

Invivo Antidiabetic activity of *Pleurospermum candollei* in STZ induced Mice

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Abstract:

Diabetes is a serious global health problem that affects millions of people worldwide. It is a chronic metabolic illness characterized by high blood sugar levels, which can lead to dangerous side effects that impact several organs and systems. Finding efficient diabetes therapies is vital, and researching the antidiabetic properties of plants is extremely important in this context. This study uses a number of metrics, such as acute toxicity, body weight changes, blood glucose levels, insulin production, and pancreatic histology, to examine the therapeutic potential of *Pleurospermum candollei* extract on diabetes treatment. The extract's safety was validated by the acute toxicity test, which revealed no mortality at levels up to 2000 mg/kg. The results demonstrated a dose-dependent antihyperglycemic effect of the *Pleurospermum candollei* extract. The 400 mg/kg dose group showed a more marked reduction in blood glucose levels compared to the low dose groups suggesting greater antidiabetic potential at higher doses with a significance of $p < 0.01$ for 400mg/kg group. Glucose tolerance was also enhanced in a dose-dependent manner, with the 400 mg/kg and 200 mg/kg groups exhibiting better glucose control than the 100 mg/kg group. The higher dose group (400 mg/kg) were more effective in mitigating the weight loss associated with diabetes compared than the lower dose (100 mg/kg, 200mg/kg) and the negative control group. The positive control group initially lost some weight but eventually stabilized. In the groups treated with the extract, those receiving 100 mg/kg and 200 mg/kg experienced a gradual decline in weight, with the 200 mg/kg group leveling off by the end of the study. Interestingly, the group receiving 400 mg/kg maintained a higher weight compared to the lower doses, with a significance of $p < 0.1$. Insulin measurements showed that the extract treatments improved insulin levels, with the group treated with 400mg/kg yielding the highest increase in extract treated groups. Histopathological analysis demonstrated that the normal control group had intact pancreatic architecture, while the STZ-induced diabetic group showed severe damage, including the absence of beta cells. In contrast, the extract-treated groups exhibited varying degrees of islet regeneration, with the 400 mg/kg dose showing the most pronounced effect. Overall, these findings indicate that *Pleurospermum candollei* extract may effectively mitigate diabetic symptoms and promote pancreatic tissue regeneration, warranting further investigation into its mechanisms and therapeutic applications.

Keywords: Streptozotocin, Diabetes Mellitus, Oral Glucose Tolerance, Glimepiride

1. Introduction

Chronic hyperglycemia is a metabolic disease that is induced either by an insufficiency in insulin secretion or impaired insulin action or both. However, insulin is also an anabolic hormone that in turn alters the metabolism of carbohydrates, fats, and proteins (Poznyak et al., 2020). Defects in insulin production and/or activity may be part of the etiology of diabetes mellitus (DM), a metabolic disease that causes persistent hyperglycemia. According to estimates, one in three Americans will have diabetes at some point in their lives. Type 2 diabetic mellitus (T2DM) is the most prevalent type of DM, accounting for over 90% of cases. T2DM is mostly caused by the body's tissues' inability to either react to insulin or produce enough of it (Yun & Ko, 2021). According to the estimates of the International Diabetes Federation, in 2019, there were 463 million suffering from diabetes. The number is expected to surge to 578 million by 2030 and reach 700 million by 2045 (International Diabetes Federation, 2019). Medicinal plants play a crucial role for over four billion people in developing countries, where they are commonly used to manage metabolic conditions like diabetes mellitus. These natural remedies provide accessible options for many who may not have access to conventional medical treatments (Choudhury et al., 2018). *Pleurospermum candollei*, (Baan) is a member of the Apiaceae family. This herb is evergreen. It occurs often (3000–5000 m) in high altitudes on interior ranges. Kumaon to Kashmir in the Alpine Himalayas. Furthermore, found in western Pakistan and the Himalayan region of India. Plants range in height from 10 to 30 cm. Tap roots spread widely. The base of the stem is covered in persistent leaf bases. It is frequently used as a vegetable and a medication by locals. Whole plant *P. candollei* used for food; roots boiled for decoction (used for respiratory and gastric disorders); leaves used for lower back pain treatment (Khan et al., 2015). Medicinal plants have an important role in human life. Medicinal plants are used as both traditional and modern medicines. According to WHO about 80% of the world population are still relying on natural resources for management of different ailments. Even in the developed world the consumer is attracted toward herbal medicine because these have lesser side effects. Less than 10% of all chemical compounds have reportedly been identified to date, with a reported 12,000 compounds. Natural resources are more readily available, more affordable, and more accessible than modern medical treatments, to name a few advantages over the latter. Medicinal plants have been used for healing since 4000-5000 B.C. To avoid difficult side effects, it is imperative that medical treatments be developed that not only cure the illness but also have a natural source. In the Karakoram and Himalayan zones, *Pleurospermum candollei* Benth. ex C.B. Clarke., also called Braq Shandun/Shoogroon/Shabdun, is a member of the Umbelliferae or Apiaceae family. It is a 30–40 cm-long herb consumed as a vegetable by native people (Ghosh and Sur 2015). *Pleurospermum candollei* also exhibits a protective reaction against cardiac issues and lowers blood pressure and reduce cholesterol levels in the body. Additionally, in Gilgit-Baltistan, the plant's powdered stem is used to treat back and joint discomfort and used for the treatment of stomach disorders and gastrointestinal difficulties. It is consumed by the Karakoram region's residents for its flavor and nutritional value. This species is available for commercial sale in these areas and has also been used to treat a variety of illnesses whereas the preparations made from this plant have also been used to treat infertility in both male and female (Abbas et al., 2022). For treating fever and cure the headaches, one teaspoon of dry plant powder is taken with milk once a day for a week. It can also be cooked with leafy vegetables (Shah et al., 2015). *Pleurospermum candollei* is also used to treat animal diarrhea (Khan et al.,

