Effects of *Polyalthia longifolia* leaves against streptozotocin-nicotinamide induced type 2 diabetes mellitus and peripheral neuropathy in rats

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ABSTRACT

Polyalthia longifolia (Sonner.) Thw. (Annonaceae) has been used in Ayurveda for treating fever, skin diseases, diabetes, hypertension and helminthiasis. This study evaluates the protection against type 2 diabetes and diabetic neuropathy by *Polyalthia longifolia* leaves. Streptozotocin-nicotinamide-induced type 2 diabetes in rat model was used. The diabetic animals were treated with the ethanol extract (PLEE) or chloroform (PLCE) extracts at two dose levels (100 and 200 mg/kg) or standard, glibenclamide (5 mg/kg) for 28 days. Diabetic neuropathy was tested by radiant heat tail-flick method. In diabetic rats the fasting blood glucose levels increased upto 296.3 ± 4.4 mg/dL which decreased to 107.5 ± 4.1 mg/dL (PLEE) and 126.3 ± 4.2 mg/dL (PLCE) at 200 mg/kg dose level. The triglycerides, total cholesterol, LDL and serum marker enzymes were elevated and HDL levels were decreased in diabetic animals and were reversed to near normal values upon treatment with extracts. The levels of enzymatic and non-enzymatic antioxidants were reduced in diabetic animals where as treatment with extracts reversed the same. Histopathological study reflected partial damage of islets of Langerhans in diabetic animals and regeneration of islets in extract- and glibenclamide-treated diabetic animals. The latency of tail flick in analgesiometer was 3.2 ± 0.3 sec in diabetic rats which increased to 10.9 ± 0.3 sec (PLEE) and 9.4 ± 0.4 sec (PLCE). The extracts showed good antidiabetic

activity and protection against diabetic neuropathy. Thus, *Polyalthia longifolia* makes a good candidate for continued exploration in this regard.

Key words:
Analgesiometer, liver enzymes, antioxidants, histopathology.

1. INTRODUCTION

The prevalence of diabetes mellitus worldwide doubled between the years 1990 and 2005 (Baloch *et al.* 2013). Estimated 366 million people worldwide (8.3% of adults) had diabetes in 2011 and was predicted to go upto 552 million people (one adult in 10) by the year 2030 (International Diabetic Federation, 2011). About 90% of them are diagnosed with type2 diabetes mellitus (Holmann *et al.* 2008). The chronic hyperglycaemia results in development of many diabetic complications, which pose a definite threat to overall quality of life and result in a significant healthcare burden (Mohan *et al.* 2013). Various diabetic complications include diabetic neuropathy, nephropathy and retinopathy (microvascular complications) and coronary artery disorder, peripheral vascular disease and stroke (macrovascular complications) (Fowler 2008). A study had reported prevalence of diabetic neuropathy at 26.1% among diabetic population (Pradeepa *et al.* 2008).

Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) injected intraperitoneally at a dose of 60 mg/kg b.w. administered 15 min after the injection of nicotinamide (NAD; nicotinamide adenine dinucleotide; 120 mg/kg b.w.) intraperitoneally produced type 2 diabetes mellitus in rats. The model has been extensively used in experimental pharmacology to study the effectiveness of various antidiabetic agents including plant extracts (Shirwaikar *et al.* 2006). The diabetic neuropathy can also be studied in these rats at the end of the treatment period by radiant heat – tail flick method using analgesiometer (Nadig *et al.* 2012).

Polyalthia longifolia (Sonner.) Thw. (Annonaceae) is a lofty evergreen tree, native to India, commonly planted due to its effectiveness in alleviating noise pollution. The plant has been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis (Kirtikar & Basu, 1995). The plant was reported to possess cytotoxic (Wu & Duh, 1990; Chen *et al.* 2000), hepatoprotective (Jain *et al.* 2006), antibacterial (Nair & Chanda, 2006a), antiulcer (Malairajan, 2008), antifungal (Nair & Chanda 2006b), antioxidant (Sivashanmugam & Chatterjee, 2011), anticataractogenesis (Sivashanmugam & Chatterjee, 2012a) and xanthine oxidase inhibitory (Sivashanmugam & Chatterjee, 2012b) activities. Earlier we have reported inhibitory effect of *Polyalthia longifolia* extracts on carbohydrate digesting enzymes α-amylase and α-glucosidase using *in vitro* models and *in vivo* antidiabetic activity against streptozotocin-induced type 1 diabetes mellitus in rats (Sivashanmugam & Chatterjee, 2013). In this study, the ethanol and chloroform extracts of *Polyalthia longifolia* leaves were used to ascertain effectiveness against streptozotocin-nicotinamide-induced type 2 diabetes mellitus and diabetic neuropathy in rats.

2. MATERIALS AND METHODS

Drugs and chemicals

Glucose assay, cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglyceride assay kits (Agappe Diagnostics, Kerala, India), Streptozotocin, nicotinamide, 3,5-dinitro salicylic acid, 5,5′-dithiobis-(2-nitro benzoic acid) (DTNB), adrenaline tartarate, NADPH, NADP, glutathione-reduced, glutathione-oxidized, (HiMedia, Mumbai, India) and bovine serum albumin (Loba Chemie, Mumbai, India) were purchased for the study. Glibenclamide was obtained as gift sample from Sanofi Aventis, Mumbai, India. All the other chemicals used in the study were of analytical grade and were obtained commercially.

