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# EVALUATION OF ANTIDIABETIC ACTIVITY OF *POLYALTHIA LONGIFOLIA* SEEDS EXTRACT ON ALLOXAN INDUCED DIABETIC RAT

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## ABSTRACT

In this research, the Pharmacognostic, Phytochemical, toxicological, and pharmacological potential of seeds from Polyalthia longifolia has been scientifically investigated with special reference to their antidiabetic activity. The aim of present study was to evaluate the potency of antdiabetic activity of seed extract. The P. longifolia seedextract was administered orally in Alloxan induced diabetic rats. However treatment with Polyalthia longifolia seed extract showed substantial improvement in body weight, especially in the medium and high dose groups. Such an improvement indicates a muscle and tissue-protective effect in diabetic conditions, as in the case of the standard treatment with metformin. At four weeks, rats that were given Polyalthia longifolia seed extract had a significant reduction in their fasting blood glucose level. The effect was dose-dependent and the maximum dose (200 mg/kg) had the largest hypoglycemic effect and was comparable to metformin. The lowering of the HbA1c level also established the long-term antidiabetic efficacy of the extract. Decreased HbA1c levels reflect improved glycemic control, lowering the risk of diabetic complications.

**KEYWORDS:** Antidiabetic, Polyalthia longifolia, Alloxan, Hypoglycemic and Metformin.

## INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by the loss of glucose with impaired carbohydrate, lipid, and protein metabolism due to defects in either insulin production or action.[1] It accomplishes therefore by damaging organs such as the heart, blood vessels, eyes, kidneys, and nerves over time. Its incidence is increasing in developing countries among younger populations.[2] Diabetes Mellitus (DM) is described by what is said to be insufficient pancreatic production of insulin causing variations in blood glucose concentration that result in vessel and nerve damage.[3] Diabetes Mellitus entailed cardiovascular morbidity and mortality.[4,5]

Diabetes has become a pandemic, with increasing cases and a decrease in treatment methods. This issue is particularly concerning for children, as hyperglycemia, dyslipidemia, and other factors contribute to micro and macro vascular complications, which are the primary cause of morbidity and death. Plant-based herbal drugs and botanicals with free radical scavenging activity are emerging as primary components of holistic approaches to diabetes management. Symptoms of high blood sugar include increased appetite, thirst, and frequency of urine production. Diabetes can result in several consequences if treatment is not received.[6] Various therapies are available, such as hypoglycemic drugs, insulin, and recently cellular therapy, but these therapies have their own limitations. Hence, alternative strategies and formulations are required to encounter this problem once the solution resides in the traditional medicines using medicinal plants that are effective against various diseases and disorders.[7]

### Worldwide Scenario of diabetes[11, 12]

In 2024, about 537 million people had been dwelling with diabetes, and this wide variety is predicted to increase up to 783 million through 2045. Growing international locations are specifically tormented by this problem, which has best been exacerbated through the

years. Type 2 diabetes bills for extra than 90% of all diabetes cases. Among its foremost complications are cardiovascular diseases which can result in kidney failure, vision loss, and decrease limb amputation.[8] To prevent ability disaster, early prognosis, and effective prevention strategies are crucial. It is anticipated that by 2023, the direct healthcare expenses related to diabetes will reach $966 billion globally. destiny treatment approaches aim to enhance disease management andclinical outcomes by using utilizing personalized medicinal drug, growing virtual health technologies and enhancing the overall shape of healthcare systems.[9]

**Indian Scenario of diabetes**

India is known as the "diabetes capital of the world." The huge urbanization, sedentary lifestyles, and food habits are contributing to difficulties faced in managing the condition, and the approaches to diabetes management in India. More recently, a few studies have documented the prevalence.[10]

**Prevalence**

The high and rapidly rising numbers of those with diabetes are now in India, which according to studies constituted about 77 million in 2020 and is projected to reach 134 million by 2045. In rural and tribal areas, initially low-risk regions, there are reports of additional diabetes cases due to lifestyle and diet changes.[11]

**Contributing Factors**

Lifestyle changes - Urbanization led to the practice of less physical activity and unhealthy diets.

Obesity - Central obesity is rapidly becoming more prevalent in both urban and rural populations and is a high-risk factor.

Socioeconomic Disparities - The degree of awareness of the disease and limited access to health care services contributes to the problem in the lower-income groups.

Genetic Pre-disposition - Compared with their BMI, South Asians have greater propensities of insulin resistance.[13, 14]

**Complications-** The high rate of uncontrolled diabetes is witnessed in complications like cardiovascular disease, diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy. It complicates the case in rural areas due to limited healthcare infrastructure.[15]

**Government Guidelines** - The National Programmed for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases, and Stroke (NPCDCS) was created in order to prevent and manage diabetes through active screening and awareness campaigns.[16] A large number of states have subsidized insulin schemes and managed diabetes management camps.

Polyalthia longifolia or Indian Mast Tree, is a commonly cultivated evergreen tree in India, Sri Lanka, and Southeast Asia. Its beautiful wood, medicinal properties, and ecological benefits make it desirable for avenues and urban spaces. The tree's seeds are small, oval, and brown and contain bioactive compounds like alkaloids, flavonoids, tannins, Saponin, glycosides, and essential oils, which yield therapeutic values.[17] The seeds contain a smooth external body and a hard seed coat, which serve the purposes of protection and dispersal. Secondary metabolites have been found in the studies to include alkaloids, flavonoids, glycosides, terpenoids, and phenolic compounds that may be therapeutic agents. The seed essential oils are known to possess antioxidant, antimicrobial, and antifungal activities, and they are important in traditional medicine and current pharmaceutical studies.[18] Polyalthia longifolia seeds are a promising source of botanical and pharmacological research. The present study was aimed to evaluate the antidiabetic activity of *Polyalthia longifolia* seeds extract on alloxan induced diabetic rats.

