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ASSESSMENT OF ANTIDIABETIC ACTIVITY OF *POLYALTHIA LONGIFOLIA* SEED EXTRACTS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS WITH HPTLC CHARACTERIZATION OF BIOACTIVE COMPOUNDS

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ABSTRACT
Diabetes mellitus is a metabolic disorder of chronic nature with hyperglycemia and complications. The present study Assessed of Antidiabetic Activity of *Polyalthia longifolia* Seed extracts in Streptozotocin-Induced Diabetic Rats with HPTLC Characterization of Bioactive Compounds. Acute toxicity studies validated the safety of *Polyalthia longifolia* (PL) extract at 2000 mg/kg. Doses of 50, 100, and 200 mg/kg were utilized for further assessment. PL treatment markedly increased body weight, decreased fasting blood glucose and HbA1c levels, and showed dose-dependent hypoglycemic efficacy similar to Metformin. A biochemical study indicated lipid profile and renal function marker normalization, particularly with the higher doses. Histopathological study presented considerable hepatic and pancreatic tissue restoration, indicating Hepatoprotective and β-cell regenerative activities of the extract. The 200 mg/kg dose revealed the most significant activity on all the parameters. These results validate the folk use of *Polyalthia longifolia* as a possible drug for diabetes and justify further research into its molecular actions and safety over the long term.

KEYWORDS: Antidiabetic, *Polyalthia longifolia,* HPTLC. Streptozotocin-Induced Diabetic Rats, bioactive compounds etc.

1. INTRODUCTION

Diabetes Mellitus is a metabolic chronic disorder that is associated with hyperglycemia resulting from insulin deficiency or impaired utilization [1]. Diabetes Mellitus is a global public health concern because of its increasing prevalence and association with severe long-term complications [2]. Among the most common manifestations of diabetes are: frequent urination, extreme hunger, thirst, weakness, weight reduction, delayed healing of wounds, blurred vision and infections [3, 4].

Complications of diabetes may be cardiovascular diseases, neuropathy, kidney damage, damage to the retina, and infection and ulcers in the foot [5]. Diabetes Mellitus (DM) is categorized into three categories: Type 1 DM, Type 2 DM, and Gestational Diabetes. Type 1 leads to hyperglycemia, altered metabolism of nutrients, and late complications [6]. Type 2 results in insulin resistance and, if left untreated, can be life-threatening [7]. Gestational diabetes occurs in females when they are pregnant, resulting in elevated glucose levels and a high risk for complications during delivery [8].

India, the "Diabetes Capital of the World," is confronted by an emerging diabetes burden owing to factors such as urbanization, lifestyle, diet, and susceptibility [9]. Streptozotocin (STZ) is an experimental diabetes chemical inducer in animal models selectively toxic to pancreatic beta cells by virtue of structural analogy with glucose. Traditional treatments include insulin therapy, oral hypoglycemic medication, lifestyle modification, and management of blood glucose levels [10]. The World Health Organization suggests the use of herbal medicine, which possesses hypoglycemic activity in over 400 plant genera, especially in areas with limited access to Western medical care.

Polyalthia longifolia also known as the mast tree or false Ashoka, is an evergreen tree native to the Indian subcontinent. It belongs to the Annonaceae family and is renowned for its ornamental value, particularly in urban

landscaping and avenue planting. The tree's tall, slender, columnar form, resembling a mast, with minimal branching, makes it a popular choice for parks, gardens, and natural hedges. Its glossy, lance-shaped leaves hang downward, adding to its aesthetic appeal. The genus *Polyalthia* comprises around 120 species, including fourteen in India [11]. *Polyalthia longifolia* is valuable not just as a decorative plant but also in traditional medicine, especially Ayurveda. Because of its therapeutic qualities, the tree's bark, leaves, and seeds have all been used to cure conditions including bacterial infections, fever, and inflammation [12]. Experiments have established the anti-inflammatory [13], antioxidant [13], antimicrobial [14], and anticancer [15] properties of the tree. Phytochemical screening of its flowers and seeds has been discovered to contain biologically active constituents, e.g., alkaloids, flavonoids, tannin, and terpenoids responsible for its medicinal activity [11]. Lack of enough literature on the role of *Polyalthia longifolia* seeds in diabetes, we in our present study have tested different parameters to find out the antidiabetic activity of this component. In this article, we studied the antihyperglycemic activity of the methanol extract of *Polyalthia longifolia* seeds in Streptozotocin-induced diabetic rats.

2. MATERIAL AND METHODS

2.1 Plant Collection and Authentication

Polyalthia longifolia seeds were obtained from the local area of Dhule, India. The plant was recognized by botanist Dr. S. R. Kshirsagar (Department of Botany) of S.S.V.P.S. Science College Dhule, India. Plant material was air-dried at room temperature until it was moisture-free after identification. Lastly, seeds were exposed to size reduction to obtain coarse powder.

2.2 Chemical and Diagnostic kits

Streptozotocin, Nicotinamide, Methanol (Loba Chem. Pvt. Ltd.), Metformin hydrochloride (Infinity Pharma), Glucometer (Gluco One), Cholesterol kit (Erba Mannheim), HDL kit (Erba Mannheim), and Triglyceride kit (Erba Mannheim) were used in this study.