Plant collection and authentification

The plant material consists of dried powdered leaves of *Polyalthia longifolia*. The leaves were collected in Tamil Nadu Agricultural University Campus, Coimbatore, India and authentificated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore, India (Ref No BSI/SRC/5/23/10-11/Tech.709).

Preparation of the extracts

The air-dried powdered leaves of *P. longifolia* (100 g) were extracted with ethanol (500 ml) by cold maceration process for 72 h (Nair & Chanda, 2006). The resultant extract was concentrated under reduced pressure to obtain a residue of *P. longifolia* leaf ethanol extract (PLEE). The same procedure was followed with chloroform to prepare *P. longifolia* leaf chloroform extract (PLCE).

Experimental animals

Healthy adult male *Wistar* rats (180-250 g) were procured from College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, India and kept in cages under ambient temperature (22 ± 3°C) with 12 h light/dark cycle. They were fed with standard rat laboratory diet (College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, India) and drinking water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee and all procedures were performed in accordance with the recommendations for the proper care and use of laboratory animals.

Acute toxicity studies and selection of doses

Acute oral toxicity testing was carried out in accordance with the OECD guideline 423 Acute Oral Toxicity - Acute Oral Toxic Class Method (Bachhawat *et al.* 2011). The results of the same had been reported by us earlier (Sivashanmugam & Chatterjee, 2013). The LD_{50} of the extracts fell under category 5 values with no death and no signs of acute toxicity even at the dose of 2000 mg/kg. The biological evaluation of the extracts was carried out at dose levels of 100 and 200 mg/kg body weight.

Experimental induction of diabetes

Type-2 diabetes mellitus was induced in overnight fasted rats by administering freshly prepared solution of streptozotocin (STZ; 60 mg/kg *b.w.*, i.p) in 0.1 mol/L cold citrate buffer (pH 4.5) 15 min after the injection of nicotinamide (NAD; 120 mg/kg *b.w.*, *i.p.*). The animals were allowed to drink 5% glucose solution over night to overcome drug-induced hypoglycemia. After 14 days of injection of STZ-NAD, rats with moderate diabetes having persistent glycosuria and hyperglycaemia (blood glucose >250 mg/dl) were used for further experimentation (Shirwaikar *et al.* 2006).

Experimental design

Sample collection during the treatment period

Blood samples were collected from tip of rat tail and blood glucose levels were estimated on 0^{th}, 7^{th}, 14^{th} and 28^{th} days of treatment using Optium Xceed Diabetes Monitoring System (Abbott Inc. USA). Body weight was measured initially and during treatment period.

Test for diabetic neuropathy - radiant heat tail flick method using analgesiometer

The test was carried out by applying radiant heat method (thermal stimulus) on the rat tail in an analgesiometer (Nadig *et al.* 2012). The animal was placed inside a restrainer which had an opening for the tail in the rear end and holes in the front allowing air into the restrainer for the animals to breathe easily. The tail of the animal was gently placed so that the middle portion of the proximal one-third of the tail touches the metal base, which attains a preset temperature of 55ºC by allowing 1.5 amperes of current to flow. The animal was allowed to acclimatise in this position for 2-3 min such that the tail stayed relatively stable over the metal base. Then the switch was put on. The latency of time taken by the animal to flick the tail in response to the obnoxious stimuli (radiant heat) was considered as the reaction time. A cut off time of 15 sec was used and animals not responding by 15 sec were removed from the source of stimuli. All the groups of animals were tested for their reaction time on the 0^{th} day and 28^{th} day of treatment period.

Terminal blood collection and collection of organs

On 29^{th} day blood was collected by retro-orbital puncture from the inner canthus of the eye under mild ether anaesthesia using capillary tubes in fresh heparinized vials and serum separated. Serum parameters like triglycerides, total cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) were determined using standard kits obtained from Agappe Diagnostics, Kerala, India using a semi autoanalyser (Mispa Excel Chemical Analyser, Mumbai, India). Immediately after the blood collection, the animals were sacrificed by cervical dislocation and pancreas and liver tissues were dissected out for further studies.

Estimation of biochemical parameters in liver homogenates

The liver tissue was removed and washed immediately with ice-cold saline to remove blood. A 10% w/v liver homogenate was prepared in ice-cold potassium phosphate buffer (100 mM, pH 7.4) followed by centrifugation at 5000 *g* for 10 min. The resulting supernatant was used for the estimation of lipid peroxidation and antioxidant activity studies.