**Figure 1: Polyalthia longifolia Seeds.**

## MATERIAL AND METHODS

**Drugs and Chemicals**

Alloxan, Metformin, Methanol, Glucose, Carboxy Methyl Cellulose, Toluene, Ethyl acetate, Formic acid.

**Collection, identification and authentication of plant material:** The fresh seeds of *Polyalthia longifolia* were collected from the field of Varanasi district in the month of their natural habitat. All seeds were cleaned of extraneous matter and ground into a fine powder. Plant and plant material was identifiedby comparing morphological features of plant. The herbarium of collected plant material was prepared & the taxonomical

identification (authentication) of the plant is done by Botanical Survey of India, Pune, Maharashtra, India.

The collected seeds were dried in shade to avoid loss of phytoconstituents. After drying, seeds were coarsely powdered with grinding mill and kept in air tight container for use in the study.

**Solubility determination:** Solubility of powder material were done by using different solvent such as Water, Ethyl alcohol, Petroleum ether, Chloroform, and Methanol. In solvent methanol powder material show good solubility than other solvent.

**Extraction**

From the result of solubility determination of powder seed material suggest that methanol is suitable solvent for extracting vital constituent required for study.

Continuous hot extraction technique was used for extraction of Polyalthia longifolia seeds powder with the help of soxhlet apparatus. Coarsely grind plant material in a paper thimble and place it in a Soxhlet apparatus. The solvent is put into the thimble and collected into the Round Bottom Flask (RBF). Extractions are carried out at elevated temperatures until the bead mark of the siphon tube is attained. The solvent vapors condense and raise the level of the solvent until all coloring material is removed. The solutions obtained are combined and the plant material is extracted using methanol. The dried extracts are weighed and placed in screw-capped vials and labeled for quantification.

**Figure 2: Extraction of Polyalthia Longifolia Seed.**

**Figure 3: Drying of Extract.**

**Pharmacognostic Evaluation**

In this evaluation we have to evaluated physicochemical parameters, ash value, moisture content and extractive value determination as per the procedure given in official compendia.

**Preliminary Phytochemical screening**

The Polyalthia longifolia seeds extract was screened for the possible presence of secondary metabolites including alkaloids, tannins, flavonoids, terpenoids, and Saponin, phenols using qualitative Phytochemical screening procedures are in K. R. Khanelwal.

**UV-visible spectroscopy**

UV-visible spectroscopy was used to examine the Polyalthia longifolia seed extract at a concentration of 1 mg/mL in methanol as the solvent. A Shimadzu UV spectrophotometer (Japan) was used for the analysis. By measuring the absorbance of the various compounds in the extract at different wavelengths, ranging from 200 to 400 nm, this approach enables both identification and quantification of the individual compounds present. The presence of flavonoids, alkaloids, phenolic compounds, and aromatic compounds all of which are recognized for their potential medical advantages, including the ability to lower blood glucose level can be inferred from the UV-visible spectrum.

**Thin Layer Chromatography (TLC)**

Thin layer chromatography (TLC) which utilizes silica gel and alumina as a stationary phase and a mobile phase, is a widely used method for the identification of phytoconstituents. Preparative commercial TLC plates are prepared using standard particle size ranges, which are formed by mixing water, an adsorbent such as silica gel, and an inert binder such as calcium sulfate.

**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 UVlight 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.

**Experimental Animals**

Male Sprague Dawley rats weighing 150 ± 250 g were grown in the animal house of the SHEAT College of Pharmacy, Varanasi, UP, India. Each animal is kept in a typical polypropylene cage with a mesh-covered top husk, bedding, and a temperature of 23 ± 2 °C and an approximate humidity of 60 ± 5 %, with a 12-hour light-dark cycle. A commercially available conventional rat pellet diet was fed to the animals. The animals had unlimited access to water. Before the testing, the animals were allowed to become used to the lab setting. All research was carried out in compliance with the guidelines set forth by the Government of India's 'Committee for the Purpose of Control and Supervision on Experiments on Animals (CCSEA), and prior approvals were obtained from the SHEAT College of Pharmacy, Varanasi.

**Acute Oral Toxicity of Polyalthia longifolia seeds extract**

The oral toxicity was performed using (OECD Guideline 423). The test group of rats received a single oral gavages dosage of the methanolic extract from Polyalthia longifolia seeds, which is equivalent to 2000 mg/kg body weight. Before the experiment started, the rats were given free access to water, fasted for the whole night, and given unrestricted access to water. Rats' eyes, hair, color, food and water intake, urine, sedation, diarrhea, and death were all noted within the first four hours of their lives and then daily for the next fourteen days.

**Induction of type 2 diabetes with Alloxan**

Hepatic glucose-6 phosphatase activity increases, insulin levels decrease, and blood free fatty acid levels rise in rats that are fasting and receive a single intraperitoneal injection of Alloxan (65 mg/kg). Low-dose Alloxan (25 mg/kg) administered in combination with NPH insulin treatment results in a transient glycosuria; islet cell degeneration and degranulation have not been shown to occur concurrently. In a series of experiments, adult animals were given a single intraperitoneal dosage of 65 mg/kg Alloxan.

