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Original Research Article

Antidiabetic activity of compounds isolated from the roots of *Premna latifolia* Roxb

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Abstract

Purpose: *To evaluate the hypoglycaemic activity of the ethanol extract, chloroform fraction, and three compounds isolated from Premna latifolia.*
Methods: *The ethanol extract, chloroform fraction, and compounds* ***1*** *–* ***3*** *(isolated from the chloroform fraction) of P. latifolia were characterised by nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR and 2D NMR). In vitro activity was assessed by α-amylase and α-glucosidase inhibitory activity assays. In vivo antidiabetic activity was evaluated in a streptozotocin-nicotinamide-induced rat model of diabetes. Fasting blood glucose (FBG), glycosylated haemoglobin (HbA_1C), serum creatinine, blood urea nitrogen (BUN), liver enzymes, and antioxidant enzymes were determined. Liver and pancreas histopathology was assessed.*
Results: *Compounds* ***2*** *and* ***3*** *exhibited significant hypoglycaemic activity by reducing elevated FBG and HbA_1C ($p \leq 0.001$) and also exerted positive effects on blood and liver enzyme profiles, which were largely altered in diabetic control group. These compounds also showed significant antioxidant activity, increasing catalase, superoxide dismutase, and glutathione reductase, while lowering malondialdehyde ($p \leq 0.001$). The histopathology results for the rats that received these compounds suggested regeneration of pancreatic β-cells.*
Conclusion: *Compounds* ***2*** *and* ***3*** *isolated from the dried roots of P. latifolia possess significant hypoglycaemic activity, reno- and hepatoprotective effects, and antioxidant activity. They show promise as potential treatments for patients with diabetes.*

Keywords: *Antioxidant, Hypoglycaemic, Premna latifolia, Stigmanstan-3β-olyl n-octadec-9', 12'-dienoate, n-Tetracosanol, n-Tridecanyl n-Tetracosanoate*

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INTRODUCTION

Diabetes mellitus is often considered the most important worldwide chronic disease of the 21st century. It is characterised by high blood glucose levels, which lead to substantial mortality and morbidity. The ultimate goals of all antidiabetic treatment are to first lower the blood glucose, then to reduce the risk of further hyperglycaemic episodes and lower the risk of cardiovascular disease, focusing on hypertension and dyslipidemia control [1].

Prevailing management strategies combine lifestyle modifications with exercise and the use of oral hypoglycaemic or insulin therapy, if needed. However, current oral hypoglycaemic medications have potential side effects, such as weight gain, gastrointestinal symptoms, hematologic adverse effects, and liver or kidney dysfunction. They may also increase free radical levels. Medicinal herbs are becoming increasingly used as more people seek relatively safe remedies [2]. Exploring these natural products through pharmacologic experiment-tation, including modification and derivatization, represents a major strategy for developing new drugs.

Belonging to the family Verbenaceae, *Premna latifolia* is a small bushy tree 20-25 feet tall found across Peninsular India, Bihar, West Bengal, and Northeastern India. It is known as *Agnimantha* in Sanskrit, *Bakar* in Hindi, *Erumaimunnai* in Tamil, and *Knappa* in Malayalam [3]. The rough, dark grey bark contains branchlets with green, cordate, simple, opposite leaves with an unpleasant and characteristic odour. The roots are woody, branched, thick, and cylindrical, whereas the bisexual flowers are creamish white to yellowish green. The fruits are tuberculate and glabrous. The leaves and tender shoots are eaten in curries and used to treat various ailments, such as fever and liver symptoms. The leaves are also used for their diuretic, cardiotonic, anticoagulant, and hepatoprotective effects [4]. Two novel icetexane diterpenes and two new glycosides have been successfully isolated from *P. latifolia* [5,6].

As part of continuing efforts directed towards the discovery of biologically active compounds from Indian medicinal plants, this study was conducted to explore the *in vitro* and *in vivo* antidiabetic potential of these compounds.

EXPERIMENTAL

Extraction, fractionation, and isolation from plant material

Coarsely powdered dried roots of *P. latifolia* (5 kg) were subjected to Soxhlet extraction with absolute ethanol (50 L). The extract was then concentrated. Gradient elution was performed by column chromatography using toluene, chloroform, ethyl acetate, and methanol in varying proportions on silica gel G (60-120), which was activated at 110 °C for 1 hour. The fractions were collected separately and combined based on their thin layer chromatography (TLC) pattern. Individual compounds were further purified separately by rechromatography using preparative TLC on silica gel G (for TLC) in the presence of toluene:ethylacetate (9.3:0.7) as the mobile phase.