The amount of protein was determined using bovine serum albumin as standard (Lowry *et al.* 1951). Lipid peroxidation was studied by estimating the levels of thiobarbituric acid reactive substances (TBARS) assay in which malondialdehyde (MDA) formed reacts with thiobarbituric acid to form a pink colored complex (Devasagayam *et al.* 2003). Another parameter for lipid peroxidation, lipid hydroperoxides (LH) resulting from oxidative damage to the polyunsaturated fatty acids in cell membranes was estimated. The study is

based on the oxidation of ferrous (II) to ferric (III) ions by hydroperoxides under acidic conditions (Devasagayam *et al.* 2003). Superoxide dismutase (SOD) activity was determined using the method of Kakkar *et al.* (1984). SOD in the homogenate inhibits the formation of autocatalyzed adrenochrome which can be measured at 480 nm. Catalase (CAT) activity was measured by the catalysis of hydrogen peroxide (H_2O_2) to H_2O in an incubation mixture adjusted to pH 7.0 and recorded at 254 nm (Sinha, 1972). Glutathione peroxidase (GPx) activity was measured by the procedure of Paglia and Valentine (1967). Gutathione reductase (GSSH) activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm (Racker, 1952). Peroxidase (Px) activity was measured spectophotometrically by following the change in absorbance at 460 nm due to *O*-dianisidine oxidation in the presence of H_2O_2 and enzyme (Lobarzewski & Ginalska, 1995).

The non-enzymatic antioxidants, reduced glutathione (GSH) and α-tocopherol also were measured. GSH was estimated based on its reaction with dithionitrobenzoic acid (DTNB) resulting in the formation of a compound that absorbs at 412 nm (Ellman, 1959). The estimation of α-tocopherol was based on its ability to reduce ferric to ferrous ion, which forms a red coloured complex with 2, 2′-dipyridyl (Baker *et al.* 1980).

Histopathological studies of pancreatic tissues

Pancreatic tissues isolated from rats were used for histopathological studies. The tissue in each group was cut into small portions measuring 1 cm, fixed with 10% formaldehyde solution, dehydrated in gradually increasing concentrations of ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections of 5 µm thickness were prepared. Haemotoxylin and eosin were used for staining and later the microscopic slides of pancreatic tissue were photographed under 100x magnification.

Statistical analysis

All determinations for *in vivo* study were carried out in triplicate and the values are expressed as mean ± SEM. The results were expressed as mean ± S.E.M. for 6 rats in each group. Statistical analysis of the results was carried out using GraphPad InStat software by one-way analysis of variance (ANOVA) followed by Dunnett's test. The level of significance was set at $P<0.05$.

3. RESULTS

Percentage yield of the extracts

The percentage yield of the ethanol extract of *P. longifolia* leaves (PLEE) was found to be 14.82% w/w and that of the chloroform extract (PLCE) was found to be 18.76% w/w.

***In vivo* antidiabetic activity**

The initial (0^{th} day) and final (28^{th} day) body weights of rats used are summarized in Table 1. Initial values in control and experimental animals were not significantly different. The body weight of diabetic control rats significantly decreased by approximately 16.1% over their initial readings after 28 days. Administration of the extracts, PLEE or PLCE, or the standard, glibenclamide in diabetic rats reversed this weight loss and there was no significant ($P>0.05$) difference between the initial and final body weight in these animals.

Untreated diabetic rats showed a significant ($P<0.01$) increase in the fasting blood glucose levels (>250 mg/dL) when compared to normal saline control rats. The fasting blood glucose levels in the rats treated with the extracts, PLEE and PLCE, showed a significant ($P<0.01$) decrease from the first week onwards and the results were comparable with that of standard, glibenclamide (Table 2).

The levels of triglycerides, total cholesterol, LDL and VLDL increased and HDL levels decreased significantly ($P<0.01$) in untreated diabetic animals when compared to normal rats. Treatment with PLEE, PLCE and glibenclamide showed a marked reversal of changes in the serum lipid parameters as compared to diabetic rats. PLCE at 100 mg/kg dose level showed a small but statistically not significant increase in HDL (Table 3).

The activities of serum marker enzymes like ALP, AST and ALT were significantly ($P<0.01$) increased in untreated diabetic rats when compared to normal rats. Diabetic rats fed simultaneously with PLEE, PLCE and glibenclamide showed a significant ($P<0.01$) reduction in the activities of ALP, AST and ALT when compared with diabetic control rats (Table 4).

There was a significant ($P<0.01$) decrease in amount of protein and an increase in the concentrations of MDA and LH in the liver tissues of diabetic rats when compared with normal saline control rats. Reversal to near normal levels was seen with diabetic rats simultaneously treated with PLEE, PLCE and glibenclamide (Table 5).

The levels of enzymatic and non-enzymatic antioxidants in the liver tissues of various groups of experimental animals are presented in Table 6. The activities of enzymatic and non-enzymatic antioxidants in the untreated diabetic rats were significantly ($P<0.01$) lower when compared with normal rats. Rats fed with the extracts and glibenclamide displayed increased activities as compared to untreated diabetic rats.

Diabetic control rats developed peripheral neuropathy which resulted in hyperlagesia and seen as the decreased reaction time for tail flick response to radiant heat tested using analgesiometer. In the diabetic rats treated with the extracts, PLEE and PLCE, and glibenclamide the reaction time for the tail flick response increased than the normal saline control rats (Table 7).