One intraperitoneal injection of newly made Alloxan at a dosage of 65 mg/kg body weight in 0.1M citrate buffer (pH 4.5) was administered to a group of rats that had fasted overnight in order to cause hyperglycemia. Rats were kept overnight in a 5% glucose solution to prevent drug-induced hypoglycemia. Using a Glucometer to measure glucose on the third day after the alloxaninjection, hyperglycemia was confirmed**.**

**Experimental Design**

Based on their fasting blood glucose levels, the diabetic rats were randomized into five groups of six rats each at 14 days following their Alloxan injection. Rats in Normal Group 1 were not treated with Alloxan. Group 2 was used to manage for diabetes. While groups 4, 5, and 6 received oral administration of methanolic extract at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg, respectively, group 3 received 100 mg/kg of metformin as a Standard. The rats were divided into several treatment groups based on table 6.3, and throughout the course of the next 28 days, they received various therapies.

By means of gastric intubation, the active fraction or Metformin were orally administered to the respective groups daily for 28 days. Both diabetic and normal control rats were treated with water for four weeks and the rest groups of animal administered PLS extract of varying Concentration dissolve in CMC (10 mg/ml) solution for 28 days body weights and Fasting Blood Glucose (FBG) of all experimental rats were recorded on a weekly interval. After the overnight fast, six groups of rats were killed on the 28 day by cervical dislocation and ether anaesthesia. The blood and tissues were subsequently removed and stored at $0^0$C until further analysis.

**Histopathological studies of rat pancreas and liver**

Rats were sacrificed using cervical dislocation and autopsy performed for the removal of liver and pancreas of rats. The tissue samples were cut and preserved in 10 per cent solution of formalin for at least One hour. The tissue samples had formalin taken out of them using running water. Acetone dehydrated fixed tissue by giving three changes (each 100 ml). Tissue cleaning from acetone was preceded by three washings of xylene (500 ml each) within a time span of three hours. The processed tissue was incubated in molten paraffin using two changes for 3-4 hours inside an incubator set at a temperature of 58-60°C. Tissue embedding within paraffin wax was proceeded with by immersing tissue within molten paraffin and subsequently chilling it to cause hardening of the paraffin. Parts of the paraffin embedded tissue were prepared by microtome set at 1-3 t thickness. The paraffin sections were taken carefully on glass slides. The sections were cleaned by submerging in xylene. The sections were stained with haematoxylin and eosin stain and screened to assess the morphology and cellular composition.

## RESULTS

### Pharmacognostic Evaluation

**Table 1: Pharmacognostic evaluation of Polyalthia longifolia Seeds.**

| Sr. No. | Parameters | Results |
|---|---|---|
| | **Physicochemical parameter** | |
| 1 | Colour | Dark green, light green |
| 2 | Shape | long and slender |
| 3 | Texture | Smooth and Shiny |
| 4 | Odour | Not particularly strong or distinctive. |
| 5 | Taste | Slightly bitter taste |
| | **Ash value** | |
| 1 | Total Ash | 9-10% |
| | **Moisture content** | |
| 1 | Moisture content | 1.492% |
| | **Extractive values** | |
| 1 | Alcohol soluble extractive value | 15.2 gm w/w |
| 2 | Water soluble extractive value | 13.6 gm w/w |

The percentage of Moisture content for Polyalthia longifolia seeds was found to be 1.492%. The percentage of alcohol soluble extractive and water soluble extractive were found to be 15.2 gm and 13.6 gm. The Polyalthia longifolia seeds extract are dissolve in Ethyl alcohol, Ethyl acetate, Chloroform, Petroleum ether and Methanol.

**Preliminary phytochemical screening of Polyalthia longifolia seeds extract**

The Phytochemical analysis of Polyalthia longifolia seeds showed the presence of phenolic compounds, flavonoids, alkaloids, Glycosides, carbohydrates, and tannins. Polyalthia longifolia extract contained inorganic components, organic acids, and vitamins. High concentrations of phenols, tannins, flavonoids, terpenoids, Glycosides and alkaloids were found in the methanolic extract of Polyalthia longifolia seeds.

**Table 2: Preliminary Phytochemical tests of extract.**

| Sr. No. | Test Compound | Test Name | Observation | Result |
|---|---|---|---|---|
| **1.** | Alkaloids | Wagner Test | Reddish brown precipitate | Alkaloid Present |
| | | Mayer Test | Yellow Precipitate occur | Alkaloid Present |
| **2.** | Carbohydrate | Molish Test | Violet ring is formed at junction | Carbohydrate Present |
| | | Seliwanoff's Test | Appearance of effervescence | Carboxylic acid Present |
| 3. | Carboxylic acid | Effervescence Test | Appearance of effervescence | Carboxylic acid Present |
| 4. | Flavonoids | Alkaline Reagent Test | An intense yellow color transfer into colorless due to diluted acid | Flavonoids Present |
| 5. | Phenol | Iodine Test | Transistant red color found | Phenol Present |

| | | | | |
|---|---|---|---|---|
| | | Ferric chloride Test | Dark green/Bluish black color occur | Phenol Present |
| 6. | Quinone | Sulfuric acid Test | No Red color appeared | Quinone Absent |
| 7. | Resins | Turbidity Test | Turbidity occurs | Resins Present |
| 8. | Steroids | Salkowski reaction | Red color is not formed | Steroid Absent |
| 9. | Saponin | Foam Test | Persistent foam obtain | Saponin Present |
| 10. | Tannin | Lead acetate Test | Creamy gelatin Precipitate | Tannin Present |
| 11. | Terpenoids | 0.5 ml Extract + 2 ml of chloroform and few ml conc. sulfuric acid | Ring formed | Terpenoids Present |
| 12. | Glycosides | NaOH Test | Yellow color occurs | Glycoside Present |
| | | Borntrager's Test | Pink color solution occur | Glycoside Present |

