. The liver function tests are commonly used in clinical practice to screen for liver disease, monitor known disease progression, and monitor the effects of potentially hepatotoxic drugs.

Hepatotoxicity leads to damage to the permeability of liver cell membranes. Consequently, cytoplasmic enzymes like transaminases leak into the bloodstream, resulting in elevated levels of these enzymes in the serum [40]. Hepatocellular necrosis or membrane damage led to very high levels of serum ALT and AST released from the liver to circulation, aligned with the increased level of these enzymes in diabetic rats [42]. Furthermore, ALP is bound to the cell membrane, and any changes to it are likely to impact membrane permeability, leading to disruptions in the transport of metabolites. The increased levels of serum marker enzymes indicate cellular leakage and loss of functional integrity of the cellular membrane in the liver. In the present study, diabetic control rats had an increase in ALT, AST, and ALP levels compared to other groups. The diabetic rats treated with 50mg/kg *P. foeitda* twigs extract showed some decrement in ALT, AST, and ALP levels compared to 100mg/kg *P. foetida* twigs extract and 300mg/kg metformin groups, which is similar to the finding by [43]. This observation may be attributed to the antioxidative property of herbal medicine or plant extracts to balance the free radicals produced, thus preventing the lipid components of the cell membrane from being peroxidized. Disruption of membrane integrity is a common relevant factor attributed to increased release or leakage of cellular contents such as these enzymes studied. This study showed the ability of the 50mg/kg *P. foetida* twig extract to balance off the free radicals generated, hence preventing peroxidation of the lipid components of the cell membrane. Reducing hyperlipidemia results in a concomitant decrease in ALT, AST, and ALP enzyme activities [1].

Oxidative stress induces the microvascular and macrovascular complications of diabetes [44]. The free radicals react with all biological substances, i. e., lipids, protein, carbohydrates, and nucleic acids. They may induce lipid peroxidation when they react with polyunsaturated fatty acids in cell membranes, which impair membrane functions by decreasing the luidity and activity of membrane-bound proteins [45,46]. STZ-induced diabetic rats may have an overproduction of free radicals, glucose autooxidation, protein glycation, formation of advanced glycation products, and polyol pathways [47]. Decreased levels of CAT and GPx and increased levels of hepatic and heart PCO and RAGE are due to the overproduction of free radicals in diabetic rats [27,36,48]. STZ-induced diabetic rats treated with different doses of *P. foetida* twigs extract decreased the level of PCO and RAGE and improved the level of CAT and GPx. These results are concordant with the previous studies that reported an improvement in the levels of antioxidant enzymes in diabetic rats treated with leaf extract of *P. foetida* [27,36].

The enzymatic antioxidant CAT is a hemoprotein that reduces hydrogen peroxide and is known to be involved in detoxifyingdrogen peroxide concentration [49]. The other antioxidant glutathione peroxidase, has reduced lipid hydroperoxidase and hydrogen peroxide levels. STZ-induced diabetic rats with a higher level of blood glucose can inactivate the SOD, CAT, and GPx by fluctuating these proteins, thus producing induced oxidative stress, which causes lipid peroxidation [50]. STZ-induced diabetic rats treated with different doses of *Paederia foetida* twigs extract brought back the level of CAT and GPx activities near to the normal control group rat, indicating the usefulness of *P. foetida* in attenuating the oxidative stress in diabetic liver.

CAT was localized in the peroxisomes or the microperoxisomes. It catalyzes the decomposition of H_2O_2 to water and oxygen, thus protecting the cell from oxidative damage produced by H_2O_2. GPx catalyzes the reaction of hydroperoxides with reduced glutathione to form glutathione disulfide (GSSG) and the reduction product of the hydroperoxide [51]. The

reduction of CAT and GPx activities in diabetes conditions was in line with the study of Abolfathi and the team [51] and Nna and her group [44]. Nokkaew and colleagues [52] stated that PCO could be a biomarker for assessing the risk of oxidative stress-induced diabetic complications. The higher PCO in diabetic rats is in agreement with the study of Nokkaew and the team [52], which reported the finding of high PCO in diabetes complications and is similar to other human and animal model studies. Da Silva and group [53] mentioned that an increase in iPCO levels is an oxidative stress parameter. AGEs are a heterogeneous group of compounds formed via non-enzymatic reactions between reducing sugars and amine residues on macromolecules [54]. RAGE is a multiligand receptor, mediating AGE interaction and acute and chronic vascular inflammation in conditions such as complications of diabetes [55,56]. The higher level of RAGE in diabetic rats agrees with the Khazaei groups [54] and Feng and colleagues [50], which reported a significant evaluation of RAGE in tissues of the diabetic group. Kay and group [57] stated that type 2 diabetes patients had been shown to have a significantly higher concentration of AGEs than the non-diabetic population. Unuofin and Lebelo [58] described four key hypotheses causing clinical trials in diabetes research to see hyperglycemia inhibitors causing type 2 diabetes. These four main hypotheses are activation of isoforms of protein kinase C (PKC), heightened formation of advanced glycation end-product (AGE), and increased biosynthetic pathway flux of hexosamine and increased pathway flux of poly(ADPribose) (PARP). The study data revealed that daily treatment of *P. foetida* twig extract markedly improves the antioxidant status of the liver and organ tissue of rats with HFD-STZ-induced diabetes.

4. Conclusions

In conclusion, this study provided evidence that oral administration of 50mg/kg *P. foetida* twig chloroform extract on HFD-STZ induced T2DM rats for 28 days improved the Lee obesity index, TC, HDL, LDL, reduced blood glucose (27.19% reduction), TG, creatinine, uric acid, AST, ALT, ASP, and oxidative stress (PCO and RAGE levels), and increased antioxidant enzymes activities (CAT and GPx activities) which also validated the traditional usage of the plant as antidiabetic. More importantly, the *P. foetida* twig extract showed no toxicity effect on the kidneys or liver. Thus, *P. foetida* twigs extract has a good potential to be developed as a therapeutic agent to treat type 2 diabetes, for which further clinical testing should be conducted on human volunteers and diabetic patients.

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Conflicts of Interest

The authors declare no conflict of interest.

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