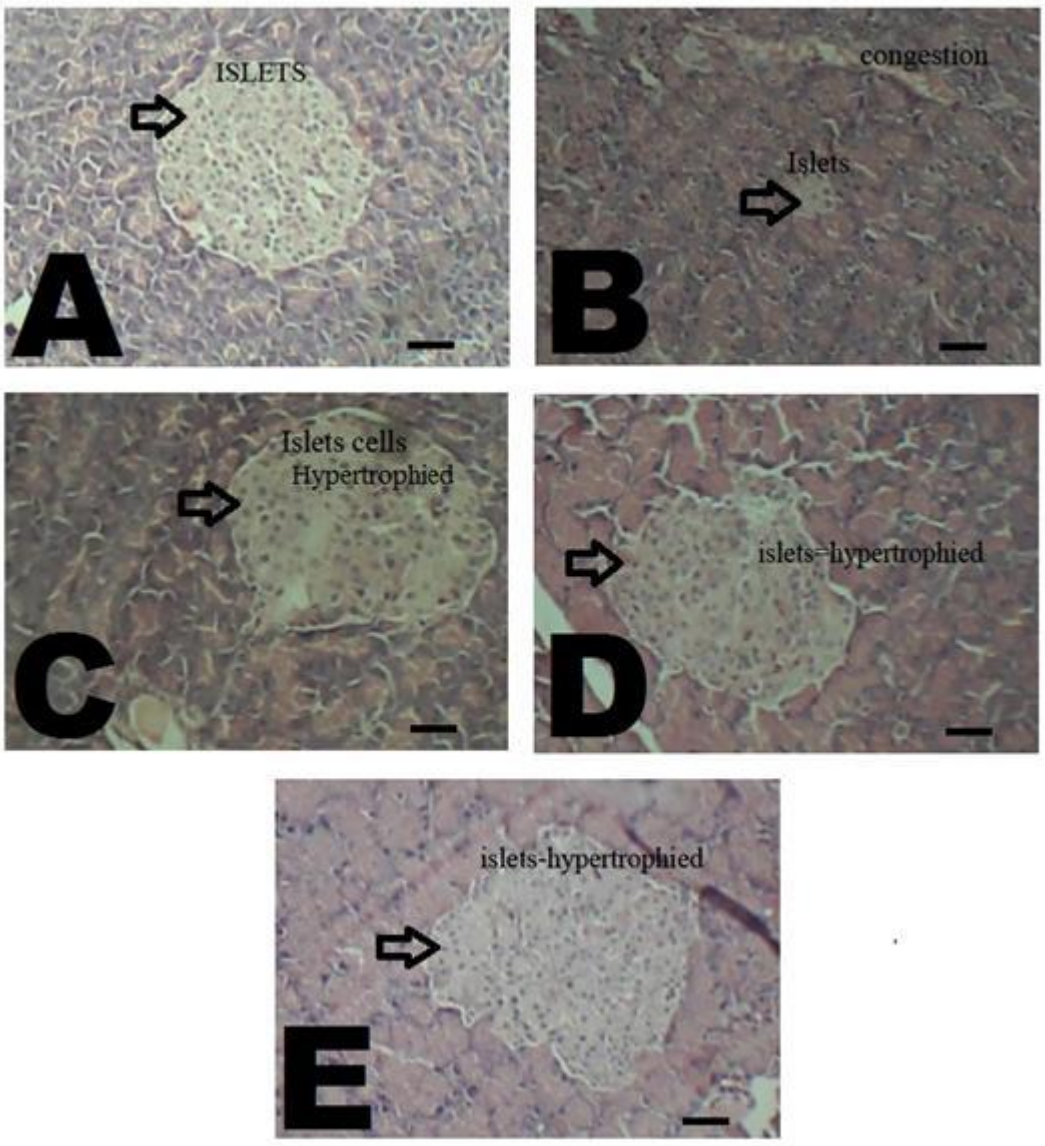

Figure 1

Histopathology of pancreatic tissue. A: Normal rats, B: Untreated streptozotocin-nicotinamide-induced type 2 diabetic rats, C: PLEE (ethanol extract of *Polyalthia longifiolia* leaves)-treated diabetic rats (200 mg/kg body weight), D: PLCE (chloroform extract of *Polyalthia longifiolia* leaves)-treated diabetic rats (200 mg/kg body weight) and E: Glibenclamide-treated diabetic rats. Arrows indicate islets of Langerhans. Scale: 100 µm

Results of the histopathological studies of the pancreatic tissues are shown in Figure 1. Normal rats showed normal architecture of the pancreas with normal well formed acini in lobules in exocrine area and prominent endocrine islands (Figure 1A). Induction of diabetes using streptozotocin-nicotinamide resulted in necrotic exocrine tissue and atrophic endocrine islands. There is evident destructive effect in both the exocrine and endocrine areas of the pancreatic tissue in the diabetic control rats (Figure 1B). The abnormal histopathology of pancreas due to streptozotocin-nicotinamide-induced type 2 diabetes mellitus was reversed in the PLEE, PLCE and glibenclamide treated diabetic animals. Treatment with PLEE (Figure 1C) and PLCE (Figure 1D) resulted in appearance of less damaged

but irregular and hypertrophied islets cells when compared to untreated diabetic rats. The recovery in glibenclamide-treated group was near normal showing well-formed prominent but hypertrophied islets showing near normalcy (Figure 1E). It can be noted that the destructive changes in islets due to streptozotocin-nicotinamide administration is reversed in the treatment groups.