2015). According to a study approximately 25- 40 species documented from Asia, Europe, and the Himalayan region. *Pleurospermum candollei* have anti-inflammatory, and antimalarial properties that contain terpenoids, steroids, saponins, glycosides, esters. *Pleurospermum rivulorum* is also utilized as a diaphoretic, analgesic, and antipyretic in Chinese traditional medicine. Bicommarins and furanocoumarins have been recorded from *Pleurospermum* species (Xiao et al., 1997). It was discovered that *P. candollei* was effective against respiratory, cerebral, and unconsciousness disorders (Ali et al., 2021). Animals with diarrhea can also be treated with this medicinal plant. According to a previous study *P. candollei* was found to contain bioactive compounds with anti-inflammatory properties, because of the *Pleurospermum* genus's biological characteristics and natural antioxidant content, its medicinal and pharmaceutical qualities are highly valued (Thiviya 2021; Kawarty et al., 2020). *P. candollei* has been traditionally used for the treatment of DM but has not been scientifically proven so the methanolic extract of this plant was used to evaluate its antidiabetic potential.

2. Materials and Methods

2.1 Chemical and Reagents

Glucose and Normal saline were purchased from Frontier Dextrose Ltd Pakistan. Glimepiride were purchased from Getz Pharma (Private) Limited Pakistan. Streptozotocin was purchased from Sigma Aldrich (USA).

2.2 Collection and authentication of plant material

In August 2023, a full fresh *Pleurospermum candollei* plant was obtained from the isolated valley of Shigar Arandoo located in Gilgit-Baltistan a region of Pakistan. After being verified by Taxonomist Dr. Muhammad Nazir (Environmental Department CUI Abbottabad Campus), the specimens were placed to the pharmacy department herbarium of COMSATS University Islamabad (Abbottabad).

2.3 Extraction and processing of Plant

The plant's delicate parts were carefully washed with fresh water to remove any dirt and impurities, then left to dry in the shade at 25°C. Each sample was dried separately to ensure quality. Once completely dry, the plant was cut into small pieces and ground into a fine powder, which was then stored in a sealed container to keep it fresh. Next, the plant powder underwent a process called maceration, where it was soaked in high-quality methanol in a closed container at room temperature for 21 days. During this time, the mixture was shaken regularly to help extract the beneficial compounds. After 21 days, the liquid was pressed and filtered to separate the solid material. To purify the liquid extract further, rotary evaporation was used a common technique for removing solvents. For the best results, the filtrate was placed in a round bottom flask, filled less than halfway, and reduced under vacuum at 40°C. Finally, any remaining water content was removed using a desiccator. This careful process ensured that the extract maintained its potency and quality (Hidayat & Wulandari, 2021).