**UV visible spectroscopy**

Massive absorbance at 220-240 nm is seen in the spectra, which is indicative of the π-π* transitions that are common in aromatic compounds. The maximum absorbance detected at 226.80 nm (Fig 7.1). At 226 nm, compounds absorb UV light for a variety of reasons. Aromatic compounds like benzene, toluene, phenol, and aniline absorb in the 200-280 nm range because of π→π* transitions in the aromatic ring. Phenolic compounds like quercetin, kaempferol, and catechin absorb UV light because of aromaticity and conjugation. Conjugated alkenes and dienes absorb UV light in the 220–300 nm range. Alkaloids like simple aromatic alkaloids, aporphine alkaloids, indole alkaloids, quinoline alkaloids, and isoquinoline alkaloids can absorb UV due to aromatic rings as well as structures containing nitrogen. Plant extracts also exhibit flavonoids and glycosides' UV absorption at peaks between 220 280 nm (Band II), among them 226 nm. Strong absorption by heterocyclic aromatic compounds occurs atthe lower part of the UV region because of electron delocalization. Carbonyl compounds, such as Acetophenone, benzaldehyde will absorb around 220–230 nm.

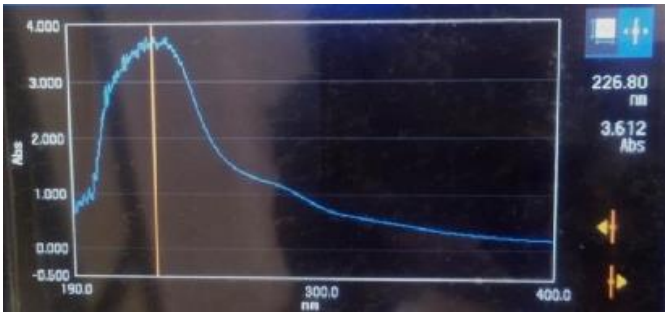



**Figure 2: UV spectral analysis at a 200 to 300 nm wavelength of Polyalthia longifolia seeds.**

**Thin Layer Chromatography of Polyalthia longifolia seeds**

Rf value of the plant extract was calculated using following formula

$$R = \frac{Distance\ travelled\ by\ the spot}{Distance\ travelled\ by\ the\ solvent}$$

**Mobile Phase:** Toluene: Ethyl acetate: Methanol: Formic acid (4:3:2:1)

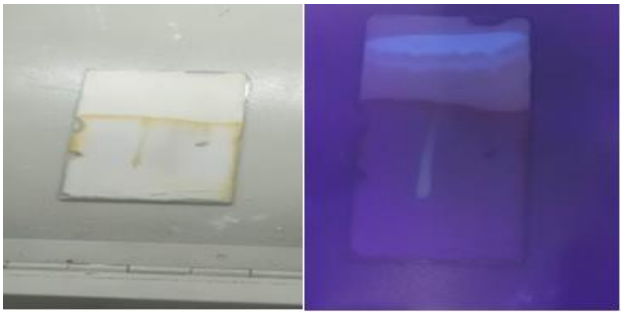


**Figure 3: Thin Layer Chromatography profile of PL seeds Extract.**

Distance Travelled by extract: 9 cm
Distance Travelled by solvent: 4 cm
Hence, Rf value of Methanolic extract of Polyalthia Longifolia seeds were found to be 0.44 respectively.

**HPTLC Fingerprinting**

HPTLC finger printing profiles of methanolic extract of Polyalthialongifolia seeds was recorded and depicted in Table 7.4 and Figure 7.3. 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.

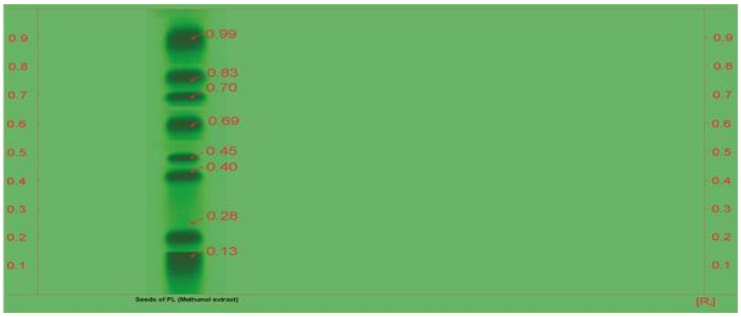



**Figure 4: HPTLC finger printing of methanolic extract of Polyalthialongifolia seeds under UV light.**

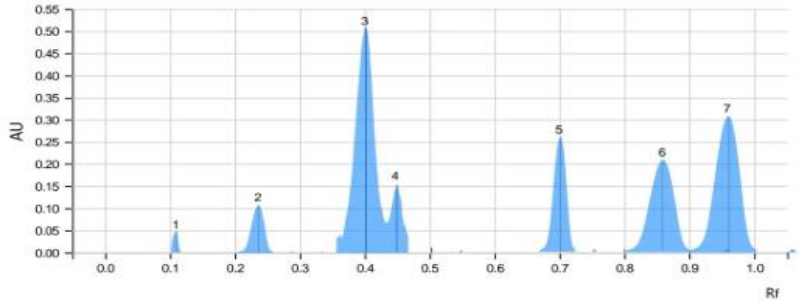



**Figure 5: HPTLC chromatogram of methanolic extract of Polyalthialongifolia seeds baseline display (scanned at 254 nm).**