Evaluation of *in vitro* antidiabetic activity

The α-amylase and α-glucosidase inhibitory activities of the ethanol extract were determined using the standard procedures of Unnikrishnan *et al* [7] and Jie *et al* [8], respectively, with minor modifications. IC_{50} was defined as the concentration of extract necessary to inhibit 50 % of the α-amylase or α-glucosidase activity under the assay conditions. The results are reported as IC_{50} ± standard error of the mean (SEM).

Determination of *in vivo* antidiabetic activity

Experiments assessing *in vivo* antidiabetic activity were approved by our Institutional Animal Ethics Committee (approval ref no.-RGIP/03/2012) and based on the Committee for the Purpose of Control and Supervision of Experiment on Animals guidelines [9]. The study was conducted in accordance with the rules and regulations of our Institutional Animal Ethics Committee. Healthy adult Wister rats of both sexes (weighing 150 – 180 g) were selected for the study. The rats were kept in clean polypropylene cages and maintained at local animal house conditions: temperature 24 ± 2 °C, humidity 45 ± 5 %, and 12-h day/12-h night cycle [10]. The animals were fed a standard pellet diet (Sai Durga Feeds and Foods, Bangalore, India), and water was available *ad libitum*.

The rats were divided into 8 groups of 6 rats each. Group I served as normal control, whereas Group II was the diabetic control; both groups were given 1 mg/kg of 0.5 % sodium carboxymethyl cellulose. Group III, the standard treatment group, was given glibenclamide (5 mg/kg), whereas Group IV and V were administered ethanolic extracts of *P. latifolia* (PLE) or chloroform fraction (PLF) at doses of 200 mg/kg and 100 mg/kg, respectively. Group VI was treated with compound **1** (PL1), Group VII was given compound **2** (PL2), and Group VIII was administered compound **3** (PL3) (15 mg/kg for PL1 – PL3).

The rats were fasted overnight before the experiments. They underwent intraperitoneal administration of 110 mg/kg nicotinamide in normal physiological saline, followed 15 minutes later by a freshly prepared solution of streptozotocin dissolved in 0.1 M citrate buffer pH 4.5 at a dose of 60 mg/kg [11-12]. Seventy-two hours after streptozotocin injection, the blood

glucose level of each rat was measured, and rats with a fasting blood glucose (FBG) level above 250 mg/dL were considered diabetic and used for this investigation [13]. One hour after administration of the control solution (sodium carboxymethyl cellulose), standard treatment (glibenclamide), or experimental treatment (PLE, PLF, PL1, PL2, or PL3), blood samples were collected using tail vein puncture technique under light anaesthesia to determine FBG level. FBG was determined by the glucose oxidase method, using a commercially available biochemical kit (Roche Diabetes Care India Pvt Ltd., Mumbai, India). The treatments were repeated daily for the next 28 days, and FBG levels were determined on days 0, 14, and 28. On day 28, the rats were sacrificed, and the liver and pancreas were removed for histopathology studies [14-15]. Other biochemical parameters, including the levels of antioxidant enzymes, were analysed on day 28 [16].

Histopathologic examination

The pancreatic and liver samples of all rats were removed and placed in 10 % formalin buffer solution. A small piece of tissue was sectioned with a microtome, fixed onto slides, stained with hematoxylin and eosin dye (H&E) and observed under an optical microscope.

Blood biochemical parameters analysis

FBG, glycosylated haemoglobin (HbA_1C), serum creatinine, blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione reductase (GSH) were analysed using standard procedures.

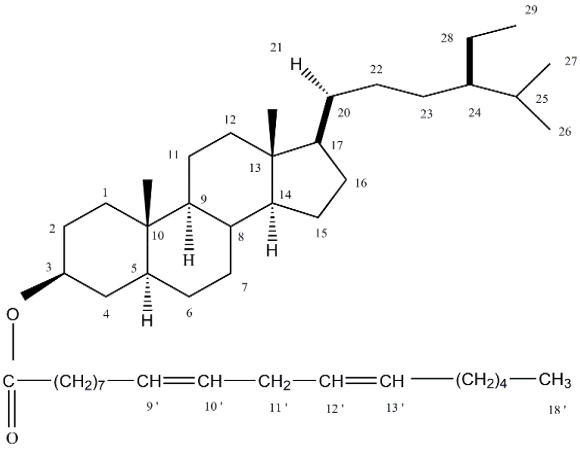
Statistical analysis

The data are expressed as mean ± SEM. One-way ANOVA, followed by Tukey's multiple comparisons test, was performed. All statistical analyses were conducted using the GraphPad Prism 5 statistical package (Graph Pad Software, USA). Comparisons with a *p* value ≤ 0.001 were regarded as significantly different.