Table 1

In vitro 12-lipoxygenase inhibitory activity of PLEE and PLCE

Test Substance	% Inhibition					IC_{50} (µg/ml)
	Concentration (µg/ml)					
	10	20	40	80	160	
PLEE	23.38 ± 2.32[a]	37.56 ± 1.67[a]	56.30 ± 1. 95[a]	62.7 ± 2.18[a]	79.6 ± 3. 72[a]	**47.5 ± 1.65**
PLCE	17.9 ± 1. 24[a]	29.8 ± 2.12[a]	40.4 ± 1.16[a]	58.6 ± 1.83[a]	71.4 ± 2.17[a]	**69.8 ± 2.42**
Indomethacin (standard)	33.84 ± 1.07	41.66 ± 1.39	63.63 ± 0.61	76.28 ± 1.10	92.85 ± 1.08	**32.2 ± 0.42**

Values are expressed as mean ± SEM of three parallel measurements. [a]P<0.01 when compared with standard.

Table 2

Effect of PLEE and PLCE on body weight in streptozotocin-nicotinamide-induced type 2 diabetic rats

Group	Body weight (g)	
	Initial	Final (% change)
Control Normal saline (10 ml/kg)	215.5 ± 3.6	224.6 ± 3.8 (↑4.2) [ns]
Diabetic control (0.5% carboxy methyl cellulose, 5 ml/kg)	218.3 ± 7.9	183.1 ± 5.5 (↓16.1)*
Diabetic + Glibenclamide 5 mg/kg	225.0 ± 9.2	216.6 ± 4.9 (↓0.3) [ns]
Diabetic + PLEE 100 mg/kg	226.6 ± 8.4	218.6 ± 7.9 (↓3.5) [ns]
Diabetic + PLEE 200 mg/kg	220.0 ± 7.7	217.8 ± 7.0 (↓1.0) [ns]
Diabetic + PLCE 100 mg/kg	225.0 ± 9.2	208.1 ± 5.9 (↓7.5) [ns]
Diabetic + PLCE 200 mg/kg	216.6 ± 6.6	209.8 ± 6.1 (↓3.1) [ns]

Values are expressed as mean ± SEM (n = 6). Values in parentheses are the percent increase (↑) or decrease (↓) from their corresponding initial readings.* denotes P < 0.01 when compared to initial readings; [ns] denotes P > 0.05 when compared to the initial readings (Student's paired *t* test). PLEE – *P. longifolia* ethanol extract PLCE – *P. longifolia* chloroform extract.

Table 3
Effect of PLEE and PLCE on fasting blood glucose levels in streptozotocin-nicotinamide-induced type 2 diabetic rats

Group	Fasting blood glucose levels (mg/dL)			
	0 day	7th day	14th day	28th day
Control Normal saline (10 ml/kg)	81.0 ± 4.1	82.5 ± 3.6^{ns}	80.5 ± 3.0 ^{ns}	80.3 ± 4.0 ^{ns}
Diabetic control (0.5% carboxy methyl cellulose, 5 ml/kg)	264.0 ± 7.1$^{\#}$	272.8 ± 4.9^{ns}	283.8 ± 3.2*	296.3 ± 4.4*
Diabetic + Glibenclamide 5 mg/kg	272.0 ± 6.0$^{\#}$	228.8 ± 6.0*	164.1 ± 10.6 *	89.1 ± 5.5 *
Diabetic + PLEE 100 mg/kg	271.0 ± 3.2$^{\#}$	227.3 ± 4.2*	193.8 ± 2.6 *	145.8 ± 3.7 *
Diabetic + PLEE 200 mg/kg	275.3 ± 4.8$^{\#}$	217.5 ± 4.4*	167.8 ± 8.5 *	107.5 ± 4.1 *
Diabetic + PLCE 100 mg/kg	262.8 ± 3.4$^{\#}$	230.5 ± 3.4*	206.6 ± 4.9 *	156.1 ± 5.6 *
Diabetic + PLCE 200 mg/kg	270.3 ± 6.1$^{\#}$	222.3± 5.7*	183.6 ± 4.0 *	126.3 ± 4.2 *

Values are expressed as mean ± SEM (n = 6 in each group).
^{ns} denotes P > 0.5 and * denotes P < 0.01 when compared to 0 day reading of respective group; $^{\#}$ denotes P< 0.01 when compared to 0 day reading of Normal saline group (One way ANOVA followed by Dunnett's test).
PLEE – *P. longifolia* ethanol extract
PLCE – *P. longifolia* chloroform extract

Table 4
Effect of PLEE and PLCE on serum lipid profile on 28th day post treatment in streptozotocin-nicotinamide-induced type 2 diabetic rats

Group	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control Normal saline (10 ml/kg)	36.1 ± 1.1	85.9 ± 2.1	60.7 ± 1.4	48.5 ± 1.5	7.2 ± 0.2
Diabetic control (0.5% carboxy methyl cellulose, 5 ml/kg)	97.7 ± 4.0$^{\#}$	252.7 ± 3.8$^{\#}$	35.9 ± 0.7$^{\#}$	182.7 ± 5.4$^{\#}$	19.5 ± 0.8$^{\#}$
Diabetic + Glibenclamide 5 mg/kg	40.0 ± 0.7*	88.7 ± 3.5*	62.1 ± 0.8*	50.6 ± 1.8*	8.0 ± 0.1*
Diabetic + PLEE 100 mg/kg	73.5 ± 0.7*	155.8 ± 16.5*	40.4 ± 1.7^{ns}	82.9 ± 2.3*	14.7 ± 0.2*