**Table 3: HPTLC finger printing profile of methanolic extract of Polyalthialongifolia seeds Rf value of various compounds.**

| Peak | Start | | Max | | | End | | Area | | Manual peak | Possible compounds |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rf | H | Rf | H | % | Rf | H | A | % | | |
| 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 |

**Acute Oral Toxicity of Polyalthialongifolia seeds extract**

The dosage safety investigation was carried out in accordance with OECD guideline 423. In Sprague Dawley rats, the estimated LD50 of the extract from Polyalthialongifolia seeds was 2000 mg/kg orally. At an oral dosage of 2000 mg/kg, no mortality was seen. The entire trial ran from nine to eighteen hours.

**Body weight Changes in diabetic rats**

As represented in Table 7.5 and Figure 7.3, Body weight of animals in all groups were performed at 1,2,3,4 Week of the study. Body weight of animals was significantly ($p<0.05$) increases in all Vehicle and PL treatment group but in Alloxan and Standard group body weight of animal was decreased.

**Table 4: Effect of Polyalthialongifolia seeds on body weight in Alloxan induced diabetes in rat in week 1, 2, 3, 4.**

| Body Weight (gm) | Week1 | Week2 | Week3 | Week4 |
|---|---|---|---|---|
| Vehicle group | 148.33 ± 2.545 | 148.33 ± 2.545 | 168.33 ± 2.545 | 173.33 ± 2.545 |
| Alloxan group (Control group) | 240 ± 3.333 ### | 243.33 ± 3.849 ### | 243.33 ± 3.849 ### | 235.33 ± 1.677 ### |
| Standard (Metformin) group [100 mg/kg] p.o. | 260 ± 8.819 * | 246.66 ± 7.698 | 253.33 ± 10.183 | 246.66 ±8.388 |
| PL Low Dose group [50 mg/kg] p.o | 130 ± 3.333 *** | 150 ± 3.333 *** | 151.66 ± 4.194 *** | 164 ± 4.807 *** |
| PL Medium Dose group [100 mg/kg] p.o. | 140.66 ± 2.009 *** | 156.66 ± 0.962 *** | 155.66 ± 3.355 *** | 167.33 ± 4.233 *** |
| PL High Dose group [200 mg/kg] p.o. | 143.33 ± 1.924 *** | 163.33 ± 1.924 *** | 167 ± 2.027 *** | 177.33 ± 2.143 *** |

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

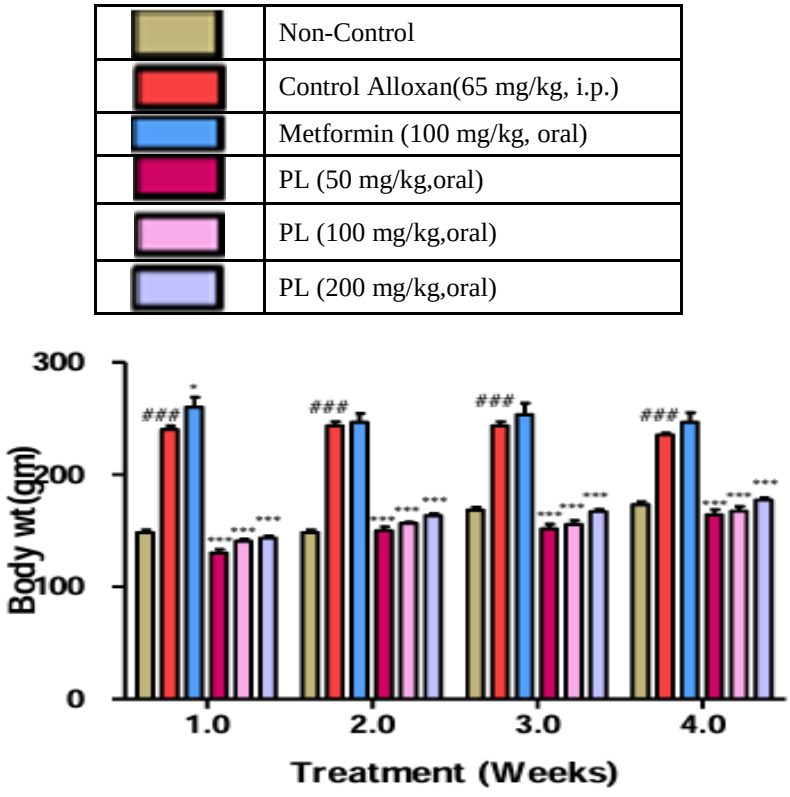



**Figure 6: Change in Body weight in Normal and Diabetic Rats.**

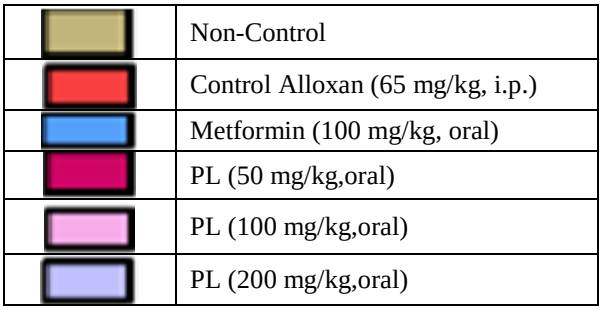
**Fasting Blood Glucose Changes in diabetic rats**

