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RESEARCH ARTICLE

Anti-hyperglycemic and ameliorative effect of concentrated hot water-infusion of *Phragmanthera incana* leaves on type 2 diabetes and indices of complications in diabetic rats

Olakunle Sanni[1] · Ochuko L. Erukainure[1,2,3] · Olajumoke Oyebode[1] · Md. Shahidul Islam[1]

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Abstract

Objectives This study investigated the anti-hyperglycemic effects of concentrated hot water infusion of *Phragmanthra incana* leaves as well as its ameliorative effect on indices related to diabetic complications in a type 2 diabetes model of rats.

Methods Type 2 diabetes was induced by feeding 10% fructose solution ad libitum for two weeks followed by an intraperitoneal injection of streptozotocin (40 mg/kg body weight (b.w.)). Concentrated plant infusion was administered orally at a dose of 150 and 300 mg/kg b.w. to two type 2 diabetes rat groups. Diabetic rats without treatment served as a negative control while the group administered with metformin was served as a positive control. The intervention lasted for 4 weeks when a single oral dose was given daily for 5 days a week. Body weight and blood glucose were determined every week. An oral glucose tolerance test was performed in the last week of treatment. The rats were sacrificed after 4 weeks of intervention, and the blood and organs were harvested for further analysis.

Results Both dosages of the plant infusion significantly improved body weight, pancreatic β-cell function (HOMA-β), insulin secretion and reduced blood glucose, insulin resistance (HOMA-IR) with concomitant reduction in the elevated level of serum α-amylase activity, fructosamine, uric acid, urea, and liver function enzymes. The liver glycogen content was significantly improved while the activity of liver glucose-6-phosphatase was significantly reduced.

Conclusion The results demonstrate the anti-hyperglycemic ability of *P. incana* and its ability to delay the onset of diabetic complications which can be exploited for the anti-diabetic drug discovery.

Keywords *Phragmanthra incana* · Antihyperglycemic · Type 2 diabetes · Glucose homeostasis

Introduction

Over the years the life expectancy and the quality of life have been decreased gradually due to the unrelenting growth in numbers of people living with diabetes. The prevalence of diabetes has increased from 239 million in 2000 to 425 million in 2017 [1]. Consequently, diabetes has directly caused 1.6 million deaths in 2016 and is the major cause of kidney failure, blindness, heart failure and other complication that impact significantly on the quality of life [2].

Maintaining a normal blood glucose level is the underlying principle of the management and treatment of diabetes. Different classes of hypoglycemic agents used in the treatment of diabetes that utilize different mechanisms to achieve this goal. For example, biguanide suppresses hepatic glucose production, thus decreases blood glucose level [3]. Sulfonylureas work by stimulating insulin secretion from pancreatic β-cells [4], while α-glucosidase inhibitors decrease postprandial hyperglycemia and delay the breakdown of carbohydrates thereby decrease small intestinal glucose absorption [5]. However, none of these drugs are without side effects and relatively expensive, particularly for the people in developing world.

On the other hand, medicinal plants provide cheaper alternative therapy for the treatment of diabetes. Several medicinal plants have been investigated for their antihyperglycemic activity using different techniques in vitro, ex vivo and in vivo

✉ Md. Shahidul Islam
islamd@ukzn.ac.za

[1] Department of Biochemistry, School of Life Sciences, University of Kwazulu-Natal (Westville Campus), Durban 4000, South Africa

[2] Nutrition and Toxicology Division, Federal Institute of Industrial Research, Lagos, Nigeria

[3] Department of Pharmacology, University of the Free State, Bloemfontein 9300, South Africa

experimental models. They modulate blood sugar levels through a variety of mechanisms such as, increasing muscle glucose uptake [6], pancreatic β-cells regeneration, increasing insulin secretion [7, 8], and inhibiting carbohydrate digesting enzymes [9–11].

Phragmanthra incana or *P. incana*, commonly known as African mistletoe, is a parasitic plant species of the Loranthaceae family. It is used traditionally in the treatment of different disease conditions and ailments including hypertension, diabetes, inflammation, cancer, and insomnia [12]. However, scientific validations of its traditional use are scanty. The methanol and ethyl acetate extracts have been reported to demonstrate antibacterial activity [13]. The methanolic extract of the leaves showed antioxidant and inhibitory activities against Fe^{2+}-induced lipid peroxidation [12]. The hepatoprotective effect of the methanol crude extract in alloxan-induced diabetes has also been reported [14]. Recently, the infusion of the leaf was shown to inhibit the carbohydrate digesting enzymes and promote glucose uptake in ex vivo model [15]. However, the antihyperglycemic effect of the leaves hot water infusion in diabetic rats has not been investigated.

Hence, this study was aimed at investigating the antihyperglycemic activities of *P. incana* in a fructose-fed streptozotocin-induced type 2 diabetes model of rats.

