



Original Research Article

Glycemic control efficacy of *Phyllanthus niruri*. Linn extract in Diabetic Mice model

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies characterized by absolute or relative deficiency of insulin secretion with or without varying degree of insulin resistance. Sedentary life style and obesity are two major epidemiological determinants of diabetes mellitus. In the present investigation hypoglycemic efficacy of *Phyllanthus niruri* methanol extract was tested on STZ induced mice diabetic models. The results clearly indicated that the diabetic control (DC) mice presented a significant lowering of body weight ($p < 0.001$) when compared with the normal control (NC) mice. The DC mice showed a significantly ($p < 0.001$) higher level of glucose (+279%), when compared with their normal control counterparts. Diabetic mice of both of the groups (DT₁₅₀ and DT₂₅₀) showed a reduction in glucose levels, when compared to the DC ones. The results clearly indicated that the *P. niruri* whole plant methanol extract is antidiabetic in nature due to the presence of different types of active phytochemicals.

Keywords

Diabetes mellitus, *Phyllanthus niruri*, Streptozotocin, Mice

Introduction

Diabetes mellitus (DM) is the third leading disease, after heart attack and cancer affecting almost every organ in the human body (Nyenwe et al., 2011) and is also called silent killer. This is a metabolic disorder of multiple etiologies (Mohler et al., 2009) characterized by absolute or relative deficiency of insulin secretion with or without varying degree of insulin resistance (Lin and Sun, 2010; Nyenwe et al., 2011).

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia with an elevated fasting (>110mg/dL of blood

and post prandial (> 130mg/dL of blood) plasma glucose level. According to WHO (2006) diagnosis a fasting plasma sugar of >126mg/dL and post prandial plasma sugar value of > 200mg/dL is considered as diabetes mellitus. There are two major forms of diabetes mellitus namely Type-1, characterized by diminished production of insulin due to degeneration of pancreatic B-cells, and Type-2, the multifactorial syndrome characterized by either hypo secretion of insulin or insulin insensitivity or sometimes both. Sedentary life style and obesity are two major epidemiological determinants of diabetes mellitus. The

current therapy of this disorder includes exogenous insulin administration (particularly in case of Type-1 diabetes mellitus), and oral hypoglycemic agents (for Type-2DM) which includes Metformin, Pioglitazone, Sulphonylurea etc. which may have adverse effects in diabetic subjects. Multiple risk factors for diabetes have been identified (WHO, 2006). The greatest risk is impaired glucose tolerance, a precursor of diabetes. Thus, a number of type 2 diabetes prevention trials have included subjects with impaired glucose tolerance. These trials compared intensive lifestyle modifications (e.g., diet, exercise and weight loss), OHAs and placebo controls (Tuomilehto et al., 2001; Knowler et al., 2002). Ayurvedic treatment known as *Apatarpana* (balanced diet with restricted calories) and *Santarpana* (highly nutritious, high-calorie diet intended to increase weight) are recommended for patients with type 2 and type 1 diabetes, respectively (Sharma and Chandola, 2011).

The Clinical Practice Guidelines for the Prevention and Management of Diabetes recommends a target glycosylated hemoglobin (HbA_{1c}) concentration of 7.0% or less for all patients with diabetes and, for those in whom it can be safely achieved, a target HbA_{1c} concentration in the normal range, usually $\leq 6.0\%$ (WHO, 2006). Although nonpharmacologic therapy (e.g., diet, exercise and weight loss) remains a critical component in the treatment of diabetes, pharmacologic therapy is often necessary to achieve optimal glycemic control. Orally administered antihyperglycemic agents (OHAs) can be used either alone or in combination with other OHAs or insulin. Various classes of OHAs are now available that target the different pathophysiologic factors contributing to diabetes: α -glucosidase inhibitors to delay intestinal carbohydrate absorption (Lebovitz, 1997; Inzucchi, 2002;