Animals in all groups were tested for fasting blood glucose at 1, 2, 3, and 4 weeks of the study, as indicated in Table 7.6 and Figure 7.4. During the study, a progressive reduction in fasting blood glucose was observed in all PL treatment groups. At the end of the trial, the blood glucose levels of the Metformin 100 mg/kg p.o. and control group is increased and Polyalthialongifolia seeds methanolic extract 50, 100, and 200 mg/kg p.o. reduced significantly ($p<0.05$) on 28th day.

**Table 5: Effect of Polyalthialongifolia seeds on blood glucose level in Streptozotocin induced diabetes in rat in week 1, 2, 3, 4.**

| Glucose level (mg/dl) | Week1 | Week2 | Week3 | Week4 |
|---|---|---|---|---|
| Vehicle group | 96 ± 2.13 | 117 ± 2.449 | 129 ± 6 | 202 ± 13.872 |
| Alloxan group (Control group) | 127.33 ± 10.404 # | 123.33 ±4.220 | 142.33 ± 9.833 | 182 ± 12.220 |
| Standard (Metformin) group [100 mg/kg] p.o. | 125.66 ± 6.167 | 99.66 ± 1.347 *** | 128.5 ± 5.919 | 165.66 ± 17.212 |
| PL Low Dose group [50 mg/kg] p.o | 114 ± 2.962 | 116 ± 2.403 | 161.33 ± 8.261 | 158.33 ± 12.098 |
| PL Medium Dose group [100 mg/kg] p.o. | 142 ± 15.677 | 123.33 ± 6.379 | 183 ± 7.310 | 169.33 ± 8.552 |
| PL High Dose group [200 mg/kg] p.o. | 133 ± 0.693 | 115.33 ± 2.912 | 231 ± 20.002 *** | 159.33 ± 7.676 |

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



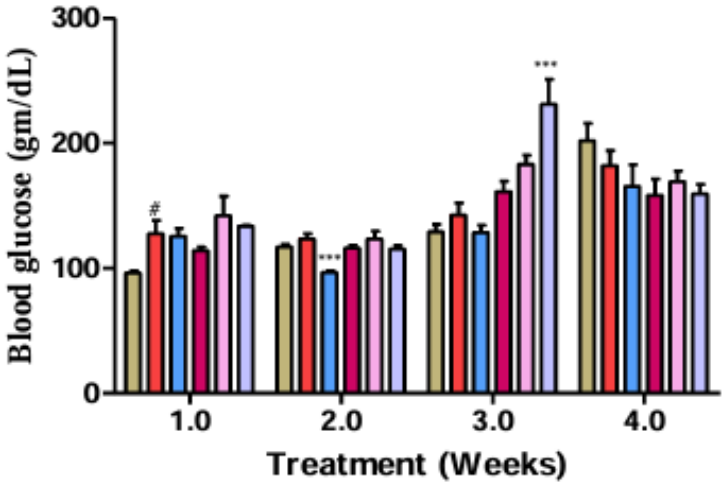




**Figure 7: Change in Fasting Blood Glucose level in normal and diabetic rats.**

**HbA1c in diabetic rats**

Animals in all groups were tested for HbA1c at 1st and 4th weeks of the study, as indicated in Table 7.7 and Figure 7.5. During the study, a progressive augmentation in HbA1c was observed in all groups. At the end of the trial, the HbA1c level of the Metformin 100 mg/kg p.o. and Polyalthialongifolia seeds methanolic extract 50, 100, and 200 mg/kg p.o. increase significantly on 28th day.

**Table 6: Effect of Polyalthialongifolia seeds on HbA1c in Alloxan induced diabetes in rat in week 1 and 4.**

| HbA1c level | Week1 | Week4 |
|---|---|---|
| Vehicle group | 4.97 ± 0.026 | 8.65 ± 0.483 |
| Alloxan group (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.

| | |
|---|---|
| | Non-Control |
| | Control Alloxan (65 mg/kg, i.p.) |
| | Metformin (100 mg/kg, oral) |
| | PL (50 mg/kg,oral) |
| | PL (100 mg/kg,oral) |
| | PL (200 mg/kg,oral) |