Materials and methods

Plant material

P. incana leaves were collected sometime in March 2016 from Owo community in Ondo State of Nigeria. The plant sample was authenticated at the Biological Science Department of Adekunle Ajasin University, Akungba, Nigeria when a voucher specimen number: PSB 178 was preserved at the same university.

Sample extraction

The leaves were washed with tap water and air-dried to a constant weight. The dried leaves were blended to fine powder using Thomas-Wiley Laboratory mill, Philadephia P.A, USA. One kilogram (1 kg) of the blended sample was infused in boiling water and allowed to stand for 20 min. The infusion was then filtered with Whatmann's filter paper (No. 1) and concentrated in a water bath at 50 °C. It was then stored at 4 °C until further use.

Experimental animals

Forty-two male Sprague-Dawley (SD) rats of six week old (mean b.w. 193 ± 11.94 g) were procured from and housed at the Biomedical Resource Unit, University of Kwazulu-Natal, Durban, South Africa. The animals were maintained according to the guidelines of the Animal Research Ethics Committee (AREC) of the University of Kwazulu-Natal, Durban, South Africa (Protocol approval number: AREC/067/017D).

Animal grouping and induction of T2D

After the acclimatization of the animals for one week, they were randomly divided into six groups of seven animal in each group as shown below;

Normal control (NC)
Normal control +300 mg/kg b.w. of the infusion (NTPI)
Diabetic control group (DBC)
Diabetic + low dose (150 mg/kg b.w.) of the infusion (DPIL)
Diabetic + high dose (300 mg/kg b.w.) of the infusion (DPIH)
Diabetic + metformin (300 mg/kg b.w.) (DBM)

After an overnight fast, animals in diabetic groups (DBC, DPIL, DPIH and DBM) were given a 10% fructose solution *ad libitum* for two weeks to induce insulin resistance followed by a single injection of streptozotocin (STZ) (40 mg/kg b.w. dissolved in citrate buffer pH 4.5) intraperitonially to cause partial destruction of pancreatic β-cells [16]. The animals in normal groups (NC and NTAI) were given water *ad libitum* for two weeks and then injected with citrate buffer (40 mg/kg b.w). After one week of STZ injection, non-fasting blood glucose (NFBG) of all the animals was measured using a portable glucometer (Glucoplus Inc., Quebec, Canada). Animals with NFBG >18 mmol/L were considered diabetic while the animals with NFBG <18 mmol/L were excluded from the study.

Intervention period

After the confirmation of diabetes, the animals were given their respective treatment (as indicated in the grouping) for five days a week using a gastric gavage needle. While animals in the control groups were treated with a similar volume of the vehicle. During the intervention period, fluid and food intake were measured daily and body weight and NFGB were measured weekly in all the groups.

Oral glucose tolerance test (OGTT)

OGTT was performed in the last week of the intervention period. The animals were given an oral dose of glucose solution (2 g/kg b.w.) after an overnight (12 h) fast. Thereafter, the blood glucose levels were measured at 0 (just before glucose ingestion), 30, 90, and 120 min after the glucose ingestion

using a portable glucometer. The area under the curve (AUC) was calculated according to the following formula:

$$AUC_{t_k} = \sum_{i=1}^{k} \left(\frac{C_{i-1} + C_i}{2} \right) (t_i - t_{i-1})$$

Where Ci is the concentration of blood glucose at time ti.

Collection of blood and organs

At the end of the experimental period, the rats were sacrificed by euthanizing with halothane. Whole blood was collected via cardiac puncture from each animal into a plain tube and centrifuge at 3000 rpm for 15 min to obtain the serum which was preserved at -20 °C for subsequent analysis. The liver and pancreas of each animal were collected, washed with normal salaine, weighed and stored at -20 °C for further analysis. A small piece of the pancreatic tissue of each animal was cut and placed in a 10% formalin solution for histopathological examination.

Biochemical analysis

Serum insulin

Serum insulin concentration was measured using an ultrasensitive rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Upsala, Sweden) according to the manufacturer's manual. Serum fructosamine, urea, and creatinine concentrations as well as liver function enzymes; aspartate and alanine aminotransferases (AST and ALT) and alkaline phosphatase (ALP) were measured with an Automated Chemistry Analyzer (Labmax Plenno, Labtest Co. Ltd., Lagoa Santa, Brazil) with compatible commercial assay kits.

Serum amylase activity

This was determined using a colorimetric method with 3,5-dinitrosalicylic acid (DNS) reagent [17]. The assay is based on the conversion of starch to maltose by α – amylase and measured by the reduction of 3,5-dinitrosalicylic acid.

