STM JOURNALS

Research & Reviews: A Journal of
Toxicology

ISSN: 2231-3834 (Online)
ISSN: 2349-1264 (Print)
Volume 14, Issue 1, 2024
January—April
DOI (Journal): 10.37591/RRJoT

http://medicaljournals.stmjournals.in/index.php/RRJoT/index

Research RRJoT

Evaluation of the Antidiabetic Effects of Ethanolic Root Extract from *Polyalthia longifolia* in Diabetic Rats Induced by Streptozotocin and Nicotinamide with Consideration of Toxicological Aspects

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Abstract

The root of Polialthia longifolia was selected for the evaluation of antidiabetic potential in STZ-nicotinamide induced diabetic rat model. The root extract was extracted by hot percolation using ethanol as a solvent in Soxhlet apparatus and the preliminary phytochemical screening was performed and it shows that the ethanolic root extract of Polialthia longifolia contains alkaloids, glycosides, flavonoids and tannins. Safety profile is essential for the drugs obtained from the plant origin. The level of toxicity can be evaluated by toxicological studies. From the results it was concluded that the ethanolic root extract of Polialthia longifolia showed significant antidiabetic activity in a dose dependent manner compared to the standard drug glibenclamide. The result also showed significant decrease in the liver Alkaline phosphatase (ALP), Aspartate amino transferase (AST), Alanine amino transferase (ALT), serum urea level, cholesterol, triglycerides, VLDL and LDL was in STZ-nicotinamide induced diabetic animals when compared to control group. It is concluded that ethanolic root extract of Polialthia longifolia showed significant effect in STZ-nicotinamide induced diabetic rats. Further studies are necessary to examine the underlying mechanism of hypoglycemic effect and to isolate the active compound (s) responsible for antidiabetic activities.

Keywords: Antidiabetic activity, ethanolic root extract, *Polialthia longifolia*, Streptozotocin, nicotinamide

INTRODUCTION

Diabetes mellitus, one of the most common endocrine metabolic disorders has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications [1]. Human bodies possess enzymatic and non-enzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes [2]. The disease is rapidly increasing worldwide and affecting all parts of the world. Due to deficiency of the insulin people suffering from diabetes have high blood glucose level [3]. Type 2 diabetes or non-insulin-dependent diabetes mellitus, is the most common form of the disease, accounting for 90%–95% of cases in which the body does not produce enough insulin or properly use it [4]. According to World Health Organization the diabetic population is likely to increase up to 300 million or more by the

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Received Date: February 08, 2024
Accepted Date: February 10, 2024
Published Date: March 05, 2024

Citation: Suresh Kumar Godasu, D. Varun, N.V. Priyarekha, K. Hemakanth. Evaluation of the Antidiabetic Effects of Ethanolic Root Extract from *Polyalthia longifolia* in Diabetic Rats Induced by Streptozotocin and Nicotinamide with Consideration of Toxicological Aspects. Research & Reviews: A Journal of Toxicology. 2024; 14(1): 37–50p.

year 2025 [5]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigation [6]. Aldose reductases, a key enzyme in the polyol pathway catalyze the reduction of glucose to sorbitol. Accumulation of sorbitol in the body causes various complications including cataract, neuropathy and nephropathy [7]. The hypoglycemic effect of several plants used as antidiabetic remedies has been confirmed, and the mechanisms of hypoglycemic activity of these plants are being studied. Natural products having antidiabetic potential which act through either insulinomimetic or secretagogues properties are reviewed here. This review also focuses on the role of traditional therapeutic and natural medicines from traditional medicinal plants for diabetes.

Traditional medicines from readily available medicinal plants offer great potential for the discovery of new antidiabetic drugs [8]. More than 400 plant species having hypoglycemic activity have been available in literature; however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect [9]. *Polyalthia longifolia* cv. pendula (Annonaceae) is native to the drier regions of India and is locally known as "Ashoka" and is commonly cultivated in India, Pakistan, and Sri Lanka. *P. longifolia*, although an ornamental tree, finds its reference in Indian medicinal literature owing to its popular Hindi name Ashoka [7]. Ashoka (Latin name: *Saraca asoka* (Roxb) De Wilde) is also a Sanskrit name in Ayurveda of a drug used for the treatment of uterine disorders [8]. However, the bark of *P. longifolia* is available as one of the adulterant and used as Ashoka due to its easy availability in nature. The aim of the study is evaluation of anti-diabetic activity of ethanolic root extract of *Polialthia longifolia* against Streptozotocin and nicotinamide in diabetic rats.

MATERIALS AND METHODS

Plant Materials

Roots of *Polialthia longifolia* was collected from Chittoor District, AP, India and authenticated by Prof. Madhav Shetty, Dept. of Botany, Taxonomist, SV University, Tirupati. A voucher was kept in the Department of Pharmacognosy for reference.

Preparation of the Extract

The powdered roots of Polialthia Longifolia were individually subjected to extraction using 70% ethanol via a Soxhlet apparatus. Subsequently, the extracts were evaporated under reduced pressure. The resulting dried extract, weighing 24.8 g, was stored in a desiccator and underwent a series of chemical tests to identify various phyto-constituents such as alkaloids, tannins, cardiac glycosides, and minor amounts of flavonoids.

Preliminary Phytochemical Screening

The ethanolic extract of roots of *Polialthia longifolia* were subjected to preliminary screening for various active phytochemical constituents (Evans WC. 1989) by the following tests.

Test for Alkaloids

The extract was treated with dilute hydrochloric acid and filtered. The filtrate is used for the following tests.

Mayer's Reagent (Potassium Mercuric Solution)

0.5 ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid

Dragendroff's Test (Potassium Bismuth Iodide)

0.5 ml of the extract was treated with Dragendroff's reagent and the appearance of reddish brown color precipitate indicates the presence of alkaloid.