2.3 Preparation of the extract

The air-dried seed powder of *Polyalthia longifolia* (500 gm) was packed separately in the Soxhlet apparatus and extracted using methanol (2 liters) until the extraction process was complete. Vacuum distillation at reduced pressure was done to distill the extract so that the solvent was completely removed, and the methanolic extract of seeds was stored in an airtight container to be used for further study.

2.4 HPTLC finger printing

The HPTLC analysis was conducted using silica gel 60 F254 TLC aluminium plates (Merck) with dimensions 200 mm × 100 mm as the stationary phase. The sample, a methanolic extract of *Polyalthia longifolia* seeds at a concentration of 100 ppm, was applied using the ATS 4 applicator system. A volume of 10 µl was spotted on the plate at a position of Y = 8.0 mm, forming bands of 8 mm in length. The tracks were applied at an X position of 20.0 mm with a distance of 15.4 mm between tracks. Sample solvent used was methanol. The mobile phase was a solvent system of Toluene: Ethyl acetate: Methanol: Formic acid in the proportion 5:3:1:1. Chamber saturation was done for 20 minutes with a saturation pad. Plate development was done in a 20 × 10 cm twin trough chamber, permitting the front to travel up to 70 mm. After development, the plate was dried in the air for 5 minutes at room temperature. The documentation was done with the TLC Visualizer under white light and UV light at 254 nm wavelengths. Densitometric scanning was conducted with a TLC Scanner 4 in absorbance mode with a 254 nm wavelength, slit dimension of 6 × 0.45 mm, and scan speed of 20 mm/s. A high SCAN of the spectrum was also conducted in the 200–450 nm range to record the fingerprint profile of the extract. Diagnostic tests confirmed the system and ensured its functionality during analysis. [16, 17].

2.5 Experimental Animals

Female Sprague Dawley rats weighing 150-250 gm were used for the present work. The animals used for the experiment were maintained under standard laboratory conditions in an animal house of DCS's A. R. A. College of Pharmacy, Dhule, India approved by the committee for control and supervision on experiments on animals (Ref. No: ARACOP/IAEC/24/10) under 12 h dark/light cycle and controlled temperature 24±20°C. They had free access to food and water ad libitum. The animals were acclimatized to the laboratory for 7 days, before the commencement of the experiment.

2.6 Acute Oral Toxicity Study

Acute oral toxicity test was performed as per the OECD guideline 423 Acute Oral Toxicity-Acute Oral Toxic Class Method [18] and [19]. Testing was performed on 3 animals per extract in each step. Healthy adult Female Sprague Dawley rats having weights of 180–250 g were obtained and housed in cages at ambient temperature (22 ± 3 °C) with a 12 h light/dark cycle. The animals were randomly chosen, tagged, and retained in their cages for 5 days before dosing for acclimatization to laboratory conditions. The animals were overnight fasted but water was given ad libitum and administered a single oral dose (2000 mg/kg, b.w., p.o.) of Methanolic extract of *Polyalthia longifolia* seeds. Food was withheld after administration of the extracts for an additional 3–4 hours. The animals

were observed singly at least once within the first 30 minutes post-dose, at irregular intervals during the first 24 hours (special attention is given during the initial 4 hours) and then daily for up to 14 days.

2.7 Induction of Diabetes

Non-insulin dependent diabetes mellitus (NIDDM) was induced [20] & [21] in overnight fasted adult Female Sprague Dawley rats weighing 180-250 g by a single intraperitoneal injection, at the dose of 65 mg/kg, b.w., i.p. Streptozotocin, 15 minutes after i.p. administration of 230 mg/kg of nicotinamide. Streptozotocin (STZ) was dissolved in a citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hours and then on day 7, after injection. The fasting plasma value of glucose to diagnose diabetes was taken as > 126 mg/dl. The rats which were found these values to have permanent NIDDM were used for the study.

2.8 Experimental Design

Female Sprague Dawley rats (weighing 180 - 250 g) were used for the study. Rats used for the study were obtained from DCS's A. R. A. College of Pharmacy, Dhule, India. All experimental procedures and protocols used in the study were reviewed by the "Institutional Animal Ethical Committee" (IAEC) and CCSEA (Committee for Control and Supervision of Experiments on Animals) rules. Animals were allowed to free access to water and standard chow diet up to the end of the experimental period and divided into following groups. Each group consists of 6 rats,

Group I: Normal/ Vehicle group of rats received distilled water.
Group II: Diabetic control/ STZ group of rats, diabetes was induced by STZ at a dose of 65 mg/kg was administered intraperitoneally.
Group III: Standard/ Metformin group of rats received Metformin at a dose of 100mg/kg through oral route along with STZ (65mg/kg, i.p.).
Group IV: Test group of rats were received Methanolic extract of *Polyalthia longifolia* seeds at a dose of 50 mg/kg orally along with STZ (65mg/kg, i.p.).
Group V: Test group of rats were received Methanolic extract of *Polyalthia longifolia* seeds at a dose of 100 mg/kg orally along with STZ (65mg/kg, i.p.).
Group VI: Test group of rats were received Methanolic extract of *Polyalthia longifolia* seeds at a dose of 200 mg/kg orally along with STZ (65mg/kg, i.p.).