Diabetic + PLEE 200 mg/kg	45.7 ± 2.9*	92.1 ± 4.3*	52.6 ± 1.5*	57.5 ± 0.8*	9.1 ± 0.6*
Diabetic + PLCE 100 mg/kg	85.9 ± 2.1**	170.7 ± 3.7*	39.0 ± 0.4^{ns}	95.0 ± 1.0 *	19.4 ± 0.5 **
Diabetic + PLCE 200 mg/kg	64.4 ± 2.0*	104.8 ± 6.4*	49.9 ± 2.1*	64.0 ± 1.0*	12.8 ± 0.4*

Values are expressed as mean ± SEM (n = 6 in each group).
$^{\#}$ denotes P< 0.01 when compared Normal saline group; * denotes P < 0.01; ** denotes P < 0.05 and ^{ns} denotes P > 0.5 when compared diabetic control group (One way ANOVA followed by Dunnett's test).
PLEE – *P. longifolia* ethanol extract
PLCE – *P. longifolia* chloroform extract

Table 5
Effect of PLEE and PLCE on serum marker enzymes on 28th day post treatment in streptozotocin-nicotinamide-induced type 2 diabetic rats

Group	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Control Normal saline (10 ml/kg)	60.6 ± 0.8	75.5 ± 1.9	39.7 ± 0.6
Diabetic control (0.5% carboxy methyl cellulose, 5 ml/kg)	140.8 ± 6.1$^{\#}$	181.0 ± 2.2$^{\#}$	127.8 ± 2.8$^{\#}$
Diabetic + Glibenclamide 5 mg/kg	67.2 ± 3.0*	78.7 ± 2.0*	42.7 ± 1.5*
Diabetic + PLEE 100 mg/kg	89.7 ± 2.5*	94.3 ± 2.2*	67.1 ± 2.0*
Diabetic + PLEE 200 mg/kg	69.3 ± 0.8*	77.6 ± 2.5*	46.2 ± 0.9*
Diabetic + PLCE 100 mg/kg	99.7 ± 4.5*	96.7 ± 1.6*	73.6 ± 1.1*
Diabetic + PLCE 200 mg/kg	75.5 ± 3.0*	84.6 ± 0.8*	53.0 ± 1.1*

Values are expressed as mean ± SEM (n = 6 in each group).
$^{\#}$ denotes P< 0.01 when compared Normal saline group; * denotes P < 0.01 when compared to diabetic control group (One way ANOVA followed by Dunnett's test).
PLEE – *P. longifolia* ethanol extract
PLCE – *P. longifolia* chloroform extract

Table 6

Effect of PLEE and PLCE on protein, MDA and LH on 28th day post treatment in the liver tissues of streptozotocin-nicotinamide-induced type 2 diabetic rats

Group	Protein (µg/mg wet tissue)	MDA (nmoles/min/mg protein)	LH (nmoles/min/mg protein)
Control Normal saline (10 ml/kg)	167.9 ± 1.5	0.278 ± 0.03	0.791 ± 0.06
Diabetic control (0.5% carboxy methyl cellulose, 5 ml/kg)	125.5 ± 3.2#	4.306 ± 0.18#	5.879 ± 0.29#
Diabetic + Glibenclamide 5 mg/kg	164.8 ± 1.7*	0.556 ± 0.10*	0.893 ± 0.29*
Diabetic + PLEE 100 mg/kg	139.4 ± 7.0*	2.047 ± 0.07*	2.498 ± 0.23*
Diabetic + PLEE 200 mg/kg	164.4 ± 1.9*	1.134 ± 0.05*	1.865 ± 0.05*
Diabetic + PLCE 100 mg/kg	138.9 ± 4.0*	1.626 ± 0.06*	1.803 ± 0.12*
Diabetic + PLCE 200 mg/kg	162. 4 ± 4.2*	0.827 ± 0.24*	1.121 ± 0.16*

Values are expressed as mean ± SEM (n = 6 in each group).

# denotes P< 0.01 when compared Normal saline group. * denotes P < 0.01 when compared to diabetic control group (One way ANOVA followed by Dunnett's test).

PLEE – *P. longifolia* ethanol extract

PLCE – *P. longifolia* chloroform extract

Table 7

Effect of PLEE and PLCE on enzymatic and non-enzymatic antioxidants on 28th day post treatment in the liver tissues of streptozotocin-nicotinamide-induced type 2 diabetic rats Values are expressed as mean ± SEM (n = 6)