Wagner's Test (Iodine-Potassium Iodide Solution)

0.5 ml of the extract was treated with Wagner's test and the appearance of brown color precipitate indicates the presence of alkaloid.

Hager's Test (Saturated Solution of Picric Acid)

0.5 ml of the extract was treated with Hager's test and the appearance of yellow color precipitate indicates the presence of alkaloid.

Test for Tannin's

1. The extract was treated with 10% lead acetate solution. The appearance of white precipitate indicates the presence of tannins.
2. The extract was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

TEST FOR FLAVONOID'S

Alkaline Reagent Test

The extract was added few drops of NaOH solution. Intense of yellow color was formed which turns to colorless on addition of few drops of dilute acid, indicated the presence of flavonoids.

Zinc Hydrochloride Test

The extract solution added mixture of zinc dust and concentrated HCL it gives red color after few minute indicates the presence of flavonoids.

Shinoda's Test

The extracts were dissolved in alcohol, to that one piece of magnesium is added followed by concentrated hydrochloric acid along the sides of the test tube drop wise. It is heated in a boiling water bath for few minutes. The appearance of magenta color indicates the presence of flavonoids.

Test for Cardiac Glycosides

Crude extract (2–5 ml) was mixed with 2 ml of glacial acid containing 1–2 drops of 2% solution of ferric chloride; the mixture was then poured into another test tube containing 2 ml of concentrated sulfuric acid. A brown ring of a deoxy-sugar, characteristic of alcoholic cardenolides at the interface indicated the presence of cardiac glycosides.

PHARMACOLOGICAL SCREENING

Experimental Animals and Ethical Consideration

Male Wistar albino rats, 9–12 weeks old with average weight of 150–180 g were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum.* The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each experiment, the animals were fasted for at least 18 h. The experimental protocols were approved by Institutional Animal Ethics Committee and were in accordance with IAEC.

Acute Toxicity Studies

The animals were divided into six separate groups and orally administered with ethanolic extracts derived from the roots of Polialthia Longifolia at varying doses of 50, 300, 500, 1000, and 2000 mg/kg body weight. Observations on the animals were made continuously for one hour after treatment, followed by frequent monitoring over the course of 14 days. During the initial 4-hour period post-administration, the animals were continuously observed, followed by intermittent monitoring for the subsequent six hours, and then again at 24 and 48 hours following the administration of the extract. Parameters such as grooming behavior, hyperactivity, sedation, loss of the righting reflex, respiratory rate, and occurrence of convulsions were assessed throughout the observation period.

Estimation of Blood Glucose Levels

Blood was collected from tip of the tail vein and fasting blood glucose levels were measured using a commercial glucometer and glucose-oxidase strips (One touch glucometer).

Streptozotocin-Nicotinamide Induced Diabetic Rats

This approach involves administering nicotinamide concurrently to partially shield β-cells from the damaging effects of streptozotocin (STZ). It is derived from the research conducted by individuals who extensively studied the initial evidence showing that nicotinamide provided protection against the diabetes-inducing effects of STZ. By following this protocol, a model of type 2 diabetes (T2D) is generated, which lacks sufficient insulin but does not exhibit resistance to it. This model is marked by stable and moderate elevation in blood sugar levels, accompanied by a 60% decline in β-cell function.

Requirements and Chemicals

Wistar albino rats: 150 to 200 g, 8 to 10 weeks old; Nicotinamide (Sigma) 0.9% (w/v) Sodium chloride 50 mM Sodium citrate buffer (enzyme grade; Fisher), pH 4.5: prepared immediately before use Streptozotocin (STZ; Sigma) 1-ml syringes 23- and 25-G needles One Touch Basic blood glucose monitoring system (Lifescan).

Prepare Animals

At least 5 days prior to the start of the experiment, house two to five male rats per cage at 24°C ± 1°C and 55% ± 5% humidity, with a 12-hour light-dark cycle (light on at 8:00 and off at 20:00). Allow the rats to have free access to food and water. Males are generally preferred for these studies, as female rats are less sensitive to STZ. While the protocol is designed to minimize variability, it is recommended that group sizes number 6 animals each. This allows for the morbidity and variance generally associated with these studies. Usually >80% of STZ-injected rats develop diabetes under this protocol).

1. Weigh all rats accurately to 1 g, and randomly divide them into control and experimental groups. (The number of rats should be the same in each group).
2. On experimental day 1, fast all rats for 6 to 8 h (from 7:00 to 13:00–15:00) prior to STZ treatment. Provide water as normal.

Induction of Diabetes with STZ and Nicotinamide

Firstly, dissolve nicotinamide in a 0.9% sodium chloride solution to achieve a concentration of 230 mg/ml. Next, weigh out 32.5 mg of streptozotocin (STZ) and place it in a 1.5 ml microcentrifuge tube, covering the tube with aluminum foil; prepare one tube for each rat. Prepare the citrate buffer solution. Administer nicotinamide intraperitoneally (i.p.) at a dosage of 230 mg/kg (1.0 ml/kg) using a 1-ml syringe and a 23-G needle. It's crucial to administer the nicotinamide injection 15 minutes prior to the intravenous (i.v.) administration of streptozotocin. Just before injection, dissolve the STZ in a 50 mM sodium citrate buffer (pH 4.5, as indicated in step 5) to achieve a final concentration of 32.5 mg/ml. Ensure that the STZ solution is freshly prepared for each injection and administered within 5 minutes of dissolution.