2.9 Effect of Methanolic seeds on Body weight, Fasting blood glucose and HbA1c

The study involved estimating body weight and blood glucose levels in rats during Weeks 1, 2, 3, and 4. The initial weight was compared to normal rats, and blood glucose levels were calculated using a Glucometer (Gluco-one). HbA1c levels were also calculated at the beginning and end of the treatment. In the fourth week, blood was collected from retro-orbital puncture and centrifuge with a centrifuge machine, and the serum for further study.

2.10 Biochemical Analysis

On 28th day of treatment, blood was taken from the retro-orbital puncture of animal and centrifuged at 4000 rpm for 10 min. to differentiate the serum. Studies were conducted to estimate the serum lipid profile of serum by various methods. Total cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL) cholesterol, Blood urea and serum creatinine were estimated colorimetrically (*Erba Mannheim*) [22].

2.11 Histopathological examination of pancreas and liver

Pancreatic and Hepatic tissue of all the groups were carried out for histopathological study. The pancreas and liver were immediately dissected on the 28th day of treatment, rinsed with cold physiological saline, and fixed with 10% formalin and immediately proceed by the paraffin technique cut sectioned, and stained with hematoxylin and eosin (H & E) to undergo histology.

2.12 Statistical analysis

The results were shown as mean ± SEM. The inter-group variation was examined using the Student's t-test between two groups and one-way analysis of variance (ANOVA), followed by Dunnett's test for more than two treatments. Statistical significance was considered at $p < 0.05$. The concentration of the material generating 50% of the maximum contraction (EC_{50}) or inhibition (IC_{50}) in each experiment was visually quantified using a concentration-response curve for the contraction response or relaxation response, respectively. Statistics were applied using GraphPad Prism 5 (GraphPad Software Inc.) version 5.00.

3. RESULTS

3.1 HPTLC Fingerprinting

HPTLC finger printing profiles of methanolic extract of *Polyalthia longifolia* seeds was recorded and depicted in Table 3.1 and Figure 3.1. Through High-Performance Thin-Layer Chromatography (HPTLC) it was easy to confirm the presence of some of the phytochemical constituents in the extract by locating peaks with classic Rf

values. From the Rf values of the extracts, I was able to identify seven different peaks from the sample, thus providing evidence some of the phytoconstituents. The Rf value peaks were 0.272, 0.406, 0.453, 0.692, 0.702, 0.839, and 0.993 which indicate a variety of polarized compounds. The main Rf values extracted, with expected kinds of chemicals from literature and expected Rf behavior in the solvent system for today was as follows:

Rf 0.272: This peak identifies the presence of low polarity flavonoids or phenolics. Polar chemical compounds like gallic acid or catechin would likely have low Rf in a polar solvent system, as the polarity of a molecule increases for motion.

Rf 0.406: This tall, fresh peak suggests that we are looking at a significant component. Possible molecules flavonoids like quercetin, or kaempferol, would often elute in the same area under the same solvent system. Another tall major peak that may be alkaloids or flavonol glycosides could belong here. Certain types of flavonoid aglycones and glycosides are able travel in the same area based on their polarity.

Rf 0.453: Another tall peak, could be typical of alkaloids or perhaps flavonol glycosides. These areas of flavonoid aglycones and glycosides will move according to their polarity.

Rf 0.692 and 0.702: These short-distance peaks will likely belong to terpenoids or steroids, which often elute at intermediate Rf values in any combined polar-nonpolar systems.

Rf 0.839: This is a higher Rf value indicating the presence of less polar substances, e.g., fatty acids, triterpenoids, or essential oil components.

Rf 0.993: This is close to the solvent front, normally pointing towards non-polar lipophilic substances, such as sterols, long-chain hydrocarbons, or fat-soluble vitamins.