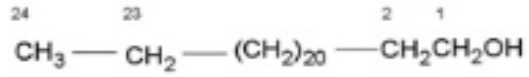
RESULTS

The three new compounds isolated from the dried roots of *P. latifolia,* designated as compounds **1** – **3**, are shown in Figure 1.

n-tridecanyl n-tetracosanoate (compound 1)



stigmanstan-3β-olyln-octadec-9',12'-dienoate (compound 2)



n-tetracosanol (compound 3)

Figure 1: Structure of three compounds isolated from the dried roots of *P. latifolia*

***In vitro* antidiabetic activity**

As glucose-lowering medications, α-amylase and α-glucosidase inhibitors delay the absorption of ingested carbohydrates, reducing postprandial glucose and insulin peaks. The ethanolic extract exhibited significant inhibition of α-glucosidase and α-amylase, with IC_{50} values of 84.57 µg/mL and 96.33 µg/mL, respectively. These effects were dose-dependent, as shown in Table 1 and Table 2. Acarbose, which was used as the positive control, had IC_{50} values of 72.30 µg/mL for α-glucosidase and 88.10 µg/ml for α-amylase, under similar assay conditions. These results reveal the *in vitro* antidiabetic activity of *P. latifolia* root extract.

Table 1: Inhibitory potency of *Premna latifolia* root extract against α-glucosidase and α-amylase

Ethanol extract	IC_{50} (µg/mL)
α-Glucosidase inhibitory activity	84.57±0.568[a]
α-amylase inhibitory activity	96.33±0.760[a]
Acarbose (standard)	
α-glucosidase inhibitory activity	72.30±0.883
α-amylase inhibitory activity	88.10±0.239

Values are mean ± SEM (n = 3). [a] *p ≤ 0.001 vs. standard.*

Effect of *Premna latifolia* on fasting blood glucose levels

Administration of streptozotocin causes widespread β-cell destruction in the pancreas via DNA strand breakage, leading to hyperglycaemia. After 28 days, no significant change in blood glucose was noted in normal rats, whereas blood glucose levels were significantly increased in diabetic rats. Treatment with the ethanolic extract and chloroform fraction of *P. latifolia* produced a slight reduction in elevated FBG. Chloroform fraction at a dose of 100 mg/kg was most effective, producing a 19.40 % decrease in elevated FBG levels on the last day. In rats administered compound **2** at a dose of 15 mg/kg, there was a dynamic decrease in FBG levels to a maximum decrease of 48.42 % decrease by day 28, which was a significantly greater change than for the diabetic control group ($p \leq 0.001$; Table 2).

Compound **1** showed no significant effects, but administration of compound **3** produced a time-dependent reduction in FBG levels to a maximum decrease of 33.71 % by day 28. Thus, these results demonstrate that compounds **2** and **3** have prominent antihyperglycemic effect.

Effect of *Premna latifolia* on HbA_1C, creatinine, and BUN

HbA_1C, formed by non-enzymatic glycation pathway in response to plasma glucose, is considered an indicator of the onset of diabetes mellitus and the degree of glucose control over time. HbA_1C levels were increased to 13.10 % in diabetic control rats on day 28, and both the ethanolic extract and chloroform fraction produced significantly lower HbA_1C levels. Compounds **2** and **3** significantly lowered HbA_1C on day 28 to 5.11 % and 6.12 %, respectively; these levels were close to those seen with standard treatment. Creatinine and BUN, two major biomarkers of renal dysfunction, increased to 1.60 mg/dL and 90.30 mg/dL, respectively, on day 28 in the diabetic control group. The PLE and PLF groups had significantly lower creatinine and BUN levels than the diabetic control group, but compound **1** had no effect. Compared with diabetic control rats, creatinine and BUN on day 28 were lower in rats treated with compound **2** (1.01 mg/dL and 62.80 mg/dL, respectively) ($p \leq 0.001$) and rats treated with compound **3** (1.12 mg/dL and 67.00 mg/dL, respectively) ($p \leq 0.001$; Table 3).

Effect of *Premna latifolia* on liver enzyme levels

The liver plays a major role in metabolism, detoxification, storage, and excretion of xenobiotics and their associated metabolites. In diabetes mellitus, liver enzymes are often raised secondary to enzymes leaking from the liver cytosol into the bloodstream; this is an indication of hepatotoxicity. Standard treatment of diabetic rats with glibenclamide reduced SGOT, SGPT, and ALP to 43.70 IU/L, 65.80 IU/L, and 61.20 IU/L, respectively, on day 28. Both ethanolic extract and chloroform fraction exerted little effect, whereas rats receiving compound **2** exhibited significantly lower levels of SGOT, SGPT and ALP on day 28 (50.10 IU/L, 67.10 IU/L, and 67.00 IU/L, respectively), compared with diabetic control rats ($p < 0.001$). Compound **3** also significantly decreased these enzymes, when compared to diabetic control rats, as shown in Table 4. Thus, compounds **2** and **3** exerted a protective effect on the liver but compound **1** had no effect.