Group	Enzymatic antioxidants						Non-enzymatic antioxidants	
	SOD (nmoles/min /mg protein)	CAT (µmoles/min /mg protein)	GPx (nmoles/min /mg protein)	GSSH (nmoles/min /mg protein)	Peroxidase (nmoles/min /mg protein)	G6PD (µmoles/min /mg protein)	GSH (nmoles/min /mg protein)	Vitamin E (mg/mg protein)
Control Normal saline (10 ml/kg)	38.7 ± 0.9	82.5 ±1.4	98.2 ± 2.3	44.4 ± 1.4	86.1 ± 0.9	8.2 ± 0.4	38.6 ± 0.7	6.0 ± 0.2
Diabetic control (0.5% carboxy methyl cellulose, 5 ml/kg)	9.9 ± 0.4#	45.0 ± 1.2#	40.0 ± 0.8#	26.2 ± 1.3#	33.2 ± 1.8#	3.1 ± 0.2#	17.3 ± 0.9#	2.5 ± 0.2#
Diabetic + Glibenclamide 5 mg/kg	37.0 ± 1.1*	81.9 ± 1.0*	95.3 ± 1.3*	42.8 ± 1.4*	86.3 ± 1.7	7. 3 ± 0.3*	41.2 ± 1.6*	6.0 ± 0.1*

Diabetic + PLEE 100 mg/kg	15.4 ± 0.9**	56.6 ± 2.6*	53.9 ± 2.3*	32.5 ± 0.8*	52.9 ± 1.4	4.1 ± 0.3**	27.8 ± 1.0*	3.7 ± 0.1*
Diabetic + PLEE 200 mg/kg	33.7 ± 2.1*	67.1 ± 1.2*	67.2 ± 3.7*	41.1 ± 1.1*	78.8 ± 2.3	5.9 ± 0.2*	37.4 ± 1.3*	6.2 ± 0.4*
Diabetic + PLCE 100 mg/kg	14.2 ± 0.9**	51.5 ± 1.9*	52.3 ± 2.9*	27.2 ± 0.7*	43.6 ± 1.3**	3.7 ± 0.1**	23.4 ± 0.8*	3.0 ± 0.1**
Diabetic + PLCE 200 mg/kg	33.5 ± 2.5*	65.0 ± 1.9*	72.4 ± 1.2*	36.8 ±1.5*	69.0 ± 4.5*	6.1 ±0.3*	29.4 ± 0.9*	4.7 ± 0.2*

[#] denotes P< 0.01 when compared Normal saline group. * denotes P < 0.01 and ** denotes P < 0.05 when compared diabetic control group (One way ANOVA followed by Dunnett's test).
PLEE – *P. longifolia* ethanol extract
PLCE – *P. longifolia* chloroform extract

Table 8
Effect of PLEE and PLCE on diabetic neuropathy tested by radiant heat – tail flick method using analgesiometer on 28th day post treatment in streptozotocin-nicotinamide-induced type 2 diabetic rats

Group	**Mean reaction time (seconds)**	
	0 day of treatment	**28th day of treatment**
Control Normal saline (10 ml/kg)	6.5 ± 0.5	7.8 ± 0.6*
Diabetic control (0.5% carboxy methyl cellulose, 5 ml/kg)	6.8 ± 0.7	3.2 ±0.3[#]
Diabetic + Glibenclamide 5 mg/kg	6.6 ± 0.3	12.8 ± 0.8*
Diabetic + PLEE 100 mg/kg	6.8 ± 0.6	9.6 ± 0.7*
Diabetic + PLEE 200 mg/kg	6.8 ± 0.4	10.9 ± 0.3*
Diabetic + PLCE 100 mg/kg	4.8 ± 0.6	8.2 ± 0.6*
Diabetic + PLCE 200 mg/kg	6.6 ± 0.4	9.4 ± 0.4*

Values are expressed as mean ± SEM (n = 6).
[#] denotes P< 0.01 when compared Normal saline group. * denotes P < 0.01 when compared diabetic control group (One way ANOVA followed by Dunnett's test).
PLEE – *P. longifolia* ethanol extract
PLCE – *P. longifolia* chloroform extract

4. DISCUSSION

Streptozotocin by its potent alkylating properties results contribute to DNA fragmentation and evoke other deleterious changes in the β-cells of pancreatic islets (Powers & D'Alessio, 2011). Oxidative stress induced by streptozotocin limit mitochondrial ATP production which is mediated by nitric oxide. It has been demonstrated that inhibition of poly ADP-ribosylation prevents effects of STZ-induced B cell damage and hyperglycemia (Pieper et al. 1999). Nicotinamide partially protects the DNA from damage by inhibiting the inducible form of nitric oxide synthase and by scavenging oxygen free radicals (Bedoya et al. 1996).

The body weight in streptozotocin-nicotinamide-induced type 2 diabetic rats can be attributed to muscle wasting, dehydration, reduced body fat, reduction in adipose tissue and excessive breakdown of proteins (Baker et al. 1980). There was reversal of this loss in body weight in the diabetic animals treated with the extracts and glibenclamide. Treatment with the extracts and glibenclamide significantly reduced the blood glucose levels in the experimental diabetic animals. The ability of the extracts to reduce the blood glucose levels can partially be attributed to the inhibition of carbohydrate digesting enzymes, alpha amylase and alpha glucosidase (Sivashanmugam & Chatterjee, 2013). The mechanism of glycaemic control could also be similar to glibenclamide, i.e., stimulating the release of insulin from the beta cells in the islets (Powers & D'Alessio, 2011) and also due to increase in peripheral utilisation of glucose (Sivashanmugam & Chatterjee 2013).