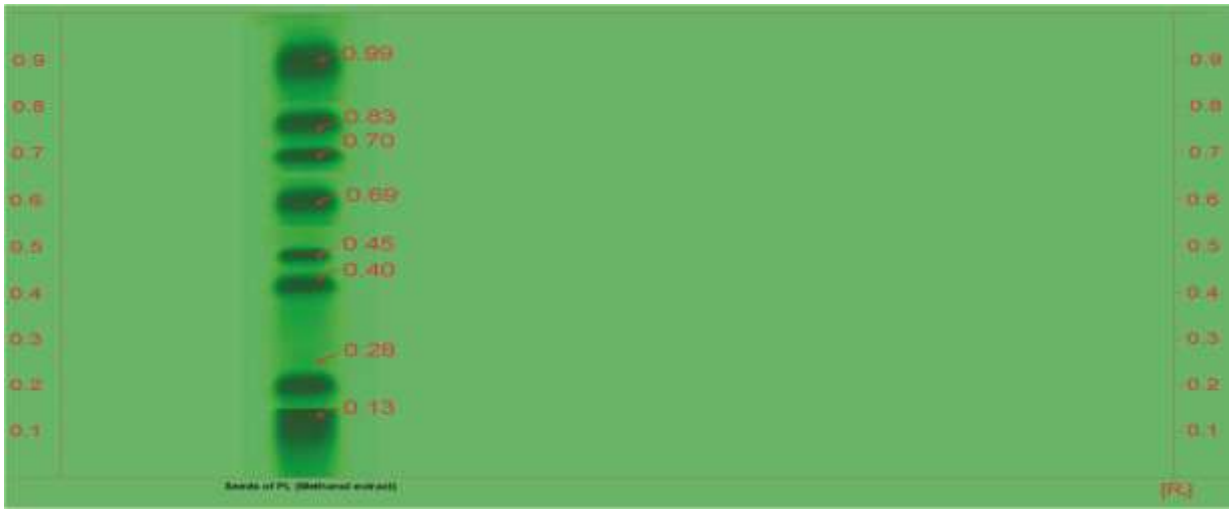


Figure 3. 1 HPTLC finger printing of methanolic extract of *Polyalthia longifolia* seeds under UV light. Solvent system used Toluene: Ethyl acetate: Methanol: Formic acid (5:3:1:1) Under UV 254 nm

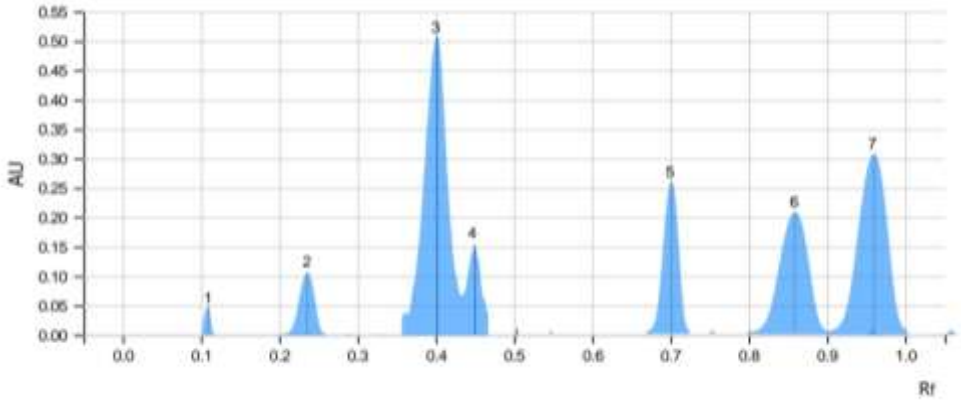


Figure 3. 2 HPTLC chromatogram of methanolic extract of *Polyalthia longifolia* seeds baseline display (scanned at 254 nm).

Table 3. 1 HPTLC finger printing profile of methanolic extract of *Polyalthia longifolia* seeds Rf value of various compounds.

Pe	Start		Max			End		Area		Manual peak	Possible compounds`
	Rf	H	Rf	H	%	Rf	H	A	%		

a k											
1	0.271	0.0032	0.272	0.0014	26.72	0.271	0.0023	0.02065	42.51	No	Possibly a flavonoids glycosides
2	0.392	0.0073	0.406	0.0528	84.31	0.416	0.0046	0.08712	89.28	No	Queracetin, Kaempferol
3	0.432	0.0165	0.453	0.2374	16.23	0.459	0.0036	0.00336	24.45	No	B-sitosterol or similar
4	0.649	0.0054	0.692	0.0034	43.87	0.698	0.0034	0.03887	54.47	No	Linalool derivatives or phenolic acids
5	0.649	0.0032	0.702	0.0254	49.22	0.729	0.0054	0.02287	44.37	No	Gallic acid or related
6	0.839	0.0049	0.839	0.0058	63.32	0.839	0.0065	0.06528	63.27	No	Possibly Solasodine or similar alkaloid
7	0.961	0.0255	0.993	0.2374	63.31	0.996	0.2337	0.08931	64.47	No	Fatty acids, Hydrocarbons

3.2 Acute Toxicity Studies

Acute Toxicity Studies on the Sprague Dawley albino rats show no mortality at a dose of 2000 mg/kg p.o., during time period of 14 days. During the study, no noticeable were seen in the rats. This help to predict that it does not contain any type of toxicity and it is full safe. So 50 mg/kg b.w. p.o., 100 mg/kg b.w. p.o. and 200 mg/kg b.w. p.o. were selected of that dose for the further study.

3.3 Effect of Methanolic Seeds extract on Body weight, Fasting blood Glucose level and HbA1c

As represented in Table 3.2, the Body weight of animals in all groups was done at 1,2,3,4 Weeks of the study. The body weight of animals was significantly ($p<0.05$) increased in all Vehicle and PL treatment groups but in the STZ and Standard group's body weight of animals was reduced. As shown in Table 3.3, fasting blood glucose was measured in animals of all groups at weeks 1, 2, 3, and 4 of trial. All treatment groups of PL showed a reduction trend of fasting blood glucose during the trial. On day 28, methanolic extract of 50, 100, and 200 mg/kg p.o. *Polyalthia longifolia* seeds fell significantly ($p<0.05$) in the control and in the Metformin 100 mg/kg p.o. Blood glucose level. Animals in all groups were subjected to HbA1c at 1st and 4th week of study, as shown in Table 3.4. Throughout the study, there was a gradual increase in HbA1c in all groups. After the trial, the level of HbA1c of the Metformin 100 mg/kg p.o. and *Polyalthia longifolia* seeds methanolic extract 50, 100, and 200 mg/kg p.o. raises significantly on day 28th.