Liver glycogen estimation

Liver glycogen was estimated according to a previously described method by Lo et al. [18], and glycogen content was calculated from the glycogen standard curve using log-log graph and expressed as μg/mg of tissue.

Determination of glucose-6-phosphatase activity

The activity of glucose-6-phosphatase was measured by spectrophotometric determination of inorganic phosphate (Pi) production according to a method as described previously by Koide [19]. The amount of liberated Pi was extrapolated from a phosphorus standard curve. Glucose-6-phophatase activity was expressed as units/min/mg of protein in tissue.

Homeostatic model assessment

Homeostatic model assessment scores are used to determine the pancreatic β-cell function (HOMA- β) and insulin resistance (HOMA- IR) from fasting serum insulin and glucose concentrations [20]. These were calculated according to the following formula:

$$HOMA{-}IR = [Fasting\ serum\ insulin\ (U/L)\ x\ Fasting\ blood\ glucose\ (mmol/L)]/22.5$$
$$HOMA{-}\beta = [20\ x\ Fasting\ serum\ insulin\ (U/L)]/Fasting\ blood\ glucose\ (mmol/L){-}3.5$$
$$Conversion\ factor: insulin\ (1\ U/L = 7.174\ pmol/L)$$

Histological examination of pancreatic tissue

Tissue sections were cut into a size of 4 μm and fixed on slides after necessary processing. They were embedded in paraffin and then subjected to hematoxylin and eosin staining. Slides were viewed using a Leica slide scanner (SCN 4000, Leica Biosystems, Germany).

Statistical analysis

Data are represented as means ± SD and analyzed with Windows SPSS statistical software package (version 26, IBM Corporation, New York, USA) using Tukey's-HSD multiple range post hoc test. $p < 0.05$ was considered as significant.

Results

Treatment with *P. incana* infusion had a significant ($p < 0.05$) effect on both fluid and food intake as shown in Fig. 1. However, there was no significant difference in the fluid intake between the low and high doses of the treatment.

As indicated in Fig. 2, the body weight of diabetic animals was significantly ($p < 0.05$) reduced a week after the induction of diabetes. But along the intervention period, DAIL, DAIH, and DBM groups had significantly ($p < 0.05$) higher body weight gain compared to the DBC group.

One week after the induction of diabetes, there was a significant ($p < 0.05$) increase in NFBG levels of diabetic groups (DBC, DPIL, DPIH and DBM) when compared to the normal control groups (NC and NTPI) as shown in Fig. 3. However, the administration of *P. incana* infusion significantly reduced the elevated blood glucose levels compared to the diabetic control group (DBC). Likewise, the calculated area under the curve (AUC) for DBC was significantly higher than

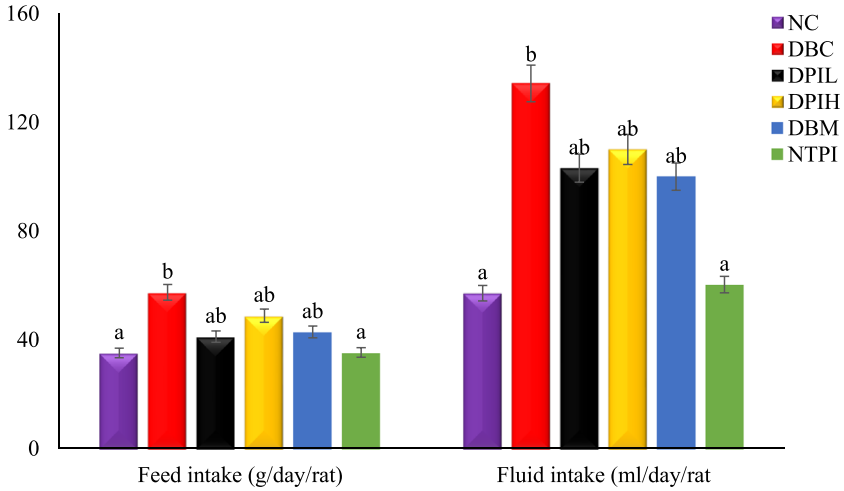

Fig. 1 Food and fluid intake in different animal groups during the experimental period. Data are presented as the mean ± SD of 5-7 animals. [a &b] Different alphabets over the bars for a given animal group represent significance of difference ($p < 0.05$). NC, Normal Control; DBC, Diabetic Control; DPIL, Diabetic *P. incana* low dose; DPIH, Diabetic *P. incana* high dose; DBM, Diabetic Metformin; NTPI, Normal *P. incana* high dose

DPIL, DPIH and DBM groups, while NC and NTAI groups were not significantly different from each other but were significantly lower than the diabetic groups (Table 1).