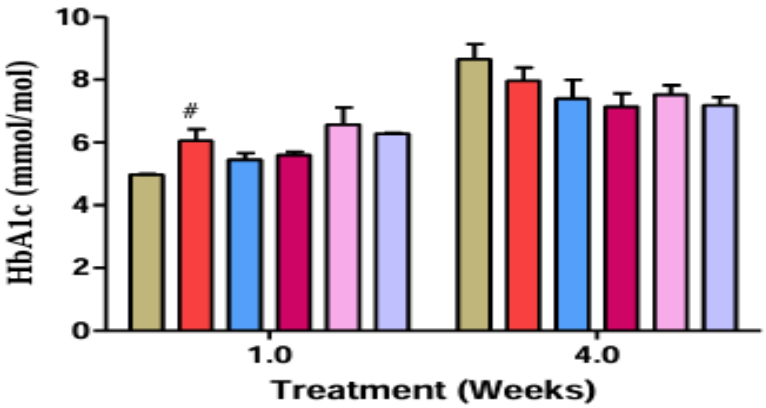
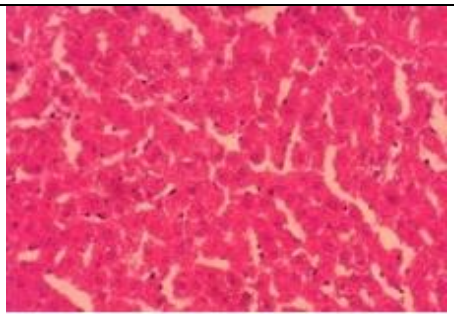


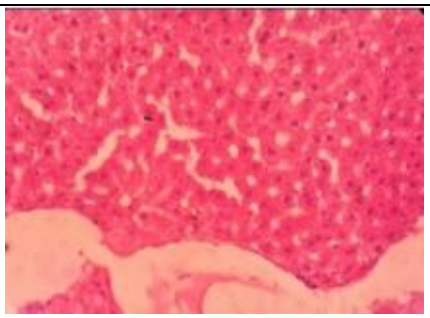

**Figure 8: Change in HbA1c Level in Normal and Diabetic Rats.**

**Histopathological studies of Rat Pancreas and Liver**

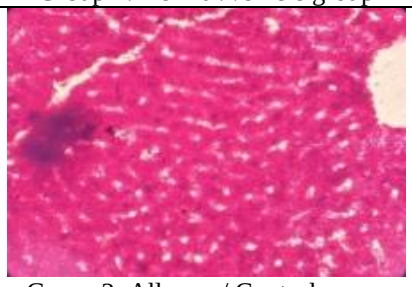
Significant difference in the pattern and number of rats hepatocytes were observed among different experimental groups, shown in Figure 7.15. Alloxan-induced diabetes resulted in severe hepatic damage, including necrosis, fatty changes, and inflammation, disrupting normal liver function. Standard treatment 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.



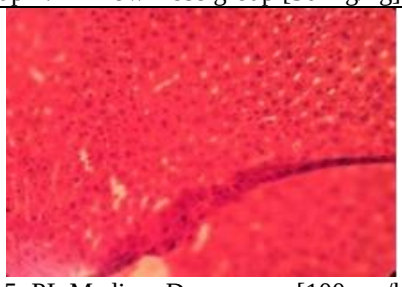
Group 1: Normal/vehicle group



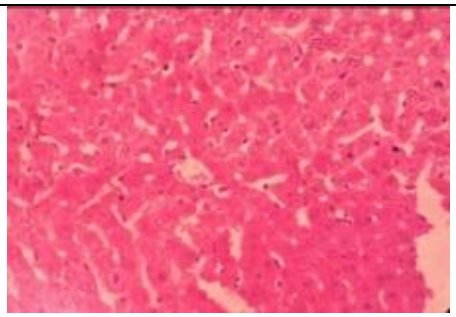
Group 4: PL Low Dose group [50 mg/kg] p.o.



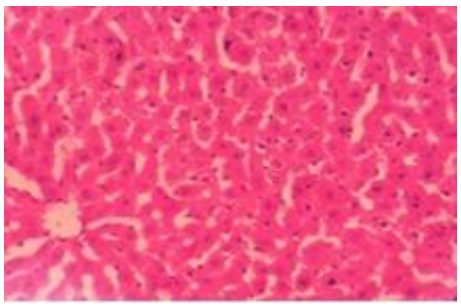
Group 2: Alloxan / Control group



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



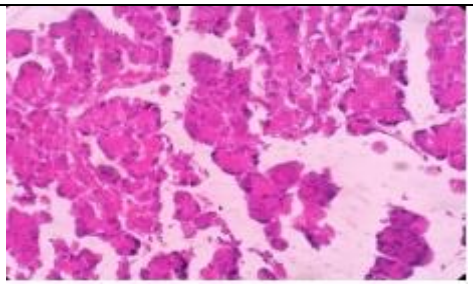
Group 3: Standard (Metformin) group [100 mg/kg] p.o.



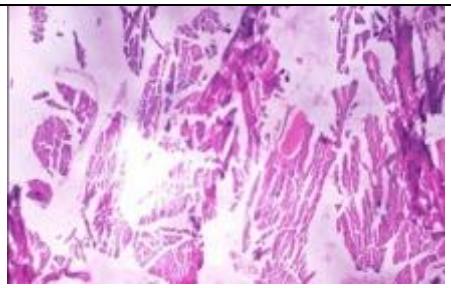
Group 5: PL Medium Dose group [200 mg/kg] p.o.

**Figure 8: Histopathological examination of liver in normal and Alloxan induce diabetic rat.**

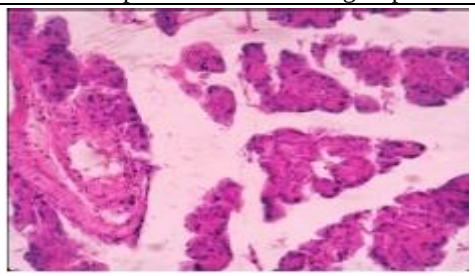
The protective effect on pancreatic islets is shown in Figure 7.16. The normal islet cells were tightly packed with distinct borders. Alloxan-induced diabetes resulted in severe pancreatic damage characterized by necrosis, edema, inflammatory infiltration and congestion. Standard treatment with Metformin 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.