Table 2: Effect of *Premna latifolia* on fasting blood glucose

Group	Mean (mg/dL)			Change in FBG on day 28 (%)[a]
	Day 0	Day 14	Day 28	
Normal	80.80±0.307	83.30±0.667	82.30±0.667	+1.86
Diabetic control	280.00±0.477[a]	282.00±0.792[a]	301.00±0.725[a]	+7.50
Diabetic+standard	264.39±0.477[b]	131.01±0.872[b]	95.70±0.577[b]	-63.80
Diabetic+PLE (200 mg/kg)	275.00±0365[bc]	244.00±0.365[bc]	240.00±0.333[bc]	-12.73
Diabetic+PLF (100 mg/kg)	268.00±0.477[bc]	231.00±0.307[bc]	216.00±0.365[bc]	-19.40
Diabetic+PL1 (15 mg/kg)	279.00±0.428	281.00±0.333	298.00±1.920	+6.81
Diabetic+PL2 (15 mg/kg)	263.02±0.577[bc]	197.00±0.422[bc]	135.66±0.428[bc]	-48.42
Diabetic+PL3 (15 mg/kg)	264.00±0.333[bc]	203.02±0.422[bc]	175.00±0.667[bc]	-33.71

*Values are mean ±SEM (n=6). [a] $p \leq 0.001$ vs. normal control; [b] $p \leq 0.001$ vs. diabetic control; [c] $p \leq 0.001$ vs. standard treatment. FBG, fasting blood glucose; PL2, chloroform fraction of P. latifolia; PLE, ethanolic extract of P. latifolia; PL1 – PL3, compounds **1** – **3** from P. latifolia*

Table 3: Effect of *Premna latifolia* on blood biochemical parameters on day 28

Group	HbA_1C (%)	Creatinine (mg/dL)	BUN (mg/dL)
Normal	3.00±0.053	0.545±0.009	43.20±0.833
Diabetic control	13.10±0.063[a]	1.600±0.014[a]	90.30±0.882
Diabetic+standard	4.83±0.106[b]	0.862±0.005[b]	56.70±0.667[b]
Diabetic+PLE (200 mg/kg)	11.00±0.040[bc]	1.47±0.007[bc]	82.30±0.494[bc]
Diabetic+PLF (100 mg/kg)	7.91±0.058[bc]	1.390±0.005[bc]	77.00±0.577[bc]
Diabetic+PL1 (200 mg/kg)	12.90±0.310	1.640±0.008	89.70±0.760
Diabetic+PL2 (15 mg/kg)	5.11±0.049[bc]	1.010±0.009[bc]	62.80±0.477[bc]
Diabetic+PL3 (15 mg/kg)	6.12±0.015[bc]	1.120±0.006[bc]	67.00±0.577[bc]

*Values are mean ± SEM (n=6). [a] p ≤ 0.001 vs. normal control; [b] p ≤ 0.001 vs. diabetic control; [c] p ≤ 0.001 vs. standard treatment. PL2, chloroform fraction of P. latifolia; PLE, ethanolic extract of P. latifolia; PL1 – PL3, compounds **1** – **3** from P. latifolia*

Table 4: Effect of *Premna latifolia* on liver enzymes on day 28

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Normal	35.60±0.050	57.90±0.028	55.20±0.010
Diabetic control	73.60±0.033[a]	93.70±0.042[a]	87.70±0.008[a]
Diabetic+standard	43.70±0.030[b]	65.80±0.016[b]	61.20±0.017[b]
Diabetic+PLE (200 mg/kg)	70.90±0.033[bc]	88.90±0.031[bc]	81.70±0.013[bc]
Diabetic+PLF (100 mg/kg)	64.60±0.031[bc]	79.70±0.023[bc]	77.30±0.010[bc]
Diabetic+PL1(15 mg/kg)	73.80±0.579	93.60±0.452	87.50±0.273
Diabetic+PL2 (15 mg/kg)	50.10±0.031[bc]	67.10±0.025[bc]	67.00±0.018[bc]
Diabetic+PL3 (15 mg/kg)	54.30±0.018[bc]	67.10±0.034[bc]	70.30±0.012[bc]

*Values are mean ± SEM (n = 6); [a] p ≤ 0.001 vs. normal control; [b] p ≤ 0.001 vs. diabetic control; [c] p ≤ 0.001 vs. compared with standard treatment; ALP, alkaline phosphatase; PL2, chloroform fraction of P. latifolia; PLE, ethanolic extract of P. latifolia; PL1 – PL3, compounds **1** – **3** from P. latifolia; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase*

Effect of *Premna latifolia* on antioxidant enzyme levels

Much evidence indicates a strong relationship between diabetes mellitus and oxidative stress induced by free radicals, which contribute to a variety of diabetic complications, such as coronary artery disease, retinopathy, and nephropathy. Studies have shown that the majority of plasma antioxidants are depleted in diabetic patients, and antioxidant therapy has been helpful in reducing complications of diabetes. In prolonged diabetes, levels of key antioxidant enzymes such as CAT, SOD, and GSH are decreased, whereas levels of lipid peroxidation markers such as MDA are increased.