Utilizing a 1-ml syringe and a 25-G needle, inject the streptozotocin (STZ) solution intraperitoneally (i.p.) at a dosage of 65 mg/kg (2.0 ml/kg) for the experimental group, as described by Donovan and Brown (2006). Control animals receive an injection of an equivalent volume of citrate buffer (pH 4.5) only, then return the rats to their cages. Ensure access to normal food and drinking water for all animals. On the 10th day of the experiment, around 8:00 a.m., measure the blood glucose level from a tail vein blood sample using a One Touch Basic blood glucose monitoring system. Following STZ administration, animals are given free access to food and water, with a 5% glucose solution provided in the subsequent 48 hours. Blood samples are collected from the tail to determine glucose levels, confirming the onset of diabetes.

The rats were divided into five groups as follows: Group I served as normal control which received vehicle, Group II served as diabetic control, Group III and IV served as tests group, received 200 and

400 mg/kg b.w. of ethanolic extract of *Polialthia longifolia,* respectively whereas Group V served as standard which received Glibenclamide (5 mg/kg b.w.). The treatment was continued daily for 21 days and the blood was collected from the tail for blood glucose estimation.

- *Group I* - Normal control animals treated with 1% CMC.
- *Group II* - Diabetic control animals treated with Streptozotocin (65 mg/kg, i.p.).
- *Group III* - Test animals treated with Streptozotocin (65 mg/kg, i.p.) and treated with low dose of EEPL 200 mg/kg b.w./ p.o.
- *Group IV* - Test animals treated with Streptozotocin (65 mg/kg, i.p.) and treated with low dose of EEPL 400 mg/kg b.w./ p.o.
- *Group V* - Standard animals treated with Streptozotocin (65 mg/kg, i.p.) and treated with Glibenclamide 0.5 mg/kg b.w./p.o.

The above mentioned treatment schedule was followed for the respective group of animals for 21 days. Blood samples were collected from tail vein in animals on 0, 3, 7, 14 and 21st day to estimate blood glucose levels using a commercial glucometer and glucose-oxidase strips. (One touch glucometer).

BIOCHEMICAL STUDIES

At the end of the study, the blood samples were collected by bleeding of retero-orbital plexus using micro capillary technique from all the groups of rats and serum was separated to study biochemical parameters such as liver parameters (AST, ALP, ALT), renal function tests (serum creatinine and blood urea) and lipid profile (Total cholesterol, Triglycerides, HDL, LDL, VLDL).

BIOCHEMICAL PARAMETERS

Estimation of Serum Triglycerides

Principle

Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphates which is oxidized by the enzyme glycerol phosphate oxidase to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the color formed is directly proportional to the amount of triglycerides present in the sample. Triglycerides kit consists of LI (triglyceride reagent) and S (triglyceride standard) 200 mg/dl.

Procedure

0.01 ml of sample was mixed with 1.0 ml of working reagent and incubated at 35ºC for 5 min. measured the absorbance of the standard and test sample at 505 nm at a light path of 1 cm against the blank, within 60 min.

Triglycerides in mg/dl=Abs. T/Abs.S X 200

Estimation of Serum Total Cholesterol

Principle

Cholesterol esterase hydrolysis esterified cholesterol to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the color formed is directly proportional to the amount of cholesterol present in the sample.

Cholesterol kit has LI reagent (cholesterol reagent) and Standard Cholesterol (200 mg/dl).

Procedure

0.01 ml sample was mixed with 1.0 ml of cholesterol reagent and incubated at 35ºC for 5 min measured the absorbance of the standard and test at 505 nm at a light path of 1 cm against the blank, within 60 min.

Calculation

Serum total cholesterol (mg/dl)=absorbance of test × conc. of standard. absorbance of standard

Estimation of Serum High-Density Lipoprotein Cholesterol

Principle

The principle behind the HDL (high density lipoprotein) estimation lies in precipitating the serum VLDL and LDL (Low-Density Lipoprotein). When the serum is reacted with the polyethylene glycol contained in the precipitating reagent, all the VLDL and LDL are precipitated. The HDL remains in the supernatant and is then assayed as a sample of cholesterol using the cholesterol reagent. HDL kit has LI, L2 (enzyme reagent), L3 (precipitating reagent) and HDL cholesterol standard (25 mg/dl).

Working reagent: L1 (4 parts): L2 (1 part)

Procedure

Precipitation of VLDL and LDL

0.1 ml of sample was mixed with 0.1 ml of precipitating reagent. Incubated at room temperature for 5 min. centrifuged at 2500–3000 rpm to obtain a clear supernatant.

HDL Assay

0.05 ml of sample was mixed with 1.0 ml of working reagent and incubate at 35ºC for 5 min. Measure the absorbance of the standard and test sample at 505 nm at a light path of 1 cm against the blank, within 60 min.

HDL Cholesterol in mg/dl=Abs.T/Abs. S X 25 X2

Where, 2 is the dilution factor due to the deproteinization step

Calculation of LDL cholesterol (mg/dl) = total cholesterol – (Triglycerides/5)-HDL cholesterol

Estimation of Serum Low-Density Lipoprotein Cholesterol (LDL-C)

Using the data obtained including total cholesterol, HDL cholesterol and VDL, the LDL cholesterol levels were calculated using the empirical equation of Friede Wald.

Calculation

Serum LDL cholesterol = total cholesterol-(Triglyceride-HDL cholesterol)

Estimation of Serum Glutamate Oxaloacetate Transaminase-SGOT Principle

Between L-Aspartate and SGOT catalyzes the transfer of amino group between L-Aspartate and alpha Ketoglutarate to form oxaloacetate and glutamate. The oxaloacetate formed reacts with NADH in the presence of malate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a SGOT kit contains L1 (enzyme reagent) and L2 (Starter reagent).

Procedure

Substrate Start Assay

0.1 ml of sample was mixed with 0.8 ml of enzyme reagent and incubated at 35ºC for 1 min and added 0.2 ml of starter reagent. Mixed well and read the initial absorbance change per minute (ΔA/min).