2.4 Experimental animals

Male BALB/c mice was chosen for the experiment instead of the female. Animals were in good health. We bought the animals from the NIH Islamabad. The CUI (Abbottabad) Department of Pharmacy's Animal House provided more bread for the animals. They were provided with water and regular meals. Every animal was kept in a standard cage with standard conditions in an animal home. REC Department of Pharmacy, CUI (Abbottabad) authorized the animal research, and the study number was PHM-Eth/CF-M22/11-01. Each animal was kept in a room with light and dark cycles, at a temperature of $25 \pm 2^{\circ}\text{C}$ and a humidity of $50 \pm 5\%$.

2.5 Acute Toxicity Test

The extract was tested for acute toxicity following the traditional guidelines set by the OECD (423). Male Balb/c mice were fasted overnight and provided only water. The extract was then administered orally at a dose of 300 mg/kg, and the mice were monitored for 24 hours. If two out of three mice died, the dose was considered hazardous. If only one mouse died, the same dose was given again to confirm the toxicity level. If there were no deaths, the procedure was repeated using a higher dose of 2000 mg/kg to further assess safety (Hsu et al., 2022).

2.6 Induction of Diabetes

Each mice was accurately weighed, and they were randomly divided into experimental and control groups. On the experimental day, all food was removed from the cages of each group four hours before the streptozotocin (STZ) therapy. Water was provided to the mice as usual. One 1.5-ml microcentrifuge tube was used for every three mice who were given a single intraperitoneal injection of 180 mg/kg of STZ to induce diabetes; four hours before the treatment, the animals were fed nothing. Immediately before injection, STZ was dissolved in cold 0.9% NaCl, with an injection volume of 450–550 μL . After three days blood glucose levels were measured by glucometer for each mouse and the readings that are above 300mg/dl were selected for protocol (Hayashi et al., 2006; Deeds et al., 2011).

2.7 Animal Grouping

Groups (n=6)	Treatment given
I	Only Vehicle
II	STZ injection (180 mg/kg, ip)
III	STZ injection (180mg/kg, ip) + Glimepiride 0.8 mg/kg
IV	STZ injection (180mg/kg, ip) + PC Extract 100 mg/kg
V	STZ injection (180mg/kg, ip) + PC Extract 200 mg/kg
VI	STZ injection (180mg/kg, ip) + PC Extract 400 mg/kg

2.8 Body weight Measurement

A notable effect on the experimental animals' body weights was noted for the duration of the treatment regimen. In particular, the mice under observation lost a significant amount of weight after receiving STZ. Precise measurements were made on days 0, 3, 6, 9, 12, and 15 to precisely monitor this impact.

2.9 Measurement of Blood Glucose levels

Accu-Check (glucometer) were used to assess blood glucose levels, on days 0, 3, 6, 9, 12, and 15 of the research. A little piece of tissue, less than 2 mm, was cut with sharp scissors or a scalpel starting at the tail's tip, precisely distal to the bone, to collect blood samples. After that, blood was drawn by letting it run straight onto a glucose test strip, into a capillary tube, or into another appropriate receptacle. In certain instances, light rubbing or "milking" of the tail was done to promote blood flow and gathering. Using these blood samples, the blood glucose levels of the mice were then monitored and recorded for the planned days of the experiment. (Alam & Saqib, 2017).

2.10 Oral glucose tolerance test (OGTT)

After completing the two-week experimental treatment, the mice were fasted overnight to prepare for the next phase of the study. A baseline blood glucose sample was taken to establish a reference point for the evaluations to come. Next, a specific dose of glucose solution, calculated at 2.5 g/kg, was administered orally to the mice to initiate the assessment. Following this, blood samples were collected at set intervals to closely monitor how the mice responded to the glucose. Blood glucose levels were measured with a glucometer at key points: 0, 30, 60, and 120 minutes after the glucose intake. This careful approach allowed researchers to thoroughly examine how the mice metabolized and regulated their blood sugar levels over time. The entire process was essential for conducting the Oral Glucose Tolerance Test (OGTT), a valuable method used to evaluate glucose tolerance and blood sugar regulation in experimental subjects (Kuo et al., 2021).