Bayraktar et al., 1996; Chiasson et al., 2002), biguanides to target hepatic insulin resistance (Bailey and Turner, 1996; Kirpichnikov et al., 2002; Zhou et al., 2001; Holmes et al., 1999; Salpeter et al., 2004), insulin secretagogues to increase pancreatic insulin secretion (Klepzig et al., 1999; Lebovitz, 2001; Strom et al., 2003; Hatorpe, 2002; McLeod, 2004), insulin sensitizers or thiazolidinediones which function as ligands for the peroxisome proliferator-activated receptor gamma (PPAR γ) to target adipocyte and muscle insulin resistance (Lister et al., 1999; Finegood et al., 2001; Bell, 2003; Bakris et al., 2003; Herz et al., 2003; Nesto et al., 2003; Kelly et al., 1999; Lee et al., 2003), and intestinal lipase inhibitor or orlistat to inhibit fat absorption and promote weight loss in obese patients (Guerciolini, 1997; Hollander et al., 1998; Hanefeld and Sachse, 2002; Kelley et al., 2002).

Despite excellent potencies, these synthetic antidiabetic drugs had presented unwanted therapeutic profiles, marked by fluid retention, hypoglycemia at higher doses, liver problems, lactic acidosis, weight gain and potential cardiac hypertrophy. There is also evidence that hyperglycaemia per se has deleterious effects on beta cell function and insulin action (glucotoxicity). Thus, a concerted effort to search more effective drugs for T2DM has become the need of the time in terms of efficacy as well as safety due to the undesirable side effects of synthetic drugs.

Over the past 25 years, 50% of prescription drugs have been developed from natural products and their derivatives. These medicines have emerged as unique, safe, effective, and relatively inexpensive remedies producing minimal or no side effects with tall claims of efficacy as add on therapy (Heinrich *et al.*, 2012). Herbal drugs

with antidiabetic activity can be classified into four categories according to their mode of action. The first group has insulinomimetic effect and includes plant like *Momordica charantia* (bitter gourd) (Grover and Yadav 2004). Second group acts on the β -cells to increase the production of insulin and include plants like *Allium cepa* (onion) and *Pterocarpus marsupium* (Vijaysaar) (Grover and Vats 2001). The third one enhances glucose utilization in diabetic patients and includes plants like *Gingiber officinale* (ginger), *Cyamospsis tetragonalobus* (Gower plant) and *Grewia asiatica* (phalsa). They increase the viscosity of gastrointestinal contents, slow gastric emptying and act as a barrier to diffusion (Grover and Vats 2001). Fourth group act by miscellaneous mechanisms and include plants like *Euphorbia prostrata*, *Fumaria parvia*, *Panax ginseng* and *Phyllanthus embelica*. They may alter the fiber content and thereby altering the rate and speed of absorption of glucose from the gut (Grover and Vats 2001). The isolation and formulation of active constituents from these plants along with their pharmacological and toxicological evaluation are the need of the modern therapeutics.

The hypoglycemic effects of *Phyllanthus niruri* extract on mice diabetic models has not been evaluated so far and hence the present investigation was undertaken.

Materials and Methods

Methanol extract of *phyllanthus niruri*. Linn (Euphorbiaceae) was used for assaying hypoglycemic activities in Streptozotocin induced mice diabetic models. The plant *Phyllanthus niruri* was collected from campus of Patna Science College, Patna and identified following relevant monographs of Indian Pharmacopoeia(2012). Freshly harvested plant materials (root, stem, leaves

and flowers) were washed under running tap water, blotted with filter paper and was dried in the shade at room temperature. The dried plant sample (2.6 kg) was then soaked with absolute methanol under reflux condition for the methanolic extract preparation. The sample was then homogenized with extraction buffer and the supernatant collected after three rounds of extraction. The solvent was evaporated under reduced pressure in a rotary evaporator at 40 °C. To this thick paste colloidal silicon dioxide was added and dried in vacuum tube dryer. The obtained methanol extract was stored in deep freezer at -20° C until further test.

Significant insights into the etiology of diabetes in human have been gained from the study of animal models. The albino mouse is an excellent model for study of human diabetes. Therefore all mice used in this study were in the albino genetic background. Adult albino mice weighing around 17–20 gram with 6.5 ± 0.5 cm length are selected for experiments. The mice were housed in shoe-box type cages under good hygienic conditions in the departmental animal house during experimental period. The mice were allowed to acclimatize for 15 days in an environmentally controlled room under standard environmental conditions (21±2°C, 55±5%Relative humidity, 12 hr Light: Dark cycle).