Sample Start Assay

1.0 ml of working reagent is incubated at 35ºC for 1 min and added 0.1 ml of starter reagent. Mixed well and read the initial absorbance A_0 and repeat the absorbance reading after every 1, 2 and 3 min. calculate the mean absorbance changer per minute (ΔA/min).

SGOT (ASAT) activity in U/L =ΔA/min X 1546

Estimation of Serum SGPT (Serum Glutamic Pyruvic Transaminase)

Principle

SGPT catalyzes the transfer of amino group between L-Alanine and α Ketoglutarate to form pyruvate and glutamate. The pyruvate formed reacts with NADH in the presence of lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT activity in the sample.

SGPT kit contains L1 (Enzyme reagent) L2 (Starter reagent)

Procedure

Substrate Start Assay

0.1 ml of sample was mixed with 0.8 ml of enzyme reagent and incubated at 35ºC for 1 min and added 0.2 ml of starter reagent. Mixed well and read the initial absorbance A_0 and repeat the absorbance reading after every 1, 2 and 3 min. Calculate the mean absorbance change per minute (ΔA/min).

Sample Start assay

1.0 ml of working reagent is incubated at 35ºC for 1 min and adds 0.1 ml of starter reagent. Mix well and read the initial absorbance A_0 and repeat the absorbance reading after every 1, 2, and 3 min. Calculate the mean absorbance change per minute(ΔA/min).

SGPT (ALAT) activity in U/L = ΔA/min X 1546

Estimation of Creatinine (Modified Jaffe's Kinetic method)

Serum creatinine levels were estimated by using test kit.

Estimation of Urea

Serum urea levels were estimated by using test kit.

THE HISTOLOGICAL STUDY

After blood sampling for the biochemical analysis, the animals were sacrificed, quickly dissected and small slices of pancreas were taken and fixed in 10% formalin. The specimens were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 6 µm in thickness were prepared and stained with Haematoxylin and Eosin then examined under microscopy.

Statistical Analysis

Data were analyzed using Statistical analysis system (SAS) and presented as means ± SEM. Comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' test. The level of significance was set at $P < 0.05$.

RESULTS

Pharmacognostical Study

Qualitative Determination

The qualitative determinations of the ethanolic extract of root of *C* were shown in Table 1. In qualitative determination, the phytoconstituents analysis was performed and which confirms the presence of alkaloids, glycosides, flavonoids and tannins.

Acute Toxicity Studies

This study helps us to determine the therapeutic index and the extract was confirmed to be safe. Acute toxicity study was performed as per the OECD guidelines. The extracts were administered orally at a doses of 50, 300, 500, 1000 and 2000 mg/kg b.w. in 0.5% carboxy methyl cellulose (CMC). No gross observational changes were recorded during the period of 14 days observation.

Pharmacological Studies

Antidiabetic Activity

The blood glucose level increased significantly in STZ and nicotinamide treated group when compared to control group. The STZ and nicotinamide induced rats were treated with the ethanolic root

extract of *Polialthia longifolia* 200 mg/kg/p.o and 400 mg/kg/p.o for the duration of 21 days. Treatment with ethanolic root extract of *Polialthia longifolia* at the dose of 200 mg/kg/p.o shows marginal reduction in the blood glucose level at second week. Treatment with ethanolic root extract of *Polialthia longifolia* at the dose of 400 mg/kg/p.o. showed significant decrease in the blood glucose level at first week ($p<0.01$), which further reduced in the second, third and fourth weeks ($p<0.001$), respectively. Treatment with glibenclamide (0.5 mg/kg b.w./p.o) produced a significant ($p<0.001$) decrease in blood glucose level from first week to fourth week as seen in Tables 2 and 3.

Estimation of Liver Enzymes

The effect of ethanolic root extract of *Polialthia longifolia* at doses of 200 and 400 mg/kg b.w. on liver enzymes. It was found that the ethanolic root extract of *Polialthia longifolia* showed significant ($p<0.001$) protection on liver parameters. The ethanolic root extract of *Polialthia longifolia* has not elevated the normal liver profiles (ALP, AST, and ALT) on dose dependent manner. The standard drug glibenclamide (0.5 mg/kg/p.o) showed significant ($p<0.001$) protection in ALP,AST,ALT when compared STZ-nicotinamide induced diabetic animals as seen in Table 4, Figure 1.

Table 1. Phytoconstituents analysis of ethanolic root extract of *Polialthia longifolia.*

S.N.	Plant constituents	*Polialthia longifolia*
1	Test for alkaloids	+
2	Test for glycosides	+
3	Test for carbohydrates	-
4	Test for Proteins and amino acids	-
5	Test for steroids	+
6	Test for flavonoids	+
7	Test for saponins	-
8	Test for tannins	+
9	Test for terpenoids	-
10	Test for fixed oils and fats	-

'-' Absent,
'+' Present

Table 2. The effects of ethanolic root extract of *Polialthia longifolia* on general behavioral observation in acute toxicity studies.

S.N.	General Behavior	Observation after drug administration
1	Sedation	+
2	Hypnosis	-
3	Convulsion	-
4	Ptosis	-
5	Analgesia	-
6	Stupar reaction	-
7	Motor activity	-
8	Muscle relaxant	-
9	CNS stimulant	+
10	CNS depressant	-
11	Pilo erection	-
12	Skin color	-
13	Lacrimation	-
14	Stool consistency	-

'-' Absent,
'+' Present

Table 3. The effect of ethanolic root extract of *Polialthia longifolia* on blood glucose levels in STZ-nicotinamide induced diabetic rats.