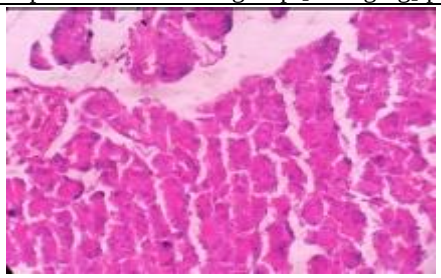
Group 1: Normal/vehicle group



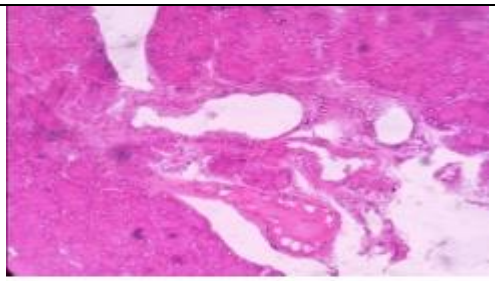
Group 4: PL Low Dose group [50 mg/kg] p.o.



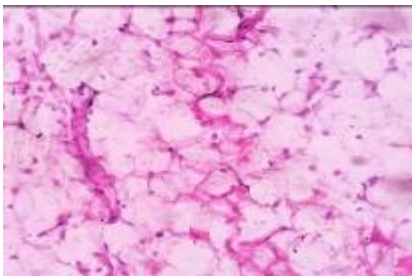
Group 2: Alloxan / Control group



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



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



Group 5: PL High Dose group [200 mg/kg] p.o.

**Figure 9: Histopathological examination of pancreas in normal and Alloxan induce diabetic rats.**

## DISCUSSION

The pharmacognostic study revealed that Polyalthia longifolia seeds possess distinct morphological and physicochemical features, including dark green color, smooth surface, and feeble bitterness. Physicochemical study confirmed low moisture level (1.492%), which means that it possesses good shelf life and stability. The plant's extractive content was found to be more in organic solvents such as ethanol, methanol, and chloroform, with Phytochemical results supporting the

occurrence of precious secondary metabolites such as phenolic compounds, flavonoids, alkaloids, carbohydrates, glycoside, tannins, terpenoids, and resins. It is well established that these compounds possess antidiabetic as well as antioxidant activity. Flavonoids and phenolic compounds have been reported to provide protection against oxidative stress and inflammatory damage, two key factors leading to diabetogenic problems.

The Polyalthia longifolia seed extract, as confirmed by a UV-visible spectroscopy study, has antioxidant activity due to its phenolic and flavonoids moieties. Absorption of UV light occurs at 226 nm for many reasons. Aromatic compounds are absorbed by 200-280 nm by $\pi \rightarrow \pi^*$ transition in the aromatic ring. Phenolic compounds absorb 220–300 nm by aromaticity and conjugation. Alkaloids such as simple aromatic alkaloids, aporphine alkaloids, indole alkaloids, quinoline alkaloids, and isoquinoline alkaloids absorb UV by aromatic rings and nitrogen structures. 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.

The OECD guideline 423-compliant acute toxicity study found that the extract is nontoxic up to a dose of 2000 mg/kg, with no mortality, behavioral alterations, or notable toxic effects on test animals. This suggests the extract has high safety margins and could be further investigated for potential therapeutic uses.

At four weeks, rats that were given Polyalthia longifolia seed extract had a significant reduction in their fasting blood glucose level. The effect was dose-dependent and the maximum dose (200 mg/kg) had the largest hypoglycemic effect and was comparable to metformin. The lowering of the HbA1c level also established the long-term antidiabetic efficacy of the extract. Decreased HbA1c levels reflect improved glycemic control, lowering the risk of diabetic complications.

Histopathological examination of the pancreas revealed that the extract of Polyalthia longifolia was able to maintain pancreatic islet cells efficiently. The research revealed that untreated diabetic rats exhibited symptoms of pancreatic injury in the form of necrosis, edema, and inflammatory infiltration. The treatment resulted in major improvements in pancreatic morphology, largely attributed to the antioxidant and anti-inflammatory effects of the extract, which suggests its efficacy for β-cell regeneration.

Hepatoprotective effect was also observed in Histopathological examination, where the diabetic rats exhibited severe hepatic injury, which included necrosis, fatty changes, and inflammation. Polyalthia longifolia extract treatment improved the integrity of the hepatocytes, reduced the inflammation, and re-established normal liver architecture, particularly with higher doses. These results point towards a protective action of the extract against diabetic liver damage.

## CONCLUSION

The research demonstrates the antidiabetic activity of Polyalthialongifolia seed extract. The extract contains bioactive compounds like flavonoids, alkaloids, phenolic, tannins, and terpenoids. It reduced fasting blood glucose and HbA1c levels considerably, comparable to metformin. Histopathological examination confirmed its protective effect on the integrity of liver and pancreatic tissues. At 2000 mg/kg, the extract was not toxic, reflecting a broad margin of safety for its potential therapeutic use. To confirm its efficacy and explore its potential as an adjuvant therapy for diabetes mellitus, further research is required for identify bioactive compound those are useful in diabetes.

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