CAT, SOD, and GSH were reduced in diabetic control rats to 52.3 nM H_2O_2 decomposed/ min/ g, 10.40 U/mg protein, and 20.80 µ mol of GSH/mg protein, respectively, on day 28, which were improved to almost normal by treatment with standard therapy (glibenclamide). CAT, SOD, and GSH levels were 69.90 nM H_2O_2 decomposed/ min/ g, 16.20 U/mg protein, and 32.10 µ mol of GSH/mg protein , respectively, in rats administered compound **2** and 88.60 µ mol of GSH/mg protein, 14.60 U/mg protein, and 31.5 µ mol of GSH/mg protein µ mol of GSH/mg protein, respectively, in rats treated with compound **3**. These values were significantly higher than those seen in the diabetic control group, although lower than those in the standard treatment group (p ≤ 0.001; Tables 5 and 6).

In diabetes mellitus, MDA, which is produced by degradation of polyunsaturated lipids by free radicals, is increased. MDA was lower with compounds **2** and **3** (36.90 nmol/mL for both) than in diabetic control rats on day 28, as shown in Table 6.

Effect of *Premna latifolia* on pancreatic and liver histopathology

The normal group rats had a normal pancreatic surface, with no traces of fibrosis. However, diabetic control rat had extensive pancreatic damage, with necrosis and fibrosis. Standard treatment rats exhibited substantially less pancreatic fibrosis and atrophy than the controls. Groups treated with extract and fraction exhibited less cellular degeneration than the diabetic control group. Rats administered compounds **2** and **3** exhibited significantly less fibrosis, which was associated with improved architecture and function of the pancreas (Figure 2). Compound **1** rats exhibited no substantial improvement, compared with diabetic control rats.

Table 5: Effect of *Premna latifolia* on levels of CAT and SOD on day 28

Group	CAT (nM H_2O_2 decomposed/ min/g)	SOD (U/mg protein)	GSH (µmol/mg protein)
Normal	78.90±0.031	20.60±0.201	39.30±0.063
Diabetic control	52.30±0.0165[a]	10.40±0.312[a]	20.80±0.016[a]
Diabetic+standard	72.70±0.016[b]	18.90±0.293[b]	34.00±0.021[b]
Diabetic+PLE (200 mg/kg)	58.10±0.020[bc]	11.60±0.056[bc]	23.60±0.050[bc]
Diabetic+PLF (100 mg/kg)	65.30±0.020[bc]	12.60±0.008[bc]	27.80±0.019[bc]
Diabetic+PL1 (15 mg/kg)	52.90±0.317	10.60±0.100	20.90±0.436
Diabetic+PL2 (15 mg/kg)	69.90±0.020[bc]	16.20±0.017[bc]	32.10±0.010[bc]
Diabetic+PL3 (15 mg/kg)	68.60±0.023[bc]	14.60±0.048[bc]	31.50±0.676[bc]

*Values are mean ± SEM (n = 6); [a] p ≤ 0.001 vs. normal control; [b] p ≤ 0.001 vs. diabetic control; [c] p ≤ 0.001 vs. standard treatment; CAT, catalase; PL2, chloroform fraction of P. latifolia; PLE, ethanolic extract of P. latifolia; PL1 – PL3, compounds **1** – **3** from P. latifolia; SOD, superoxide dismutase*

Table 6: Effect of *Premna latifolia* on MDA and GSH levels on day 28

Group	MDA (nmol/mL)
Normal	30.10±0.017
Diabetic control	49.60±0.025[a]
Diabetic+standard	34.00±0.013[b]
Diabetic+PLE (200 mg/kg)	46.90±0.154[bc]
Diabetic+PLF (100 mg/kg)	43.60±0.019[bc]
Diabetic+PL1 (200 mg/kg)	48.90±0.305
Diabetic+PL2 (15 mg/kg)	36.90±0.019[bc]
Diabetic+PL3 (15 mg/kg)	36.90±0.152[bc]

*Values are mean ± SEM (n = 6); [a] p ≤ 0.001 vs. normal control; [b] p ≤ 0.001 significant difference vs. diabetic control; [c] p ≤ 0.001 vs. standard treatment; GSH, glutathione reductase; MDA, malondialdehyde; PL2, chloroform fraction of P. latifolia; PLE, ethanolic extract of P. latifolia; PL1 – PL3, compounds **1** – **3** from P. latifolia*