The result of OGTT are presented in Fig. 4. The glucose tolerance ability of the animals in normal control groups (NC and NTPI) was significantly ($p < 0.05$) better than the diabetic groups. However, diabetic groups treated with hot water infusion of *P. incana* were significantly ($p < 0.05$) better than the diabetic control group. Besides, the calculated AUC for non-diabetic groups (NC and NTPI) were significantly lower than the diabetic groups (Table 1), while there was no significant difference between the DPIL, DPIH and DBM groups but were significantly ($p < 0.05$) lower than the DBC group.

The results of serum insulin concentration, fructosamine and homeostatic model assessment are presented in Table 2. The concentration of serum insulin and HOMA-β scores were significantly lower ($p < 0.05$) and the concentration of fructosamine and HOMA-IR scores were significantly higher ($p < 0.05$) in the DBC group when compared with other groups. Upon treatment with hot water infusion of *P. incana*, the serum insulin concentration and HOMA-β scores were significantly increased ($p < 0.05$) while fructosamine concentration and HOMA-IR scores were significantly ($p < 0.05$) decreased in DPIL, DPIH, and DBM groups when compared to the DBC group.

The liver glycogen and, serum α-amylase and glucose-6-phosphatase activities are presented in Table 3. The liver glycogen content was significantly ($p < 0.05$) depleted in the DBC group but upon treatment with the hot water infusion of *P. incana*, the glycogen contents of the liver of DPIL and DPIH groups were significantly ($p < 0.05$) increased. Both the α-amylase and liver glucose-6-phosphatase activities were significantly increased in the DBC ($p < 0.05$) group, when the treatment with the infusion significantly reduced the activities of these enzymes.

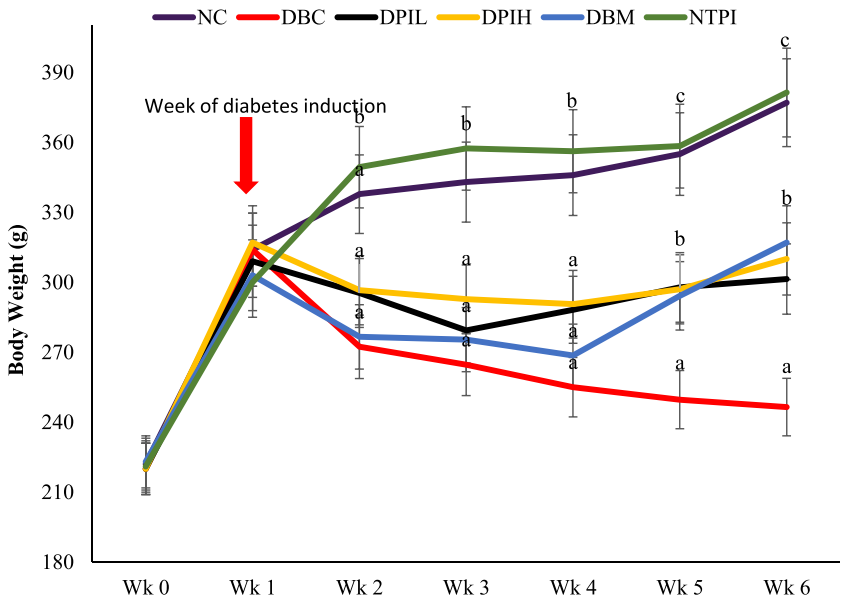

Fig. 2 Effect of oral treatment of hot water infusion of *P. incana* on body weight in different animal groups during the experimental period. Data are presented as the mean ± SD of 5-7 animals. [a - c] Different alphabets over the bars for a given animal group represent significance of difference ($p < 0.05$). NC, Normal Control; DBC, Diabetic Control; DPIL, Diabetic *P. incana* low dose; DPIH, Diabetic *P. incana* high dose; DBM, Diabetic Metformin; NTPI, Normal *P. incana* high dose