Table 3. 2 The effect of *Polyalthia longifolia* seeds extract on Body weight

Body Weight (gm)	Week1	Week2	Week3	Week4
Vehicle group	148.33 ± 2.54	148.33 ± 2.54	168.33 ± 2.54	173.33 ± 2.54
Control group	240 ± 3.33 ###	243.33 ± 3.84 ###	243.33 ± 3.84 ###	235.33 ± 1.67 ###
Standard (Metformin) group [100 mg/kg] p.o.	260 ± 8.81 *	246.66 ± 7.69	253.33 ± 10.18	246.66 ± 8.38
PL Low Dose group [50 mg/kg] p.o.	130 ± 3.33 ***	150 ± 3.33 ***	151.66 ± 4.19 ***	164 ± 4.80 ***
PL Medium Dose group [100 mg/kg] p.o.	140.66 ± 2.00 ***	156.66 ± 0.96 ***	155.66 ± 3.35 ***	167.33 ± 4.23 ***
PL High Dose group [200 mg/kg] p.o.	143.33 ± 1.92 ***	163.33 ± 1.92 ***	167 ± 2.02 ***	177.33 ± 2.14 ***

Each Value represents Mean ± SEM, n = 6, # $p< 0.0001$ as compared to vehicle group, * $p< 0.0001$ as compared with STZ Control group.

Table 3. 3 Effect of *Polyalthia longifolia* seeds extract on fasting blood glucose level

Glucose level (mg/dl)	Week1	Week2	Week3	Week4
Vehicle group	96 ± 2.13	117 ± 2.44	129 ± 60	202 ± 13.87

Control group	127.33 ± 10.40 #	123.33 ± 4.22	142.33 ± 9.83	182 ± 12.22
Standard (Metformin) group [100 mg/kg] p.o.	125.66 ± 6.16	99.66 ± 1.34 ***	128.5 ± 5.91	165.66 ±17.21
PL Low Dose group [50 mg/kg] p.o.	114 ± 2.96	116 ± 2.403	161.33 ± 8.26	158.33 ±12.09
PL Medium Dose group [100 mg/kg] p.o.	142 ± 15.67	123.33 ± 6.37	183 ± 7.31	169.33 ± 8.55
PL High Dose group [200 mg/kg] p.o.	133 ± 0.69	115.33 ± 2.91	231 ± 20.00 ***	159.33 ± 7.67

Each Value represents Mean ± SEM, n = 6, # p< 0.001 as compared to vehicle group, * p< 0.001 as compared with STZ Control group.

Table 3. 4 Effect of *Polyalthia longifolia* seeds on HbA1c level

HbA1c level	**week 1**	**week 4**
Vehicle group	4.97 ± 0.026	8.65 ± 0.483
Control group	6.05 ± 0.362 #	7.96 ± 0.425
Standard (Metformin) group [100 mg/kg] p.o.	5.45 ± 0.214	7.39 ± 0.600
PL Low Dose group [50 mg/kg] p.o.	5.59 ± 0.102	7.14 ± 0.421
PL Medium Dose group [100 mg/kg] p.o.	6.57 ± 0.545	7.52 ± 0.297
PL High Dose group [200 mg/kg] p.o.	6.28 ± 0.024	7.17 ± 0.267

Each Value represents Mean ± SEM, n = 6, # p< 0.001 as compared to vehicle group, * p< 0.001 as compared with STZ Control group.

3.4 Biochemical Analysis

The level of Triglycerides, Total Cholesterol, Creatinine and HDL, Blood Urea Nitrogen (BUN) level decreased in untreated animals when compared to normal rats. Treatment with *Polyalthia longifolia* seeds (PLS) extract and Metformin mark reversal of changes in the serum lipid parameters as compared to diabetic rats Shown in Table 3.5.

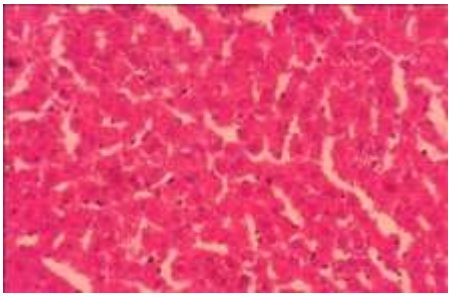
Table 3.5 changes in the serum lipid parameters as compared to diabetic rats

Sr. No.	**Groups**	**Total Cholesterol (mg/dl)**	**Triglycerides (mg/dl)**	**HDL (mg/dl)**	**Serum Creatinine (mg/dl)**	**BUN (mg/dl)**
1.	Vehicle	72.96 ± 13.022	106.08 ± 4.049	25.53 ± 0.619	0.64 ± 0.22	98.01 ± 3.91
2.	Control	86.92 ± 6.643	151.20 ± 2.882 ###	22.15 ± 2.857	1.30 ± 0.24	117.11 ± 7.54 #
3.	Standard	60.98 ± 2.32 *	156.21 ± 2.417	13.733 ± 1.711 *	1.14 ± 0.197	58.02 ± 3.64 ***
4.	PL Low Dose group [50 mg/kg] p.o.	80.89 ± 8.194	97.08 ± 1.857 ***	3.58 ± 0.611 ***	0.72 ± 0.297	65.17 ± 3.39 ***
5.	PL Medium Dose group [100 mg/kg] p.o.	82.56 ± 8.155	138.14 ± 4.597 *	7.63 ± 1.977 ***	0.86 ± 0.431	85.02 ± 6.21 **
6.	PL High Dose group [200 mg/kg] p.o.	59.21 ± 3.821 *	115.37 ± 2.451 ***	12.73 ± 1.109 **	0.61 ± 0.21	92.76 ± 5.56 *