Altered serum triglyceride and cholesterol levels in diabetic rats can be due to abnormalities in the processes of metabolism (Pushparaj et al. 2007). Increased plasma glucose and deficit in glycogen storage which would stimulate hepatic triglyceride formation effecting an increase in triglycerides and VLDL levels. Higher ratio of HDL_2/HDL_3 due to reduced activity of hepatic lipase is seen as decrease in the levels of HDL. Over production of LDL from VLDL, reduced clearance and reduced affinity to LDL receptor might have contributed to the increased serum levels of LDL (Rajkumar et al. 1991). Treatment of diabetic rats with extracts effectively reversed the abnormal changes in the lipid profile and the reversal was comparable with that of glibenclamide.

Enzymes like ALP, AST and ALT may leak from the hepatocytes into the circulation where their levels become elevated (Harris, 2005). This is particularly seen in nonalcoholic fatty liver disease seen in individuals with diabetes mellitus in which altered fat metabolism producing excess free fatty acids prove toxic to hepatocytes (Smith & Adams, 2011). In this study these elevated enzymes reduced to near normal values when simultaneously treated with the extracts and glibenclamide.

The decrease in protein content observed in diabetic rats is due to a net increase in protein breakdown and not necessarily due to decline in protein synthesis (O'Brien & Granner, 1991). Increased lipid peroxidation is observed by increased thiobarbituric acid reactive substances (TBARS), indexed by MDA production, LH in untreated diabetic rats. MDA and LH are the biomarkers of oxidative stress and found after 4 weeks in liver and kidney of diabetic rats (Moller & Nair, 2008). In the present study the decrease in protein content in diabetic rats is reversed when treated with extracts or glibenclamide.

Advanced glycation end products (AGEs) are attributed to the development of oxidative stress indiacted by the accumulation of reactive oxygen species (ROS) (Sun et al. 1999). Increased ROS levels increases the activities of intracellular antioxidant enzymes and other non-enzymatic antioxidant systems (Brownlee et al. 1988). There was an evident decrease in the levels of these components in the diabetic animals. However, the treatment groups showed a recovery in the levels of these components.

Histopathologically the partial protection of nicotinamide against streptozotocin induced damage in beta cells was observed. The streptozotocin damage was observed in our earlier study, which showed complete loss of beta islets in pancreas (Sivashanmugam & Chatterjee, 2013). Injection of nicotinamide prior to streptozotocin protected partially which is evident in presence of atrophic islets. There was partial to near normal reversal of damage as evident in the presence of hypertrophied and small islets in the case of extract treated diabetic rats and prominent hypertrophied islets in glibenclamide treated diabetic rats.

Hyperalgesia is seen in rats with diabetic neuropathy can be attributed to the hyperactivity of C nociceptive fibres. The effect is manifested as reduced latent period (reaction time) for tail flick in response to radiant heat by the analgesiometer (Ilnytska et al. 2006). Significant hyperalgesia develops 3 weeks after induction of diabetes (Nadig et al. 2012). In the present study the extracts prevented the development of hyperalgesia and the same might be due to control of blood glucose levels in the diabetic animals.

5. CONCLUSION

In conclusion, the results of this present study support the use of leaves of *Polyalthia longifolia* for the treatment of diabetes mellitus in traditional medicine. The possible mechanisms of antidiabetic effect can be attributed to reduced oxidative stress and glibenclamide like insulin secretagogue effect in addition to our earlier claim that the leaves of the *Polyalthia longifolia* to have inhibitory effect on carbohydrate digesting enzymes and increased peripheral utilisation of glucose. Further investigations both preclinical and clinical research are necessary to ascertain the exact mechanism and usefulness of this plant for the treatment of diabetes. Thus the plant makes a good candidate for continued exploration in this regard.

SUMMARY IN POINTS

1. Acute toxicity studies performed showed that the extracts can be considered as non-toxic as per the OECD guidelines and can be included in the Category 5 or unclassified category of Globally Harmonized System.
2. The extracts reversed the loss in body weight seen in the animals with diabetes mellitus.
3. Good *in vivo* anti-diabetic activity in type 2 diabetes mellitus rats was observed which was confirmed by optimal fasting blood glucose levels in treated diabetic rats.
4. The extracts prevented the development of hyperalgesia, an index for diabetic neuropathy and the same might be due to control of blood glucose levels in the treated diabetic rats.

FUTURE ISSUES

1. The possible mechanism(s) of actions were assumed and the same can be studied using appropriate techniques.
2. Further investigations both preclinical and clinical research are necessary to ascertain the exact mechanism and usefulness of this plant for the treatment of diabetes.

List of abbreviations

PLEE – *Polyalthia longifolia* leaves ethanol extract; PLCE – *Polyalthia longifolia* leaves chloroform extract; LDL – Low density lipoproteins; HDL – High density lipoproteins; NAD – Nicotinamide adenine dinucleotide; DTNB – dithio nitrobenzoic acid; OECD – Organisation of Economic Cooperation and Development; STZ – Streptozotocin ; ALP – Alkaline phosphatase; AST – Aspartate transaminase; ALT – Alanine transaminase; TBARS – Thiobarbaturic acid reactive substances; MDA – Malondialdehyde; LH – Lipid hydroperoxide; SOD – Superoxide dismutase; CAT – Catalase; GPx – Glutathione peroxidase; GSSH – Glutathione reductase; GSH – Reduced glutathione; VLDL – Very low density lipoproteins

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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