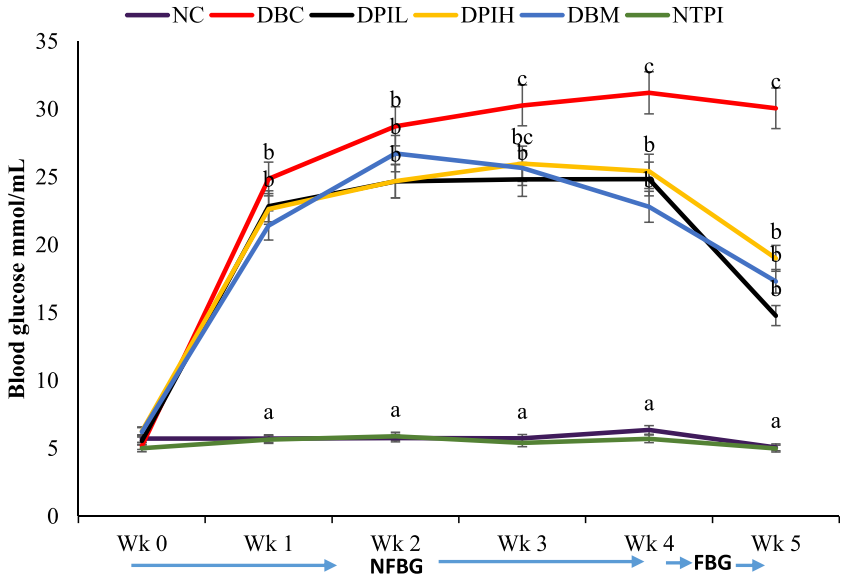

Fig. 3 The effects of oral treatment of hot water infusion of *P. incana* leaves on weekly blood glucose concentrations in different animal groups during the intervention period. Data are presented as the mean ± SD of 5-7 animals. [a-c]Values with different letters for a given week are significantly different from one another (Tukey's-HSD multiple range post hoc test, $p < 0.05$). NC, Normal Control; DBC, Diabetic Control; DPIL, Diabetic *P. incana* low dose; DPIH, Diabetic *P. incana* high dose; DBM, Diabetic Metformin; NTPI, Normal *P. incana* high dose; NFGB, non-fasting blood glucose; FGB, fasting blood glucose

The indices of hepatic and renal damages are presented in Table 3. The serum level of creatine was not affected in all the experimental groups, but serum urea, uric acid, AST, ALT and ALP levels were significantly elevated ($p < 0.05$) in the DBC group compared to the NC group. However, hot infusion treatments of *P. incana* significantly ($p < 0.05$) reduced their levels in diabetic rats compared to the untreated DBC group (Table 3). No significant differences were observed between the NC and NTPI groups for all these parameters.

The results of the histopathological examination of pancreas are presented in Fig. 5. DBC group showed a reduction in the size of the pancreatic islet with a concomitant reduction in the number of β-cells when compared with NC group. However, no difference was observed in the number of β-cells in the islet of the pancreas in DPIL, DPIH and DBM groups. DBC group showed a lower number of β-cells in the islet of pancreas when compared with DPIL, DPIH and DBM groups.

Discussion

Medicinal plants have over the years played a crucial role in the health care delivery system all over the world [21]. However, the use of herbal medicine in the management of diseases has raised questions about the efficacy and safety [21, 22]. Our previous study revealed that the hot water infusion of the leaves of *P. incana* exhibited a potent α-glucosidase and α-amylase inhibitory activity and increased muscle glucose uptake [15]. This prompted us to further investigate its antihyperglycemic activity in a type 2 diabetes model of rats.

Table 1 Serum insulin and fructosamine concentrations as well as computed HOMA-IR and HOMA-β scores for different animal groups at the end of the experiment

	NC	DBC	DPIL	DPIH	DBM	NTPI
Insulin (ρmol/L)	72.60 ± 2.47^{c}	31.61 ± 3.20^{a}	70.62 ± 3.29^{bc}	42.72 ± 4.35^{ab}	59.23 ± 1.70^{b}	80.97 ± 0.72^{c}
Fructosamine (μmol/L)	119 ± 5.39^{a}	867 ± 19.38^{c}	387 ± 11.04^{b}	442 ± 12.58^{b}	633 ± 13.18^{b}	118 ± 2.88^{a}
HOMA-IR	1.85 ± 0.76^{a}	8.71 ± 1.54^{c}	3.95 ± 1.1^{ab}	4.46 ± 0.46^{b}	4.32 ± 0.97^{b}	2.2 ± 0.54^{a}
HOMA-β	64.34 ± 2.45^{d}	2.9 ± 0.32^{a}	32.01 ± 1.97^{c}	18.93 ± 3.67^{b}	13.59 ± 2.34^{b}	67.11 ± 1.23^{d}

Results are expressed as mean ± SD of 5-7 rats

NC Normal Control, *DBC* Diabetic Control, *DPIL* Diabetic *P. incana* low dose, *DPIH* Diabetic *P. incana* high dose, *DBM* Diabetic Metformin, *NTPI* Normal *P. incana* high dose, *HOMA-IR* Homeostatic model assessment - insulin resistant, *HOMA-beta* Homeostatic model assessment - beta cell function

[a-d] Different superscripts alphabets along a row indicate significant difference (Tukey's-HSD multiple range post hoc test, $p < 0.05$)