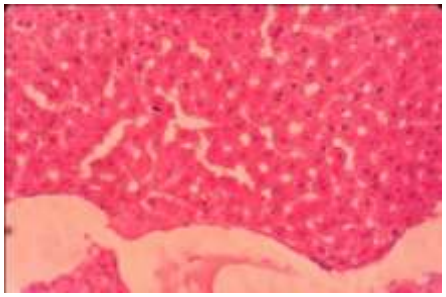
Each Value represents Mean ± SEM, n = 6, # p< 0.0001 as compared to vehicle group by unpaired T test, * p< 0.0001 as compared with STZ Control group by One Way ANOVA.

3.5 Histopathological studies of Rats Pancreas and Liver

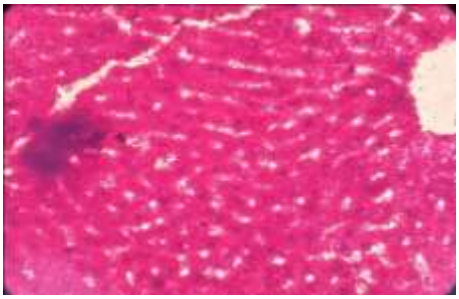
Significant difference in the pattern and number of rats hepatocytes were observed among different experimental groups, shown in Figure 3.2. STZ-induced diabetes resulted in severe hepatic damage, including necrosis, fatty changes, and inflammation, disrupting normal liver function. Standard treatment with Metformin demonstrated effective recovery, improving hepatocyte integrity and reducing fatty changes. Among the dose groups, the high dose treatment showed the best recovery, with well-arranged hepatocytes and minimal pathological changes. The medium dose treatment provided significant recovery, while the low dose treatment resulted in partial improvement but persistent vacuous areas and inflammation.



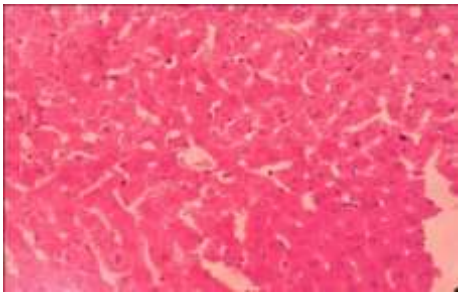
Group 1: Normal/vehicle group



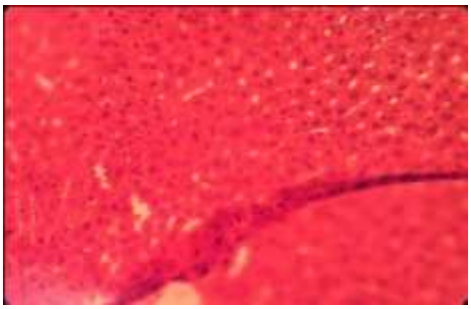
Group 2: STZ / Control group



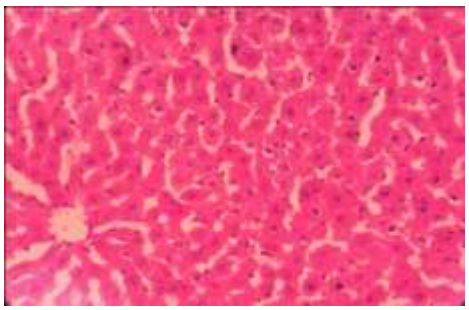
Group 3: Standard (Metformin)



Group 4: PL Low Dose group [50 mg/kg]p.o.



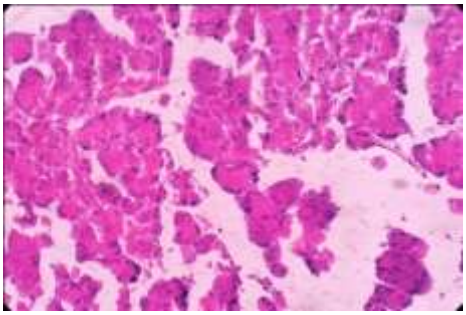
Group 5: PL Medium Dose group [100 mg/kg] p.o.



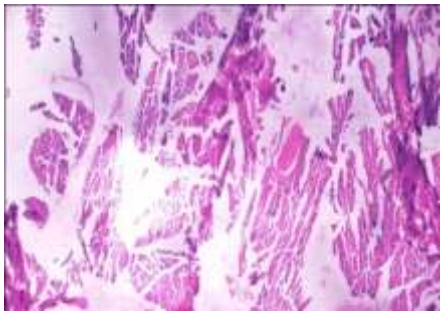
Group 6: PL High Dose group [200mg/kg] p.o.