Liver samples from normal rats exhibited distinct lobulation with normal portal triads and central veins. Samples from diabetic control rats exhibited severe fatty changes of hepatocytes, with collections of inflammatory cells and hepatocytes. Livers from standard treatment rats exhibited minimal periportal fatty infiltration and focal necrosis of hepatocytes. Groups treated with extract and fraction exhibited a minimal percentage of hepatocytes with fatty changes (20 – 30 %). Samples from rats treated with compounds **2** and **3** at a dose of 15 mg/kg had minimal hepatocyte damage, with less aggregation of inflammatory cells and hepatocytes and less focal necrosis (Figure 3). Liver samples from the compound **1** group showed no difference in liver architecture, when compared with samples from diabetic control rats.

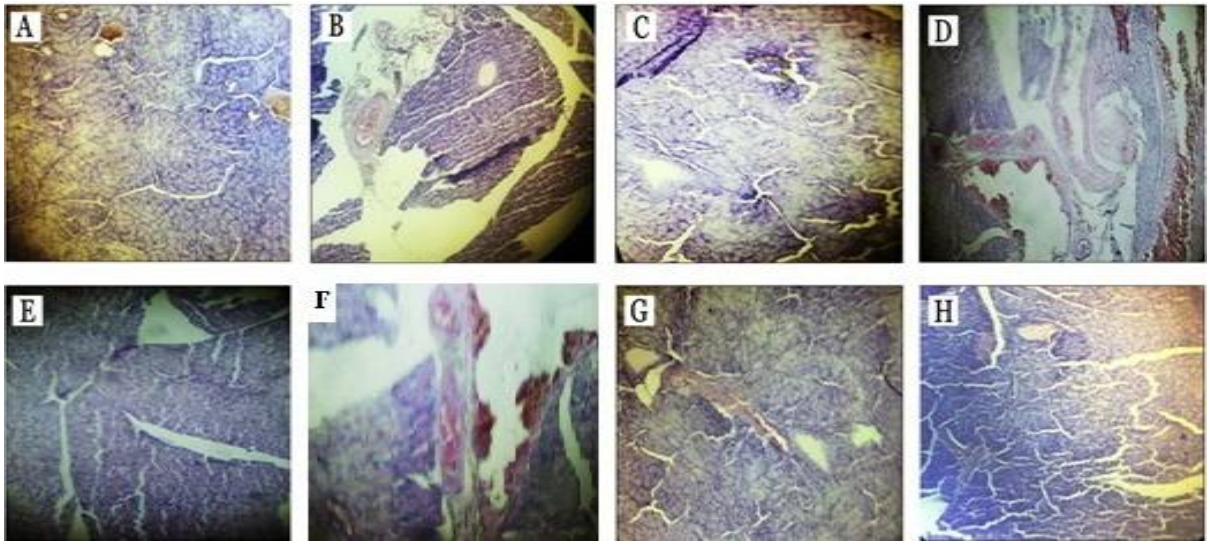


Figure 2: Histopathologic examination of pancreatic tissues of different experimental groups: (A) normal, (B) diabetic control, (C) diabetic+standard, (D) diabetic+PLE (200 mg/kg), (E) diabetic+PLF (100 mg/kg), (F) diabetic+PL1 (15 mg/kg), (G) diabetic+PL2 (15 mg/kg), and (H) diabetic+PL3 (15 mg/kg). Slides were stained with H&E dye. Magnification: X100. *PL2, chloroform fraction of P. latifolia; PLE, ethanolic extract of P. latifolia; PL1 – PL3, compounds **1** – **3** from P. latifolia*