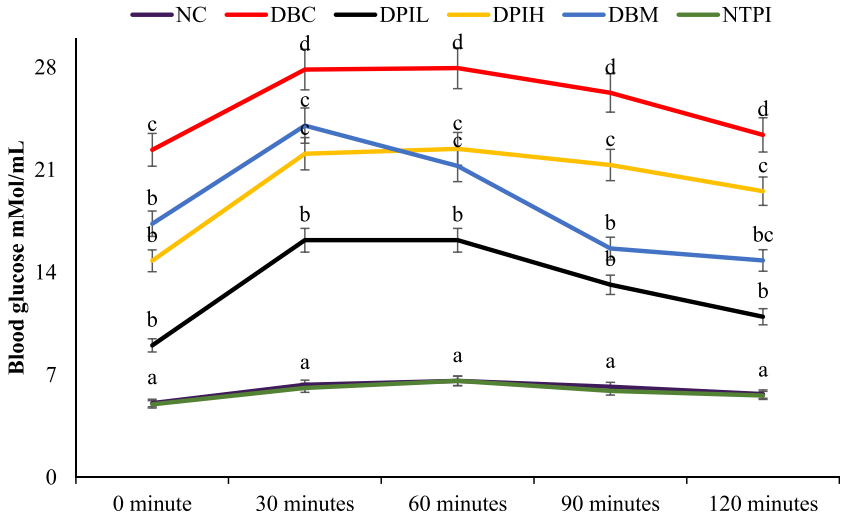

Fig. 4 Oral glucose tolerance test (OGTT) of different animal groups in the last week of experimental period. Data are presented as the mean ± SD of 5-7 animals. [a - d] Different alphabets over the lines for a given time represent significance of difference ($p < 0.05$). NC, Normal Control; DBC, Diabetic Control; DPIL, Diabetic *P. incana* low dose; DPIH, Diabetic *P. incana* high dose; DBM, Diabetic Metformin; NTPI, Normal *P. incana* high dose

Obvious symptoms of diabetes mellitus include polydipsia and polyphagia with concomitant reduction of body weight [23], which were also observed in the diabetic groups of our experiment. Several studies have linked these parameters to prolong and stable diabetic conditions [16, 24, 25]. In the present study, treatment with the *P. incana* infusion although could not completely reverse the lost body weight (Fig. 2), polydipsia and polyphagia, its effect on theses parameters was better than the diabetic group without treatment. This may be as a result of the longer duration of the intervention period.

Elevated postprandial blood glucose is an indication of T2D. Therefore, maintaining glucose homeostasis is a major strategy for preventing complications associated with diabetes [25, 26]. Studies have attributed chronic hyperglycemia to insulin insufficiency followed by reduction of pancreatic β-cell mass and the insensitivity of peripheral tissues and cells to the action of insulin [27, 28]. This concurs with the elevated blood glucose (Fig. 3), low serum insulin (Table 1) and the disgruntled pancreatic morphology (Fig. 5) of the untreated diabetic rats in our study. The ability of the infusion to reduce blood glucose (Fig. 3) with concordant improvement of β-cell functions and increase in serum insulin (Table 1) depict antihyperglycemic effect of *P. incana*. Also, the regeneration of pancreatic β-cell and improved morphology (Fig. 5) further explain the antihyperglycemic potential of *P. incana* hot water infusion.

Apart from pancreatic β-cell dysfunction, the insensitivity of peripheral tissues and cells to insulin is the major characteristic of glucose intolerance in type 2 diabetes. Several studies have indicated the use of oral glucose tolerance test (OGTT) to evaluate glucose intolerance [29–31]. The glucose intolerance (Fig. 4) of the untreated diabetic rats further supports the pancreatic β-cell dysfunction and insulin insensitivity. The ability of the *P. incana* infusion to improve glucose tolerance and utilisation (Fig. 4) further portrays its antihyperglycemic ability. This is also evident by the low

Table 2 Liver glycogen concentrations and glucose-6-phosphatse as well as serum α-amylase activity in different animal groups at the end of the experimental period

	NC	DBC	DPIL	DPIH	DBM	NTPI
Liver glycogen (mg/g tissue)	5.36 ± 0.31^{d}	1.63 ± 0.88^{a}	3.58 ± 0.94^{c}	2.51 ± 0.58^{b}	3.58 ± 0.78^{c}	4.94 ± 0.88^{d}
Serum α-amylase activity (U)	58.40 ± 1.76^{a}	86.60 ± 1.73^{c}	62.7 ± 4.65^{ab}	64.3 ± 2.81^{b}	68.6 ± 2.10^{b}	60 ± 1.73^{a}
Glucose-6-phosphatase (U)	1.92 ± 0.03^{a}	6.21 ± 0.08^{d}	2.27 ± 0.22^{b}	3.24 ± 0.03^{c}	2.01 ± 0.03^{ab}	1.87 ± 0.04^{a}

Results are presented as mean ± SD of 5-7 animals

α-amylase activity 1 U = 1 μmoles maltose formed per min per ml

Glucose-6-phosphatase 1 U = 1 μmole phosphate liberated per min per mg of the liver tissue