Figure 3. 3 Histopathological examination of liver in normal and STZ induce diabetic rat

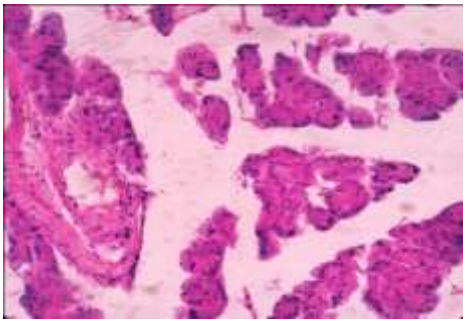
The protective effect on pancreatic islets is shown in Figure 3.3. The normal islet cells were tightly packed with distinct borders. STZ-induced diabetes resulted in severe pancreatic damage characterized by necrosis, inflammatory infiltration and congestion. Standard treatment with Metformin showed good recovery, reducing pathological changes effectively. Among the dose groups, the high dose treatment exhibited the best recovery, with well-arranged pancreatic islet cells and no evidence of necrosis or inflammation. The medium dose showed moderate recovery, while the low dose provided only partial improvement, with persistent vacuous areas and inflammation.



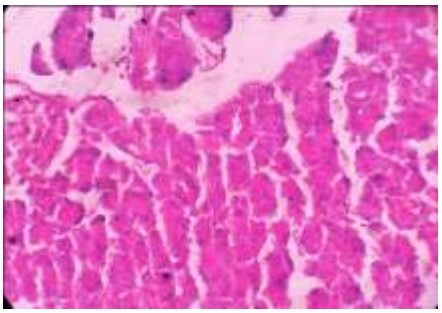
Group 1: Normal/vehicle group



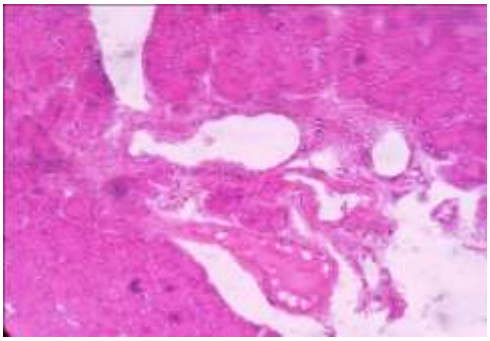
Group 4: PL Low Dose group [50 mg/kg] p.o.



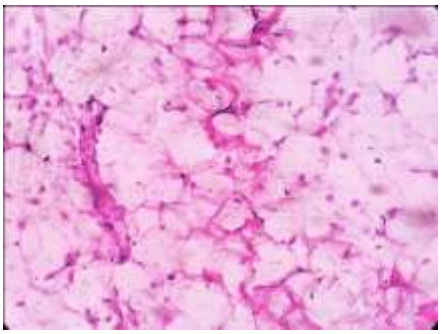
Group 2: STZ / Control group



Group 5: PL Medium Dose group [100 mg/kg] p.o.



Group 3: Standard (Metformin) group [100mg/kg]p.o.



Group 6: PL High Dose group

Figure 3. 4 Histopathological examination of pancreas in normal and STZ induce diabetic rats

3. DISCUSSION

Increased incidence of diabetes has prompted scientists to look for newer treatments against the disease. In the current study, methanolic seed extract of *Polyalthia longifolia* (PL) was employed to treat diabetic Sprague Dawley rats induced by Streptozotocin (STZ), and the acute toxicity, antidiabetic activity, biochemical profile, and histological changes upon this treatment were assessed. The findings lend weight to the literature indicating that plant-based drugs can be a safe and effective treatment for diabetes.

HPTLC fingerprinting of *Polyalthia longifolia* seeds methanolic extract revealed seven Rf peaks indicating chemical diversity, ranging from very polar to non-polar compounds, confirming the existence of various phytochemical constituents.

Most notably, prominent peaks were observed at Rf 0.406 and 0.453, which are likely to correspond to flavonoids such as quercetin and kaempferol, which are known for their antioxidant and antidiabetic effects. The low value of Rf at 0.272 indicates polar flavonoid glycosides or phenolics, which may confer bioactivity to the extract. Intermediate Rf values of 0.692 and 0.702 can be indicative of terpenoids or steroids, whereas high Rf values of 0.839 and 0.993 are likely to be non-polar compounds like Alkaloids, fatty acids, sterols, or essential oil constituents. These results establish the phytochemical wealth of *Polyalthia longifolia* seed extract and validate its traditional application towards the management of oxidative stress and metabolic diseases like diabetes. This chemical diversity is also the basis for further isolation and identification of bioactive compounds.

Acute toxicity studies revealed that *Polyalthia longifolia* seed extract was tolerated well at a dose of 2000 mg/kg body weight; no abnormality or death was observed within a period of 14 days. This result concurs with accounts

of recent phytopharmacological studies confirming the relative safety of various plant extracts employed in traditional medicine [23]. According to OECD guideline 423 for acute oral toxicity, those chemicals that show no indication of toxicity up to this point are considered practically non-toxic [19]. Thus, 50, 100, and 200 mg/kg sub-toxic doses were employed for subsequent pharmacological assessment.

The PL extract administration resulted in a significant improvement in body weight in diabetic rats, which was in contrast to weight loss in STZ-induced diabetic controls. Weight loss in diabetic animals is a recognized effect of decreased utilization of glucose and augmented muscle proteolysis [24]. Dose-dependent reversal of weight gain in PL-treated groups suggests increased metabolic activity and better glycemic control, possibly through increased insulin sensitivity or conservation of pancreatic β-cells.