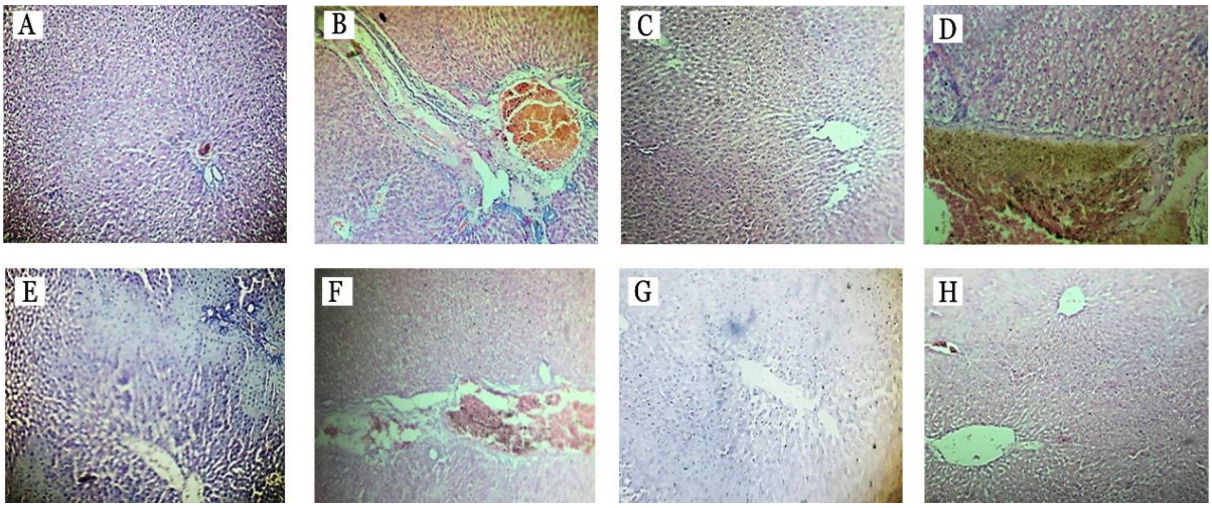


Figure 3: Histopathologic examination of liver tissues of different experimental groups: (A) normal, (B) diabetic control, (C) diabetic+standard, (D) diabetic+PLE (200 mg/kg), (E) diabetic+PLF (100 mg/kg), (F) diabetic+PL1 (15 mg/kg), (G) diabetic+PL2 (15 mg/kg), and (H) diabetic+PL3 (15 mg/kg). Slides were stained with H&E dye. Magnification: X100. *PL2, chloroform fraction of P. latifolia; PLE, ethanolic extract of P. latifolia; PL1 – PL3, compounds **1** – **3** from P. latifolia*

DISCUSSION

The present study focused on evaluating the hypoglycaemic activity of three compounds isolated from *P. latifolia,* along with its ethanolic extract and chloroform fraction. As diabetes mellitus is associated with various metabolic disturbances, we also extensively evaluated the effects of these substances on various blood biochemical parameters and histopathology of the pancreas and liver.

First, we confirmed the *in vitro* antidiabetic activity of the ethanolic extract using α-amylase and α-glucosidase inhibitory assays; the extract produced inhibition of both assays, when compared with the standard acarbose. We subsequently used a streptozotocin-nicotinamide-induced rat model of diabetes to evaluate the *in vivo* antidiabetic effects of the three isolated compounds, ethanolic extract, and chloroform fraction. Compound **2** (15 mg/kg) reduced the elevated FBG levels by 48.42 %, suggesting that it improved insulin secretion from remnant pancreatic β-cells or promoted regeneration of destroyed β-cells. Histopathologic examination of pancreatic tissue suggested that regeneration occurred. Compound **3** (15 mg/kg) also reduced FBG levels, albeit by a smaller amount (33.71 %). Similarly, administration of compounds **2** and **3** to diabetic rats decreased HbA_1C levels from 13.10 % (diabetic control group) to 5.11 % and 6.12 %, respectively, representing excellent control of the disease.

We also studied the *in vivo* effects of the *P. latifolia* substances on kidney and liver function. Creatinine and BUN levels, as biomarkers of renal dysfunction, are increased with prolonged diabetes [17], and elevated levels were markedly reduced with compound **2**, compared to diabetic controls. Compound **3** exhibited a similar (although less obvious) effect, clearly indicating that *P. latifolia* has renoprotective effects in diabetic rats. Serum levels of liver enzymes, representing hepatic damage, are also increased in diabetes mellitus [18]. Rats treated with ethanolic extract, chloroform fraction, and compounds **2** and **3** had significantly lower enzyme levels than diabetic control rats. Minimal liver damage was noted on histopathologic examination in rats treated with compounds **2** and **3**, in contrast to the severe changes observed in control diabetic rats.

In diabetic patients, auto-oxidation of glucose leads to the production of free radicals [19]. If levels of free radicals exceed the scavenging ability of antioxidant enzymes in the body, harmful oxidative stress occurs [20]. In diabetes, levels of CAT, SOD, and GSH are reduced, while levels of MDA are elevated. Compound **2** increased CAT, SOD, and GSH and decreased MDA. Thus, *P. latifolia* not only has significant hypoglycaemic effects, but it also has substantial antioxidant activity.

CONCLUSION

The findings of this study demonstrate that two new compounds from *P. latifolia*, compounds **2**

and **3**, show substantial promise as potential therapeutic agents, exemplified by their significant antidiabetic activity, reno- and hepatoprotective effects, and antioxidant activity. The antioxidant property may reduce oxidative stress in patients with diabetes mellitus, thereby reducing the risk or progression of diabetic complications.

DECLARATIONS

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Conflict of interest

The authors declare that they have no conflicts of interest with regard to this work.