NC Normal Control, *DBC* Diabetic Control, *DPIL* Diabetic *P. incana* low dose, *DPIH* Diabetic *P. incana* high dose, *DBM* Diabetic Metformin, *NTPI* Normal *P. incana* high dose

[a-d] Values with different alphabets along a row are significantly different from each other (Tukey's-HSD multiple range post hoc test, $p < 0.05$)

Table 3 Indices of hepatic and renal damages for different animal groups at the end of the experiment

	NC	DBC	DPIL	DPIH	DBM	NTPI
Urea (mg/dL)	54.75 ± 5.21^{a}	75.00 ± 3.12^{d}	60.00 ± 2.27^{bc}	65.33 ± 3.04^{c}	61.25 ± 1.25^{c}	56.33 ± 4.48^{ab}
Uric acid (mg/dL)	1.14 ± 0.17^{a}	4.80 ± 0.92^{c}	2.85 ± 0.54^{b}	3.03 ± 0.21^{b}	1.95 ± 0.14^{a}	2.11 ± 0.53^{ab}
CK-MB (U/L)	2.13 ± 0.03	2.81 ± 0.08	2.45 ± 0.22	2.75 ± 0.03	2.61 ± 0.03	2.21 ± 0.04
AST (U/L)	61.25 ± 1.87^{a}	121.67 ± 2.54^{c}	73.50 ± 4.67^{b}	88.67 ± 1.57^{b}	78.33 ± 3.12^{b}	64.00 ± 2.67^{a}
ALT (U/L)	59.50 ± 5.23^{b}	93.50 ± 6.47^{a}	86.23 ± 3.25^{b}	87.00 ± 4.26^{b}	64.00 ± 12.24^{b}	61.67 ± 10.21^{b}
ALP (U/L)	109.5 ± 21.14^{a}	513.00 ± 13.41^{d}	159.00 ± 27.21^{c}	269.50 ± 14.21^{b}	207.00 ± 11.42^{b}	102.00 ± 11.25^{a}

Results are presented as mean ± SD of 5-7 animals

NC Normal Control, *DBC* Diabetic Control, *DPIL* Diabetic *P. incana* low dose, *DPIH* Diabetic *P. incana* high dose, *DBM* Diabetic Metformin, *NTPI* Normal *P. incana* high dose

[a-d] Values with different alphabets along a row are significantly different from each other (Tukey's-HSD multiple range post hoc test, $p < 0.05$)

HOMA-IR scores (Table 1) of the *P. incana* treated groups of diabetic rats.

The liver plays a major role in overall glucose disposal and insulin sensitivity. In normal metabolic condition, elevated glucose is stored as glycogen in response to insulin action. In type 2 diabetes, the excess glucose disposal as glycogen is compromised which may further impact hepatic steatosis by diverting excess glucose to de novo fatty acid synthesis [32]. The depletion of liver glycogen in untreated diabetic rats (Table 2) indicates that both hepatosteatosis and insulin resistance have compromised glycogen synthesis [31]. The ability of the infusion to increase liver glycogen (Table 2) indicates improved glucose tolerance independent of insulin signalling [33] which further explain its antidiabetic potentials.

Furthermore, hepatic glucose production contributes significantly to the elevated postprandial hyperglycemia in diabetic subjects. It is well established that insulin inhibits hepatic glucose production [34–36]. This is evident in our study as the blood glucose level is higher in diabetic rats (Fig. 3) with concomitant reduction of serum insulin (Table 1). The processes of de novo glucose production (gluconeogenesis) involve in the activation of glucose-6-phosphatase which is one