The mice were fed on diet consisted of wheat grains-1Kg, Choker wheat-250gm, Gram grains-250gm, Maize grains-250gm, Soybean grains-250gm, Sundrop oil-50gm, Milk powder-2 table spoon and Jaggery-50gm. This diet provided carbohydrate 48.3%, crude protein 23.5%, crude fat 5.9% crude ash 5.9% and crude fibre 3.9% (W/W).

In each cage one pellet of feed per mice was given. The diet was palatable to the animal as evidenced by feeding success. It has been

observed that an adult mice normally intakes 4 to 5 gram of diet per day. The daily food consumption of the mice varied depending upon the physiological and health status of the mice as well as the environmental temperature. The consumption of food increased considerably when the mice were pregnant or at lactating stage and decreased considerably with the dose-duration and increased temperature in summer.

The animal model for the present study was based on multiple administration of low dose of freshly prepared streptozotocin (STZ). For induction of diabetes, initially the normal mice were kept 24 hours without food and water. The weight of normal mice was determined. Diabetes was induced by multiple intra-peritoneal injection of freshly prepared STZ solution in 0.05 M sodium citrate (pH 4.5) at the dose of 35 mg/kg body weight followed by an hour of fasting. The mice were then allowed to access the respective food and water *ad libitum*. Mice with fasting blood glucose level of 200 mg/dl (7.8 mmol/l) or higher were considered to be diabetic and were used in the study. A parallel set of control mice (non-diabetic) were injected with citrate buffer only.

The mice were grouped into five categories viz., Normal control (NC), Diabetic Control (DC), Diabetic Treated (DT₁₅₀), Diabetic Treated₂₅₀) and Diabetic Treated (DT_{RZG}). NC received only citrate buffer solution. DC group was STZ induced which received citrate buffer only. DT₁₅₀ and DT₂₅₀ received 150mg/Kg and 250mg/Kg body weight of methanol extract respectively. DT_{RZG} received rosiglitazone at a dose of 2mg/Kg of body weight. All the mice were fed with common pellet diets for 2 weeks after arrival, and then randomly divided into two groups. One group continued to receive common pellet diets and constituted the

normal group; the other was fed with diets high in fat and fructose, in order to induce type-2 diabetes. All the mice had free access to food and water.

For the experiment, the mice were divided into five groups having six mice in each group: DC group (diabetic control mice), NC group (non-diabetic control mice) and three DT group (diabetic mice treated with two different doses of extract as well as rosiglitazone/ kg body weight). Body weights were recorded weekly during the experimental period. Treatment with extracts was started after one week of STZ treatment, which was considered as the 1st day of treatment. Blood samples were taken after 8 hrs fasting from the retro-orbital sinus vein prior to the administration of test substances or the buffer and 4 weeks after the treatment under mild ether anesthesia and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimations were carried out.

Data were statically analyzed by mean \pm S.E and by one-way ANOVA.

The results related to body weight change and blood glucose level in mmol/l in mice during present course of investigation have been presented in Table-1 and 2.

Result and Discussion

The whole plant extract of *Phyllanthus niruri* has been reported to be effective in alleviating diabetes mellitus through its antioxidant and insulin- potentiating activities (Okoli et al., 2010). In the present investigation the effect of methanol extract of *Phyllanthus niruri* on body weight of mice was studied. The results clearly

indicated that the diabetic control (DC) mice presented a significant lowering of body weight ($p < 0.001$) when compared with the normal control (NC) mice (Table- 1& Fig. 1). A significant gain in body weight was observed in the treated groups of diabetic mice (DT₁₅₀ and DT₂₅₀) as compared to the DC ones. The DT₁₅₀ and DT₂₅₀ group showed an increase of 28% and 39% in body weight respectively after 15 days of treatment. Contrary to this, DT_{RGZ} group mice showed an increase of 50% in body weight after 15 days of treatment (Table-1& Fig. 1).