2.11 Insulin Measurement Test

To measure insulin levels in the mice, blood samples were collected, typically through cardiac puncture, and centrifuged to obtain plasma. Insulin levels were quantified using an enzyme-linked immunosorbent assay (ELISA). Plasma samples were added to pre-coated ELISA plates along with control samples and standards, followed by incubation to allow binding to the antibodies. After washing to remove any unbound substances, a secondary enzyme-conjugated antibody was introduced, producing a measurable color change upon substrate addition. The absorbance was then read with a plate reader, and insulin concentrations were calculated against a standard curve. Evaluation of this test was done at Azaan Diagnostic Laboratory Haripur (MacDonald & Gapinski, 1989).

2.12. Histopathology

After the experiment concluded, histological analysis was carried out on the pancreatic tissues from all the mice groups. Once the animals were humanely sacrificed, the entire pancreas was carefully extracted under intraperitoneal chloral hydrate anesthesia (400 mg/kg). The pancreas was then placed in a formaldehyde solution to preserve it, and histological preparations were made immediately. For the analysis, thin slices (5 micrometers) of the tissue were cut and stained with eosin and hematoxylin. This staining process allowed for detailed microscopic examination of the pancreatic tissues, providing valuable insights into their structure and any potential changes resulting from the treatments (Veite-Schmahl et al., 2017).

3. Results

3.1. Acute Toxicity Test

The results of the acute toxicity test showed no mortality up to the dose of 2000mg/kg body weight. Mice were observed for 15 days, and no severe symptoms were observed. So, the extract was found to be safe for the experiment.

3.2 Effect of *Pleurospermum candollei* extract on body weight.

The normal control group shows an increase in weight, with blood glucose levels steadily rising to 39.6 ± 1.1 by day 15. In contrast, the negative control group experiences a significant decline in glucose levels, dropping to 17.9 ± 0.5 . The positive control group initially shows a decrease but stabilizes with minor fluctuations, ending at 27.3 ± 1.4 . Among the extract-treated groups, those receiving 100 mg/kg and 200 mg/kg see a gradual decline in glucose levels, with the 200 mg/kg group stabilizing around 24.1 ± 0.6 by day 15. Notably, the 400 mg/kg extract treatment maintains higher glucose levels compared to the lower doses, finishing at 29.1 ± 0.9 , indicating a potentially beneficial effect (Fig 2).

Table 1: Effect of extract of *Pleurospermum candollei* and glimepiride on body weights

Groups	0 th day	3 rd day	6 th day	9 th day	12 th day	15 th day
Normal control	30.3 ± 0.8	31.6 ± 0.3	34.5 ± 0.4	35.6 ± 0.8	35.6 ± 0.3	39.6 ± 1.1
Negative control	29.3 ± 0.4	25.8 ± 0.7	23.6 ± 0.4	21.8 ± 0.7	20.1 ± 0.6	17.9 ± 0.5
Positive control	$28.6 \pm 0.8^*$	$25.5 \pm 0.6^*$	$23.5 \pm 0.9^*$	$24.8 \pm 0.9^*$	$26.5 \pm 1.0^*$	$27.3 \pm 1.4^*$
Extract treated 100mg/kg	30.5 ± 0.9	27.0 ± 0.8	25.1 ± 1.0	23.3 ± 0.9	23.1 ± 1.2	22.5 ± 0.7
Extract treated 200mg/kg	29.0 ± 0.7	25.8 ± 1.2	24.8 ± 0.7	23.5 ± 0.7	23.5 ± 0.4	24.1 ± 0.6
Extract treated 400mg/kg	$31.5 \pm 0.8^*$	$28.0 \pm 0.5^*$	$26.5 \pm 0.5^*$	$25.6 \pm 0.6^*$	$26.8 \pm 0.6^*$	$29.1 \pm 0.9^*$

Data represents as mean of $\pm$ SEM values of 6 animals in each group. * $p < 0.1$ (One-way ANNOVA followed by Dunnet's test, Graph pad prism software) -ive control was compared with all other groups.