Groups Treatment/ Dose	0 day (mg/dL)	After 3days (mg/dL)	After 7 days (mg/dL)	After 14 days (mg/dL)	After 21days (mg/dL)
Normal control	96.32±1.12	95.71±0.96***	96.08±1.35***	96.54±1.24***	98.92±0.97***
Diabetic control	264.18± 2.64	281.06±1.98	271.12±2.54	219.37±2.36	209.14±1.97
EEPL (200 mg/kg b.w.)	263.16± 2.91	247.74±2.23**	208.17±3.06**	176.51±3.27**	130.36±2.97**
EEPL (400 mg/kg b.w.)	261.49± 3.82	221.12±3.54**	160.75±2.59***	130.50±2.89***	110.69±3.06***
Standard |Glibencla mide (5 mg/kg b.w.)	256.13± 3.29	200.12±3.07***	152.43±2.99***	118.74±2.86***	98.16±3.04***

*Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001Vs control. Statistical significance test for comparison was done by one way ANOVA followed by Dunnett's 't' test*

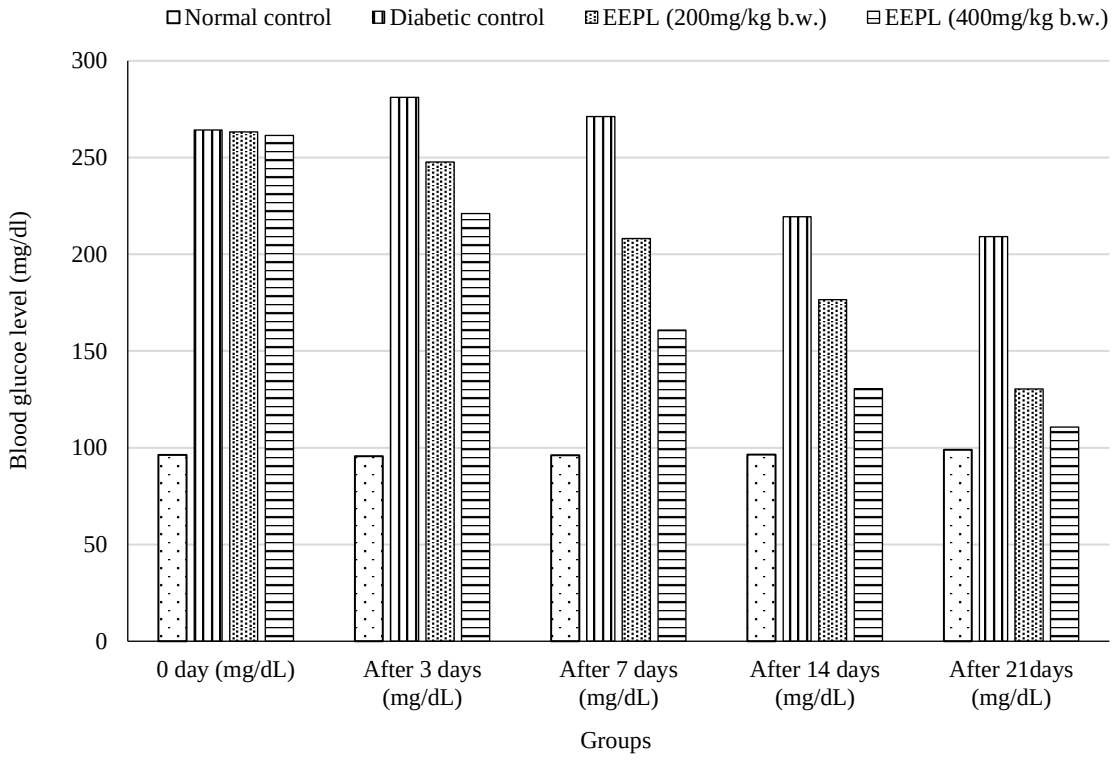


Figure 1. Effect of ethanolic root extract of *Polialthia longifolia* on blood glucose level in STZ and nicotinamide induced diabetic rats.

Table 4. Effect of Ethanolic root extract of *Polialthia longifolia* on liver profile on STZ-nicotinamide induced diabetic rats.

Groups Treatment/Dose	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Normal control	89.35±7.12***	83.75±7.36***	25.52±1.65***
Diabetic control	168.96±9.02	161.92±9.07	128.37±7.92
EEPL (200 mg/kg b.w.)	121.66±8.12*	87.31±7.10**	44.91±2.36*
EEPL (400 mg/kg b.w.)	100.99±7.96**	89.18±6.87***	34.68±2.72**
Standard |Glibenclamide (5 mg/kg b.w.)	85.94±6.06***	89.85±6.84***	28.96±1.82***

*Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001Vs control. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.*