The changes in the blood glucose levels before and after receiving the treatment in normal and diabetic mice have been presented in Table -2 and Figure 2. As expected, the DC mice showed a significantly ($p < 0.001$) higher level of glucose (+279%), when compared with their normal control counterparts. Diabetic mice of both of the groups (DT₁₅₀ and DT₂₅₀) showed a reduction in glucose levels, when compared to the DC ones; nevertheless, the reduction was particularly evident in the DT₂₅₀ mice (-44%; $p < 0.001$). When compared, the glucose levels of the DT₂₅₀ versus the DC group mice during the 4-week treatment program, a significant lower value in the first was also found (-45%; $p < 0.001$) respectively (Table-2& Fig. 2). Nevertheless, this decline in the glucose levels was less evident in the DT₁₅₀ mice (-38%) than in the DT₂₅₀ mice. In contrast to this, DT_{RGZ} group mice showed almost 67% decline in glucose level after 4-weeks of treatment program (Table -2& Fig. 2).

Phytochemicals from natural products possess potent antioxidant activity that are capable of prevention of the onset and/or progression of many human diseases by counteracting reactive oxygen species (ROS) (Palasuwan et al., 2005; Cai et al.,

2006; Bouayed et al., 2007; Liu et al., 2007). It has been reported that the *Phyllanthus niruri* plant is a key source of moieties such as phyllanthin and hypophyllanthin (Mellinger et al., 2005 and 2008).

An alcoholic extract of *P. niruri* was found to reduce significantly the blood sugar in normal mice and in STZ induced diabetes mice. In normal rats, administration of *P. niruri* 200mg/kg body weight reduced the blood sugar by 34.5 percent and to 47.4 percent at the concentration of 1000mg/kg by weight at 1 hour. However at 6th hour, values are almost similar to normal value. Continuous administration of the drug produced significant reduction in normal blood sugar in rats, which on 15th day was also found to reduce the blood sugar in alloxan diabetic rats. In short term experiment, drug was found to reduce the blood sugar at 4th hour by 6.07 percent at dose level of 200mg/kg by weight and 18.7 percent at concentration of 1000mg/kg by weight. Continuous administration of drug produced significant reduction in blood sugar in alloxan diabetic rats. On 15th day values were almost similar to normal in the group taking 1000 mg/kg by weight. Plant extract did not produce any toxicity as seen from liver and kidney function test and in hematological parameters. The results indicate potential antidiabetic action of *P. niruri* (Raphael et al., 2000).

Diabetes has a significant impact on the health, quality of life and life expectancy of patients as well as healthcare expenditure. With increasing incidence and mortality from its complications, prompt and adequate glycemic control in diabetes is paramount if management can meaningfully improve the quality of life and increase life expectancy (Nyenwe et al. 2011).

Several studies to test new drugs with potential antidiabetic activity were used in animal models of streptozotocin (STZ)-induced diabetes (Fröde and Madeiros, 2008). Although none of the chemically-induced diabetic models can reproduce the complexity of the human disease, they can be helpful to understand at least some aspects of the potential bioactivities of natural or synthetic products. We used diabetic albino mice induced by STZ (35 mg/kg body wt.), which was sufficient to induce a stable state of diabetic condition in this animal species.

Induction of diabetes with streptozotocin is associated with a characteristic loss of body weight, which is probably due to muscle wasting. In our study there was a significant weight loss in the vehicle treated diabetic mice, where as treatment with the *P. niruri* extract at three doses showed improvement in their body weight, indicating that the methanolic extract of *P. niruri* had

beneficial effect in preventing loss of body

weight of diabetic mice. The probable

mechanism of this benefit is due to its effect

in controlling muscle wasting, i.e., by reversal of antagonism. The metabolic disturbances were corrected after the plant extract was administered at the two different dose of 150 and 250 mg/kg body weight for four weeks as shown by a reduction in biochemical parameters in diabetic mice treated with plant extract. This result is in accordance with Lenzen S. (2008) and Chung et al. (2003). They have found that multiple low dose of STZ sufficiently induce stabilized acute diabetes in which there is a

progressive deterioration in the glucose tolerance and insulin secretion after the STZ injection. It ultimately causes increased oxidative stress, which play an important role in the pathogenesis of various complications.

At present, several drugs are available for the management of hyperglycemia but they are expensive and possess side effects also. Therefore, search for a suitable alternative is continued. For the developing countries herbal plants may be the most attractive target for their availability, low cost and better safety margin. The hypoglycemic activity of *Phyllanthus niruri* L. has attracted many researchers to prove it scientifically and to investigate its mechanisms of actions. Hence, in the present study, the two defined doses of methanolic extract of whole plant of *P. niruri* have been investigated for their antidiabetic potential.