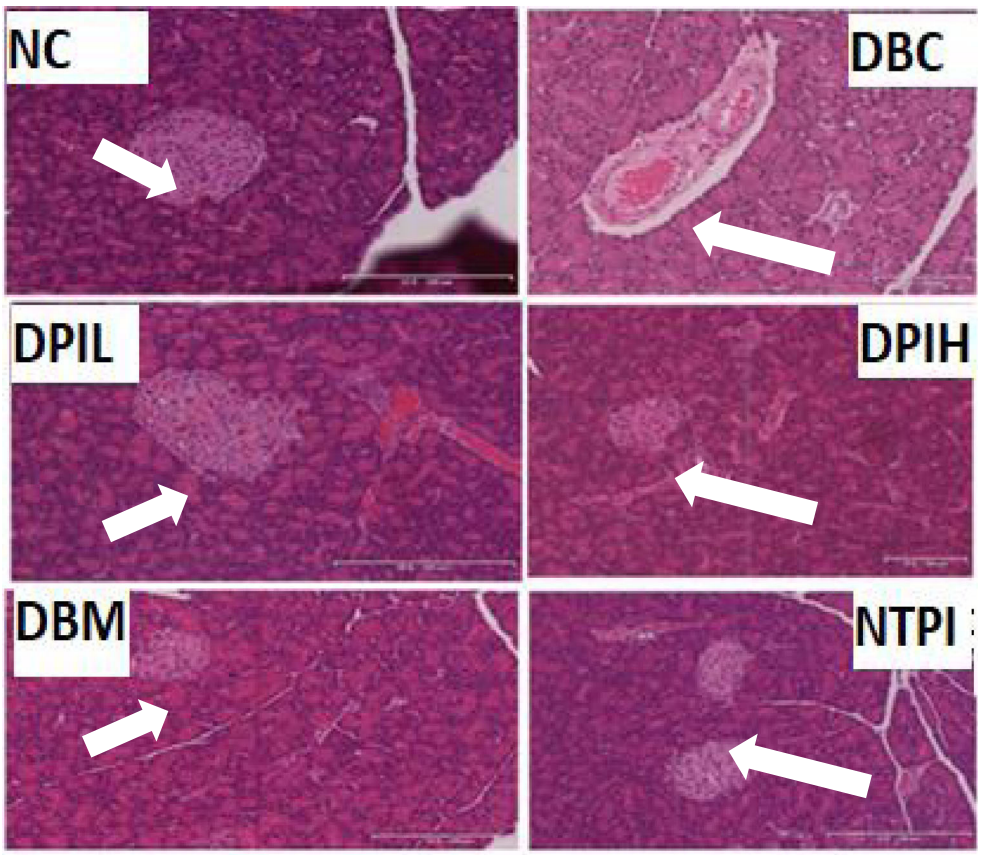

Fig. 5 Effect of oral treatments of hot water infusion of *P. incana* on histological examinations of the pancreas in different animal groups during the experimental period. The NC and NPAI group have the highest number of β-cells per islet. The islet of DBC group is distorted with fewest number of β-cells. DPIL, DPIH and DBM groups showed regeneration of the islet with more β-cells as compared to DBC. NC, Normal Control; DBC, Diabetic Control; DPIL, Diabetic *P.incana* low dose; DPIH, Diabetic *P. incana* high dose; DBM, Diabetic Metformin; NTPI, Normal *P. incana* high dose

of the rate-limiting steps of gluconeogenesis [37]. The increased glucose-6-phosphatase as seen in the untreated diabetic rats (Table 2), indicates an activation of hepatic glucose production. The ability of the infusion to decrease the glucose-6-phosphatase activity (Table 2) indicates a suppressive effect of gluconeogenesis.

Delay in postprandial glucose absorption from the small intestine is one of the ways of managing diabetes [38]. Pancreatic α-amylase play an essential role in the breakdown of dietary carbohydrates to glucose for subsequent absorption by the intestine. The increased serum α-amylase activity in the untreated diabetic rats (Table 2) may contribute to their elevated blood glucose level (Fig. 3). This correlates with previous reports that increased serum amylase activity in diabetic ketoacidosis might be enhanced by the rate of α-amylase release [39]. The reduced activity of the enzyme in diabetic rats treated with the *P. incana* infusion connotes an inhibitory effect. This further corroborates our previous report on in vitro inhibitory potential of the plant on carbohydrate digestive enzymes [15].

Persistent hyperglycemia have been implicated in many of the diabetic complications such as retinopathy, nephropathy, atherosclerosis and neuropathy [40] which involve multiple cascade of reaction such as advanced glycation end product, hexosamine pathway, polyol pathway and activation of protein kinase C. Fructosamine is a product of glycated protein in an early stage which later form advanced glycation end product. It has been used as a glycemic biomarker for the detection and control of diabetes [41]. The increased level of fructosamine in the untreated diabetic rats corresponds with its increased blood glucose (Fig. 3), which indicates a glycation cascade reaction and the onset of diabetic complications (Table 1). The reduced level of fructosamine in the *P. incana* infusion treated rats, suggests the attenuation of the glycation cascade.

Likewise, elevated serum uric acid and urea have been linked with the development of diabetic nephropathy [42]. Their reduced levels in the treatment groups further supports the ability of the infusion to ameliorate the progression of diabetic complications in type 2 diabetes. Elevated liver function enzymes levels in hyperglycemia has been linked to lipid peroxidation and recruited inflammatory cells as a result of oxidative stress and increase proinflammatory cytokines, which may cause hepatocellular injury [43]. The depleted serum level of these enzymes in normal and diabetic rats treated with the *P. incana* infusion (Table 3) also indicates its safety on healthy tissues as well as on diabetics.

Conclusion

The results of this study indicate the antihyperglycemic potentials of concentrated hot water infusion of *P. icana* thus, give credence to its folkloric claims. Our future work will focus on the detailed studies on the molecular mechanism(s) and metabolic pathways that may be involved behind its antihyperglycemic activity.

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Conflict of interest The authors declared that they have no conflict of interest.

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