Contribution of authors

The authors declare that this work was conducted by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

1. *Dubey NK, Kumar R, Tripathi P. Global promotion of herbal medicine: India's opportunity. Current Science 2004; 86: 37–41.*
2. *Kayser O, Kidderlen AF, Croft SL. Natural products as potential anti-parasitic drugs. Acta Trop 2000; 77: 307–314.*
3. *Aravindakshan C, Bai JN. Effect of Premna latifolia Roxb and Imperate A Rudinacea on the in vitro oxalate crystal growth. Ind J of Clin Biochem 1995; 2(1): 42-45.*
4. *Anbazhakan S, Babu S. Indegenous knowledge on the Indian medicinal plant Premna tomentosa. Plant Archives 2007; 7(1): 197-198.*
5. *Suresh G, Suresh Babu K, Rama SRV, Suri ARM, Lakshma NV, Sistla R. Novel cytotoxic icetexane diterpenes from Premna latifolia Roxb. Tetrahedron Letters 2011; 52: 1273–1276.*
6. *Parthasarathi G, Niranjan D, Biswanath D. Antioxidant flavones glycosides and other constituents from Premna latifolia leaves. Indian J Chemistry 2014; 53: 746-749.*
7. *Unnikrishnan PS, Sudhindhiran K, Jayasri MA. Alpha-amylase inhibition and antioxidant activity of marine green algae and its possible role in diabetes management. Pharmacogn Mag 2015; 11(44): 511-515.*
8. *Jie T, Jun C, Shuyun Z, Chunxiao Z, Hua C, Youbing L. Inhibition of wheat bran and its active components on α-glucosidase in vitro. Pharmacogn Mag 2013; 9(36): 309-314.*
9. *CPCSEA. CPCSEA Guidelines for laboratory animal facility, Indian J Pharmacol 2003; 35(4): 257-274.*
10. *Sathish SD, Sivagnanam K, Subramanian S. Antidiabetic activity of Momordica charantia seeds on streptozotocin induced diabetic rats. Pharmazie 2004; 60: 383-387.*
11. *Badole SL, Bagul PP, Mahamuni SP. Oral L-glutamine increases active GLP-1 (7-36) amide secretion and improves glycemic control in stretpozotocin-nicotinamide induced diabetic rats. Chem Biol Interact 2013; 203(2): 530–541.*
12. *Annadurai T, Muralidharan AR, Joseph T, Hsu MJ, Thomas PA, Geraldine P. Antihyperglycemic and antioxidant effects of a flavanone, naringenin, in streptozotocin-nicotinamide-induced experimental diabetic rats. J Physiol Biochem 2012; 68: 307–318.*
13. *Atsuo T, Akiko MY, Ryosuke N, Yuka S, Masayuki S. Hypoglycemic effects of antidiabetic drugs in streptozotocin-nicotinamide induced mildly diabetic and streptozotocin-induced severely diabetic rats. Basic Clin Pharmacol Toxicol 2008; 103:560-568.*
14. *Saravanan R, Pari L. Succinic acid monoethyl ester prevents oxidative stress in sterptozotocin-nicotinamide induced type-2 diabetic rats. J Basic Clin Physiol Pharmacol 2006; 17:115-132.*
15. *Ananda PK, Kumarappan CT, Christudas S, Kalaichelvan VK. Effects of Biophytum sensitivum on streptozotocin and nicotinamide induced diabetic rats. Asian Pac J Trop Biomed 2012; 2:31-35.*
16. *Ujjwal K, Vidhu A, Showkat RM, Mohammad A. Cucurbitane-type triterpenoids from the blood glucose-lowering extracts of Coccinia indica and Momordica balsamina fruits. Pharmacogn Mag 2017; 13(49): 115-121.*
17. *Sefi M, Fetoui H, Soudani N, Chtourou Y, Makni M, Zeghal N. Artemisia campestris leaf extract alleviates early diabetic nephropathy in rats by inhibiting protein oxidation and nitric oxide end products. Pathol-Res Pract 2012; 208: 157-162.*
18. *Farokhi F, Farkhad NK, Togmechi A, Soltani band K. Preventive effects of Prangos ferulacea (L.) Lindle on liver damage of diabetic rats induced by alloxan. Avicenna J Phytomed 2012; 2: 63-71.*
19. *Karaaga N, Salman F, Dogru-Abbasoglu S. Changes in prooxidant-antioxidant balance in tissues of rats following long-term hyperglycemic status. Endocr Res 2011; 36: 124–133.*
20. *Qi X-Y, CW, Zhang Xie B. Magrocides extract from Siratia Grosveuori scavenges free radicals in vitro acid lowers oxidative stress serum glucose and lipid levels in alloxan induced diabetic mice. Nutr Res 2008; 28: 278-284.*