Chronic hyperglycaemia in diabetes is a risk factor constantly fuelled by postprandial elevation of blood glucose. Control of postprandial hyperglycemia in diabetes is of great importance due to its close relation to the risk of micro and macro-vascular complications and death (Nyenwe et al. 2011). In this study, experimental evaluation of the hypoglycemic potentials of *P. niruri* has shown that the higher dose of extract (250 mg/kg body wt.) suppress postprandial rise in blood glucose levels more effectively than lower dose of extract (150 mg/kg body wt.) which is the index of effectual glycemic control. It may be due to alteration in the fiber content as well as phytochemical interactions which thereby altering the rate and speed of absorption of glucose from the gut. These favorable effects of *P. niruri* extract may be attributed to higher affinity and synergistic action of their phytochemicals on multiple targets

including PPAR- γ activation and DPP-IV inhibition which may therefore regulate the hyperglycemia, lipogenesis and hypertriglyceridemia associated with

diabetes (Shimizu *et al.*, 2003; Barnett, 2006).

Table.1 Showing body weight changes in mice during and after treatment of methanol extract of *P. niruri*

Non Diabetic Normal Control (NC)	Day 0	Day7	Day15
	18.95 \pm 2.76	20.80 \pm 2.39	23.89 \pm 2.20
Diabetic Mice			
Diabetic Control (DC)	10.70 \pm 1.05	09.45 \pm 0.95	8.47 \pm 1.32
<i>P. niruri</i> extract (150mg/Kg) (DT ₁₅₀)	10.71 \pm 2.03*	12.11 \pm 1.67*	13.72 \pm 1.53*
<i>P. niruri</i> extract (250mg/Kg) (DT ₂₅₀)	10.68 \pm 1.63*	12.74 \pm 2.37*	14.84 \pm 2.67*
Rosiglitazone (2mg/Kg) (DT _{RGZ})	10.70 \pm 3.74*	14.82 \pm 3.91*	16.04 \pm 1.84*

Table.2 Showing effects of different doses of *P.niruri* extract and rosiglitazone on blood glucose levels in mice

Mice Groups	Blood glucose levels in (mmol/l) in four different weeks				
	Pretreatment	Post-treatment			
	0	1	2	3	4
Normal control (NC)	3.95 \pm 0.13**	4.07 \pm 0.14**	4.06 \pm 0.25**	4.05 \pm 0.16**	3.99 \pm 0.19**
Diabetic control (DC)	14.96 \pm 1.55*	14.91 \pm 1.48*	14.78 \pm 1.59*	14.96 \pm 1.49*	14.94 \pm 1.48*
<i>P.niruri</i> extract (150mg/Kg) DT ₁₅₀	14.97 \pm 1.40	13.04 \pm 1.18*	10.64 \pm 2.09**	9.69 \pm 1.28**	9.27 \pm 1.79**
<i>P.niruri</i> extract (250mg/Kg) DT ₂₅₀	14.64 \pm 1.59	11.86 \pm 1.38**	9.65 \pm 1.28**	8.27 \pm 1.74**	8.15 \pm 1.28**
Rosiglitazone (2mg/Kg) DT _{RGZ}	15.03 \pm 1.49	9.84 \pm 1.48**	5.57 \pm 1.28**	4.97 \pm 1.35**	4.94 \pm 0.97**

*p<0.05 as compared with normal control. **p<0.001 as compared with diabetic control.

Fig.1 showing change in body weight of mice after administration if *P. niruri* extract and RGZ
X axis: Mice groups; Y axis body weight changes after administration of *P. niruri* extract and RGZ