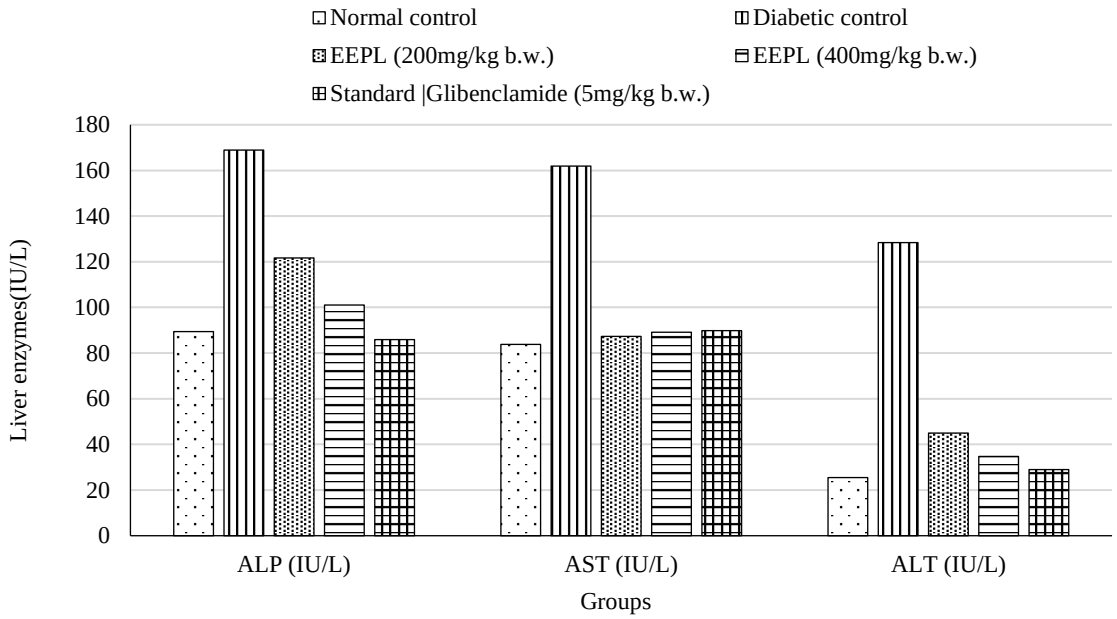


Figure 2. Effect of ethanolic root extract of *Polialthia longifolia* on liver profile in STZ-nicotinamide induced diabetic rats.

Table 5. Effect of ethanolic root extract of *Polialthia longifolia* on renal profile in STZ-nicotinamide in diabetes induced rat.

Groups Treatment/Dose	Serum Creatinine (mg/dl)	Blood Urea (mg/dl)
Normal control	1.18±0.08***	25.73±2.26***
Diabetic control	2.58±0.16	41.14±2.61
EEPL (200 mg/kg b.w.)	1.42±0.12**	30.61±2.84*
EEPL (400 mg/kg b.w.)	1.32±0.09***	27.86±2.51**
Standard |Glibenclamide (5 mg/kg b.w.)	1.27±0.10***	23.94±2.08***

*Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001Vs control. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.*

Estimation of Renal Profile

The serum urea level was significantly ($p<0.001$) increased in STZ-nicotinamide induced diabetic rats when compared to control rats. Serum urea level of diabetic rat treated with EEPL 200 mg/kg/p.o and 400 mg/kg/p.o showed significant decrease ($p<0.001$) and ($p<0.001$) in serum urea level when compared to STZ-nicotinamide induced diabetic rat. Glibenclamide (0.5 mg/kg b.w./p.o) treatment showed significant ($p<0.001$) decrease in serum urea when compared to STZ-nicotinamide induced diabetic animals as seen in Table 5, Figure 2.

Estimation of Lipid Profile

The serum total cholesterol, triglyceride, LDL, VLDL level was significantly increased whereas HDL was significantly decreased in STZ-nicotinamide induced diabetic rat when compared to control rats. Serum total cholesterol, triglyceride, LDL, VLDL level of diabetic animal treated with EEPL 200 mg/kg/p.o and 400 mg/kg/p.o showed significant decrease ($p<0.001$) and HDL level of diabetic animal treated with EEPL showed significant increase ($p<0.01$), when compared to STZ-nicotinamide induced diabetic animals. Glibenclamide (0.5 mg/kg/p.o) also showed a significant decrease ($p<0.001$) in serum total cholesterol, triglyceride, LDL,VLDL level and HDL was significantly increased when compared to STZ-nicotinamide induced diabetic rat as seen in Table 6, Figure 3.

HISTOPATHOLOGY OBSERVATION

Examination of pancreatic tissue of diabetic rats treated with *Polialthia longifolia* indicated that pancreatic section appeared more (or) less like control as seen in Figure 4.

Group I: Normal Control

Microscopy: Multiple section studied shows cells of pancreas with normal architecture and proportion. The acinar cells which stained strongly are arranged in lobules. The islet cells are embedded within acinar cells surrounded by thin fibrous capsule. No evidence of any inflammatory changes or malignancy.

Impression: Normal Histology-Pancreas biopsy.

Group II: Diabetic Control

Microscopy: Multiple section studied shows pancreatic tissue with hyalinization of islets of langerhans cells with focal mild degenerative changes. Interstitium showing mild fibrosis, dilated, thick walled and congested blood vessels along with focal chronic inflammatory cell infiltrate.

Impression: Features shows strong pancreatic inflammation-Pancreas biopsy

Group III: Diabetes Group with Glibenclamide

Microscopy: Multiple section studied shows cells of pancreas with marked atrophy of pancreatic isle cells and minimal degenerative changes in acinar population. The within shows admixed eosinophilic material, pericapsular fibrosis, congested blood vessels and scattered mononuclear inflammatory cell infiltration surrounded by thin fibrous capsule. No evidence of any inflammatory changes or malignancy.

Impression: Features are that of insulinitis-pancreas biopsy.

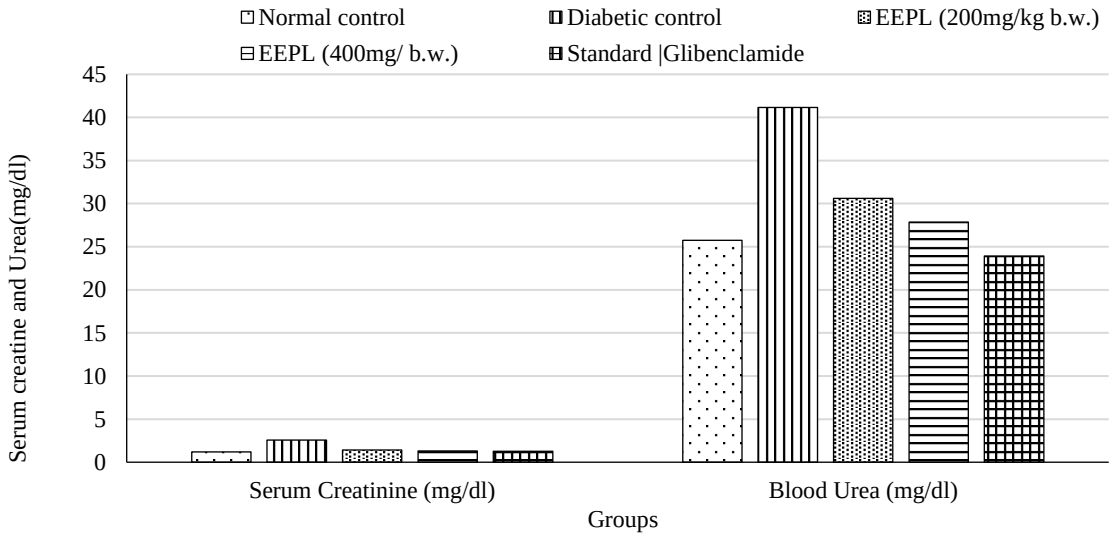


Figure 3. Effect of ethanolic root extract of *Polialthia longifolia* on renal profile in STZ-nicotinamide induced diabetic rats.