Fasting blood glucose levels were markedly decreased in all PL-treated groups at the end of the four-week treatment period, reflecting the hypoglycemic activity of the extract of particular interest was the 200 mg/kg dose group, which showed glucose-lowering activity similar to that of the reference drug metformin. These results are consistent with previous research conducted by [25], which showed the effectiveness of *Polyalthia longifolia* bark extract in modulating the glycemia level of animal models.

Surprisingly, the level of HbA1c, an indicator of long-term glycemic control, increased in the PL-treated rats. Even though there was some increase in HbA1c in all groups between week 1 and week 4 (presumably due to continuing diabetic stress), this increase was much less in the PL and metformin groups than in untreated controls. This suggests that PL extract exerts a protective effect against chronic hyperglycemia, potentially by antioxidant mechanisms or inhibition of glycation processes, in accordance with the findings of [26], who found similar trends with other antidiabetic Phytochemicals.

Lipid Profile and Renal Biomarkers Diabetes is linked with dyslipidemia and renal impairment, as evidenced by increased total cholesterol, triglycerides, serum creatinine, and BUN in the control group. The PL extract showed considerable improvements in these parameters, especially at the highest dose. The lowering of triglycerides and cholesterol noted here suggests the lipid-lowering activity of the extract, which may be mediated by inhibition of lipid peroxidation or modulation of hepatic enzymes of lipid metabolism. Besides, PL therapy also corrected HDL levels that had been reduced in the diabetic control group. HDL is essential in reverse cholesterol transport and is inversely correlated with cardiovascular risk in diabetic patients [27]. The return of HDL levels by PL extract reflects a cardioprotective action, by previous findings for its antioxidant and Hepatoprotective activities [28]. Serum creatinine and BUN are important markers of renal function, which are usually increased in diabetic nephropathy as a result of glomerular hyper filtration and oxidative injury.

Normalization of these biomarkers with PL extract treatment, especially with the high-dose group, may be reflective of nephroprotective actions. This may be due to the high flavonoids and alkaloid content of the extract that is free radical scavenging and with renal tissue protective properties [11]. Histopathological examinations yielded solid morphological proof to validate the biochemical results. Liver tissues of STZ-diabetic rats exhibited extensive cellular injury, necrosis, and inflammation along with hepatic complications associated with diabetes [29]. The liver tissue in PL-treated groups, particularly at the dose rate of 200 mg/kg, was greatly recovered with well-preserved hepatocytes and minimal fatty infiltration. This Hepatoprotective effect can be attributed to the antioxidant profile of the extract that is protective against lipid peroxidation and membrane stabilization.

Diabetic control animal pancreas sections had islet degeneration, necrosis, and inflammatory cell infiltration, characteristic of STZ-induced damage. PL extract, especially at higher doses, maintained islet cell architecture and inhibited necrotic changes, suggesting potential β-cell regeneration or protection. This agrees with results by [30], which demonstrated similar pancreatic protective effect in herbal extracts with polyphenol and flavonoids content. The structural improvements in the pancreas indicate that PL extract can either stimulate endogenous insulin release or increase the survival of remaining β-cells. This step is specifically critical in type 2 diabetes, where β-cell failure occurs simultaneously with insulin resistance [31]. Overall Implications cumulative evidence from biochemical, physiological, and histological studies reveals the antidiabetic activity of *Polyalthia longifolia* seed extract on a wide spectrum. Although the extract not only alleviates hyperglycemia, it also corrects lipid disturbances, protects renal and hepatic tissue, and enhances pancreatic activity. His activity was dose-dependent, and the optimum dose was 200 mg/kg. These findings justify the traditional ethnomedicinal practice of using *Polyalthia longifolia* and give a scientific basis for further development as an adjunct therapy in diabetes, but in-depth studies with elucidation of molecular mechanisms, bioavailability profiling and safety evaluation in the long term are needed to facilitate clinical translation.

5. CONCLUSION

The study tested the antidiabetic activity and safety of *Polyalthia longifolia* seed's methanolic extract in STZ-induced diabetic rats. Results showed no toxic signs or mortality, indicating the potential for further investigation at doses of 50, 100, and 200 mg/kg. The extract lowered fasting blood glucose, increased HbA1c, regained weight, and markedly improved diabetes indicators.

The previously mentioned results have been confirmed by biochemical analysis, which revealed enhanced renal function tests (raised blood creatinine) and lipid profiles (decreased triglycerides, cholesterol, and BUN), especially at medium and high dosages. Histopathological examination of pancreatic and liver tissues further confirmed the protective effect of the extract, with the highest tissue regeneration and normalization being recorded in the high-dose treatment group. In general, the methanolic seed extract of *Polyalthia longifolia* had potential antidiabetic and tissue-protective activity, particularly at the 200 mg/kg dose, similar to the standard

drug Metformin. Such findings indicate that *Polyalthia longifolia* seed extract could be a lead compound for the design of safe and effective plant therapeutics for the treatment of diabetes.

Conflict of Interest
Authors declared no any conflict of interest.

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