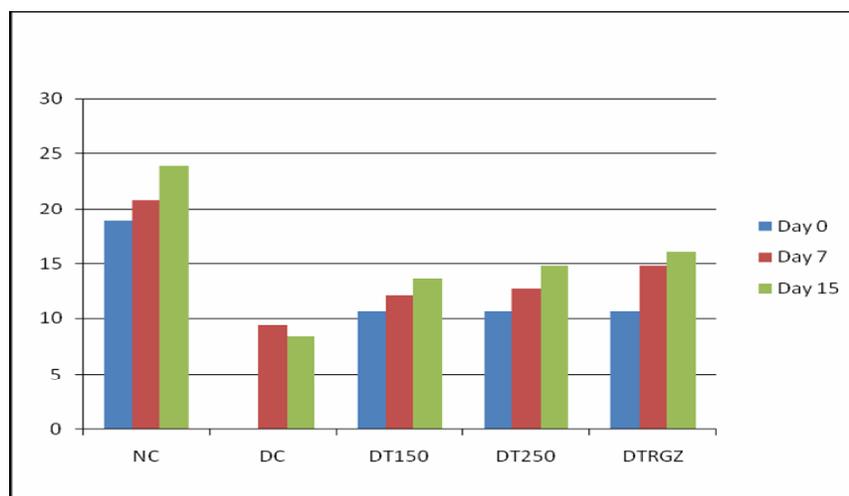
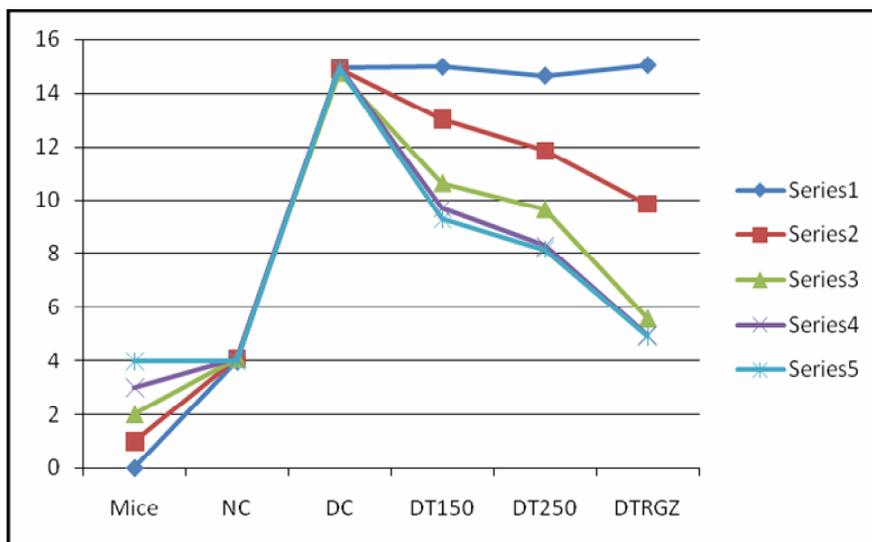


Fig.2 Showing effects of different doses of *P. niruri* extract and rosiglitazone on blood glucose levels in mice before and four weeks after administration.



X axis: Mice groups, series 1 to 5

Y axis: Blood glucose levels in mmol/l in four different weeks.

The anti-oxidant and antidiabetic activity of *P. niruri* is due to the presence of its active phytoconstituents (Jada et al., 2006). It has been well documented that *P. niruri* contain phyllanthin and hypophyllanthin as a major active constituent (Bagalkotkar et al., 2006).

In the hypoglycemic activity studies of methanol extract, daily oral administration of the extract for 28 days produced a gradual but sustained reduction in blood glucose levels in diabetic treated mice. Streptozotocin causes hyperglycaemia and glucose intolerance or syndromes similar to either type 1 or type 2 diabetes (Frode and Medeiros, 2008). Effective and sustained reduction in blood glucose levels of treated diabetic mice by the extract indicates that it may be useful in overt cases of diabetes. Treatment with the two doses of extract also

reduced mortality of diabetic mice from hyperglycaemia and prolonged their survival. In this study, some of the diabetic non-treated control animals all died on day 10 post-induction of diabetes whereas the extract-treated group survived beyond the period of the experiment. Effective control of blood glucose level is a key step in preventing and reversing diabetic complications, and improving the quality of life of diabetic patients (Bavarva and Narasimhacharya, 2008). Hence, chronic administration of the extract may cause a progressively sustained reduction in hyperglycaemia known to reduce the risk of complications associated with the disease.

From the results it can be concluded that the *P. niruri* whole plant methanol extract is antidiabetic in nature due to the presence of different types of active phytochemicals,

which may have different mechanism of action. The combination of these phytochemicals, therefore, might be beneficial as hypoglycemic agents. The *P. niruri* plant extract might be considered as a safe supplementary therapy for long-term and effective management of diabetic patients.

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