Table 6. Effect of ethanolic root extract of *Polialthia longifolia* on lipid profile in STZ-nicotinamide induced diabetic rats.

Groups Treatment/ Dose	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal control	66.68±1.95***	34.32±1.72***	13.75±0.82***	59.62±3.16***	6.85±0.22***
Diabetic control	77.61±1.56	44.11±2.16	12.21±0.95	74.25±2.94	9.49±0.30
EEPL (200 mg/kg b.w.)	71.74±1.47**	36.33±1.94**	15.16±0.82*	64.35±3.32**	7.88±0.28*
EEPL (400 mg/kg b.w.)	68.18±1.78***	35.53±2.21***	14.08±0.91***	63.35±2.68***	7.09±0.32**
Standard |Glibenclamide (5 mg/kg b.w.)	67.41±3.23***	35.64±2.62***	14.30±0.76***	63.25±2.94***	7.50±0.28***

*Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001Vs control. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.*

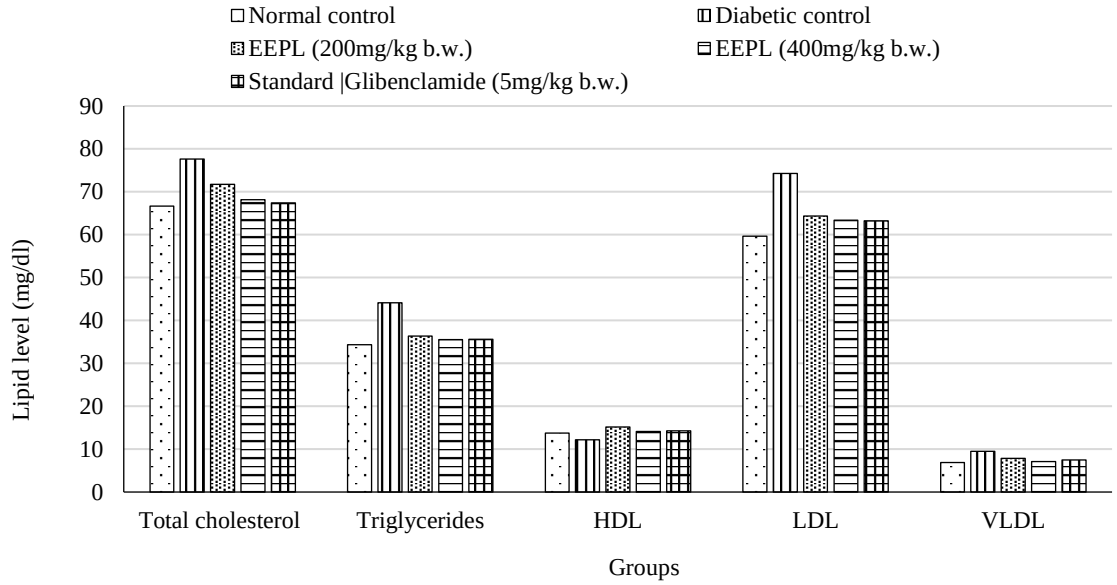


Figure 4. Effect of ethanolic root extract of *Polialthia longifolia* on serum total cholesterol, triglycerides, LDL, HDL, VLDL in STZ-nicotinamide induced diabetic rats.

Group IV: Diabetes Group with 200 mg/kg EEPL

Microscopy: Multiple section studied shows cells of pancreas with mild atrophy of pancreatic isle cells and minimal degenerative changes in acinar population. The acinar cells show dark staining and are arranged in lobules. The islet cells are embedded within acinar cells which show minimal pericapsular fibrosis, congested blood vessels and scattered mononuclear inflammatory cell infiltration surrounded by thin fibrous capsule. No evidence of any inflammatory changes or malignancy.

Impression: Features show mild decrease in pancreatic inflammation-pancreas biopsy.

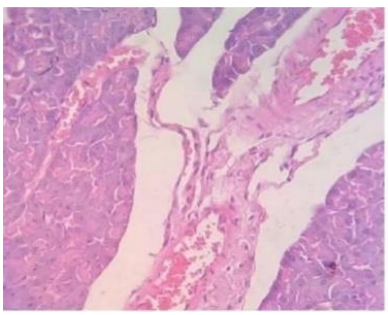
Group V: Diabetes Group with 400 mg/kg EEPL

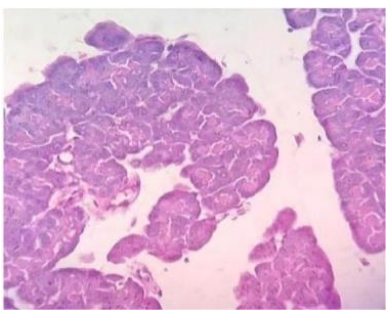
Microscopy: Multiple section studied shows cells of pancreas with mild atrophy of pancreatic isle cells and normal acinar population. The acinar cells show dark staining and are arranged in lobules. The islet cells are embedded within acinar cells which show minimal pericapsular fibrosis, edema, congested blood vessels and very few scattered mononuclear inflammatory cell infiltrations surrounded by thin fibrous capsule. No evidence of any inflammatory changes or malignancy as seen in Figure 5.

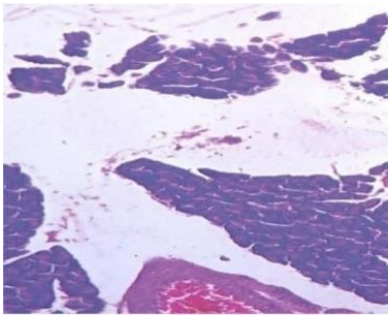
Impression: Features show marked decrease in pancreatic inflammation-pancreas biopsy.

DISCUSSION

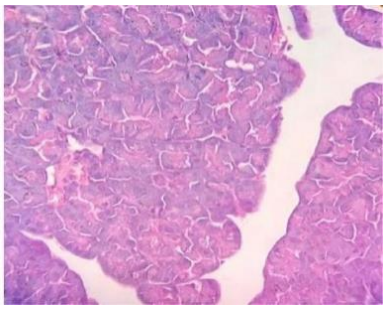
Medicinal plants due to their natural origin play a major role in treatment of diabetes, especially who have limited resources. Report of ethnobotany revealed that about 800 medicinal plants have


Normal control

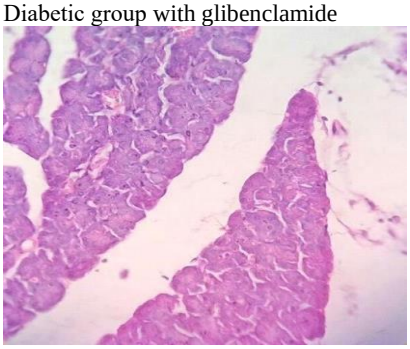

Diabetic control



Diabetic group with glibenclamide



Diabetic group with 200 mg EEPL



Diabetic group with 400 mg EEPL

Figure 5. Marked decrease in pancreatic inflammation-pancreas biopsy.

antidiabetic activity(I-4) and their respective phytochemical constituents have alkaloids, glycosides, terpenoids, flavonoids etc. are very effective in both preclinical and clinical studies (I-5,6). The present investigation showed that the ethanolic extract of *Polialthia longifolia* is nontoxic by oral route in Wistar albino rats, up to a maximum of 2000 mg/kg b.w. acutely.

Literature review and phytochemical evaluation showed the presence of flavonoids, alkaloids, glycosides, sterols. It is possible that the antidiabetic property of ethanolic extract of *Polialthia longifolia* could be mediated by the synergistic effect of these phytochemicals. The STZ induced diabetic model is one of the best model in which Streptozotocin is an alkylating agent which causes DNA damage which results in the activation of poly(ADP-ribose) synthetase that leads to the depletion of NAD and ATP virtually causes beta cell necrosis in the experimental rats. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyper glycemic state. STZ is the most commonly used chemical for the induction of experimental diabetes for both IDDM and NIDDM.

The present study was conducted to evaluate the anti-diabetic potential of *Polialthia longifolia* in normal as well as STZ-nicotinamide induced diabetic rat. The study indicated that the ethanolic extract of *Polialthia longifolia* possess blood glucose lowering property in STZ–nicotinamide induced diabetic rats. Oral administration of ethanolic extract of *Polialthia longifolia* protected against STZ–nicotinamide induced diabetes mellitus. Histopathological studies on isolated pancreas revealed that ethanolic extract of *Polialthia longifolia* reversed the changes which produced due to diabetes. The normal pattern of histology of pancreas was observed.

The normal level blood urea and serum creatinine indicates that the ethanolic extract of *Polialthia longifolia* did not interfere with the renal function and renal integrity was preserved and also there were no significant abnormalities. The study also revealed that there were no significant changes in liver function tests. The ethanolic extract of *Polialthia longifolia* does not affect the normal values of ALP, AST, ALT and it confirms that the extract doesn't have any hepatotoxic nature. The study also reveals that the ethanolic extract of *Polialthia longifolia* does not affect the lipid profile such as triglycerides, total cholesterol, HDL, LDL, and VLDL.

SUMMARY AND CONCLUSION

Several factors underlie the growing popularity of herbal treatments for a variety of chronic conditions. Interestingly, people who utilize alternative therapies are not necessarily uniform. Many people using herbal medicines find the health care alternatives are more congruent with their own values, beliefs and philosophical orientation towards health and life.

The root of *Polialthia longifolia* was selected for the evaluation of antidiabetic potential in STZ-nicotinamide induced diabetic rat model. The root extract was extracted by hot percolation using ethanol as a solvent in Soxhlet apparatus and the preliminary phytochemical screening was performed and it shows that the ethanolic root extract of *Polialthia longifolia* contains alkaloids, glycosides, flavonoids and tannins.

Safety profile is essential for the drugs obtained from the plant origin. The level of toxicity can be evaluated by toxicological studies. From the results it was concluded that the ethanolic root extract of *Polialthia longifolia* showed significant antidiabetic activity in a dose dependent manner compared to the standard drug glibenclamide.

The result also showed significant decrease in the liver Alkaline phosphatase (ALP), Aspartate amino transferase (AST), Alanine amino transferase (ALT), serum urea level, cholesterol, triglycerides, VLDL and LDL was in STZ-nicotinamide induced diabetic animals when compared to control group. It is concluded that ethanolic root extract of *Polialthia longifolia* showed significant effect in STZ-nicotinamide induced diabetic rats. Further studies are necessary to examine the underlying mechanism of hypoglycemic effect and to isolate the active compound (s) responsible for antidiabetic activities. Histopathological studies on isolated pancreas revealed that ethanolic extract of *Polialthia longifolia* reversed the changes which are produced due to diabetes. The normal pattern of histology of pancreas was observed.

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