3.3 Effect of *Pleurospermum candollei* extract on blood glucose levels.

The data presents blood glucose levels in mice across different groups over a 15-day period. The normal control group showed relatively stable levels, starting at 90.1 ± 2 and ending at 92.5 ± 3 . In contrast, the negative control group exhibited a significant increase in glucose levels, peaking at 492.1 ± 6 by day 15. The positive control group demonstrated a marked decrease, starting from 356.6 ± 6 and dropping to 112.3 ± 5 by day 15. Among the extract-treated groups, the 100 mg/kg treatment showed a reduction from 341.8 ± 7.7 to 292.5 ± 5 . The 200 mg/kg group decreased from 344.1 ± 6 to 217.8 ± 6 , while the 400 mg/kg group exhibited the most significant decline, from 332.5 ± 5 to 194.1 ± 4 . Overall, the extract treatments effectively reduced blood glucose levels compared to the negative control (Fig 3).

Table 2: Effect of extract of *Pleurospermum candollei* and glimepiride on blood glucose levels

Groups	RBG, s						16 th day FBG, s
	0 th day	3 rd day	6 th day	9 th day	12 th day	15 th day	
Normal control	90.1 ± 2	94.1 ± 4	94.1 ± 6	96.6 ± 2	95.3 ± 1	92.5 ± 3	75 ± 4
Negative control	341.2 ± 8	365.5 ± 6	387.5 ± 8	436.5 ± 14	468.6 ± 5	492.1 ± 6	380 ± 10
Positive control	$356.6 \pm 6^{***}$	$269.1 \pm 12^{***}$	$204.2 \pm 16^{***}$	$152.6 \pm 7^{***}$	$132.6 \pm 4^{***}$	$112.3 \pm 5^{***}$	108 ± 6
Extract treated 100mg/kg	$341.8 \pm 7.7^{**}$	$321.8 \pm 9^{**}$	$305.5 \pm 5.7^{**}$	$308.1 \pm 4^{**}$	$292.1 \pm 5^{**}$	$292.5 \pm 5^{**}$	240 ± 8
Extract treated 200mg/kg	$344.1 \pm 6^{**}$	$325.5 \pm 5^{**}$	$295.6 \pm 3^{**}$	$261.3 \pm 8^{**}$	$242.5 \pm 7^{**}$	$217.8 \pm 6^{**}$	186 ± 4
Extract treated 400mg/kg	$332.5 \pm 5^{**}$	$291.6 \pm 5^{**}$	$254.1 \pm 6^{**}$	$231.8 \pm 10^{**}$	$194.5 \pm 13^{**}$	$194.1 \pm 4^{**}$	160 ± 11

Data represents as mean of $\pm$ S.D values of 6 animals each $^{**}p < 0.01$, $^{***}p < 0.001$. (One-way ANNOVA followed by Dunnet's test, Graph pad prism software) -ive control was compared with all other groups.

3.4 Effect of PC extract on oral glucose tolerance test

The blood glucose levels were seen to increase significantly and quickly after the oral administration of glucose into each group. Normal control animals showed lower blood glucose concentrations after 2 h than diabetic negative group animals, and these normal mice returned to their baseline levels. Diabetic negative mice showed a significant increase in their glucose intolerance compared to controls and extract treated groups. (Fig 1)

3.5 Insulin Measurement Test

The data presents insulin levels measured in μ IU/ml across six treatment groups. The normal control group shows a healthy baseline with a high insulin level of 5.57μ IU/ml. In contrast, the

negative control group has a significantly lower insulin level of $1.90 \mu \text{ IU/ml}$, indicating reduced insulin production, which is often seen in diabetes. The positive control group shows some improvement with an insulin level of $3.94 \mu \text{ IU/ml}$, although this is still below the normal range. Among the extract-treated groups, G100 demonstrates a slight increase in insulin levels at $2.23 \mu \text{ IU/ml}$. G200 shows a more substantial rise to $3.19 \mu \text{ IU/ml}$, while G400 reaches $3.44 \mu \text{ IU/ml}$, suggesting that higher doses of the extract may enhance insulin production. Overall, these findings indicate that while normal control maintains healthy insulin levels, the extract treatments have the potential to improve insulin secretion in the treated subjects. (Fig 4)

3.5 Histopathology of Pancreas

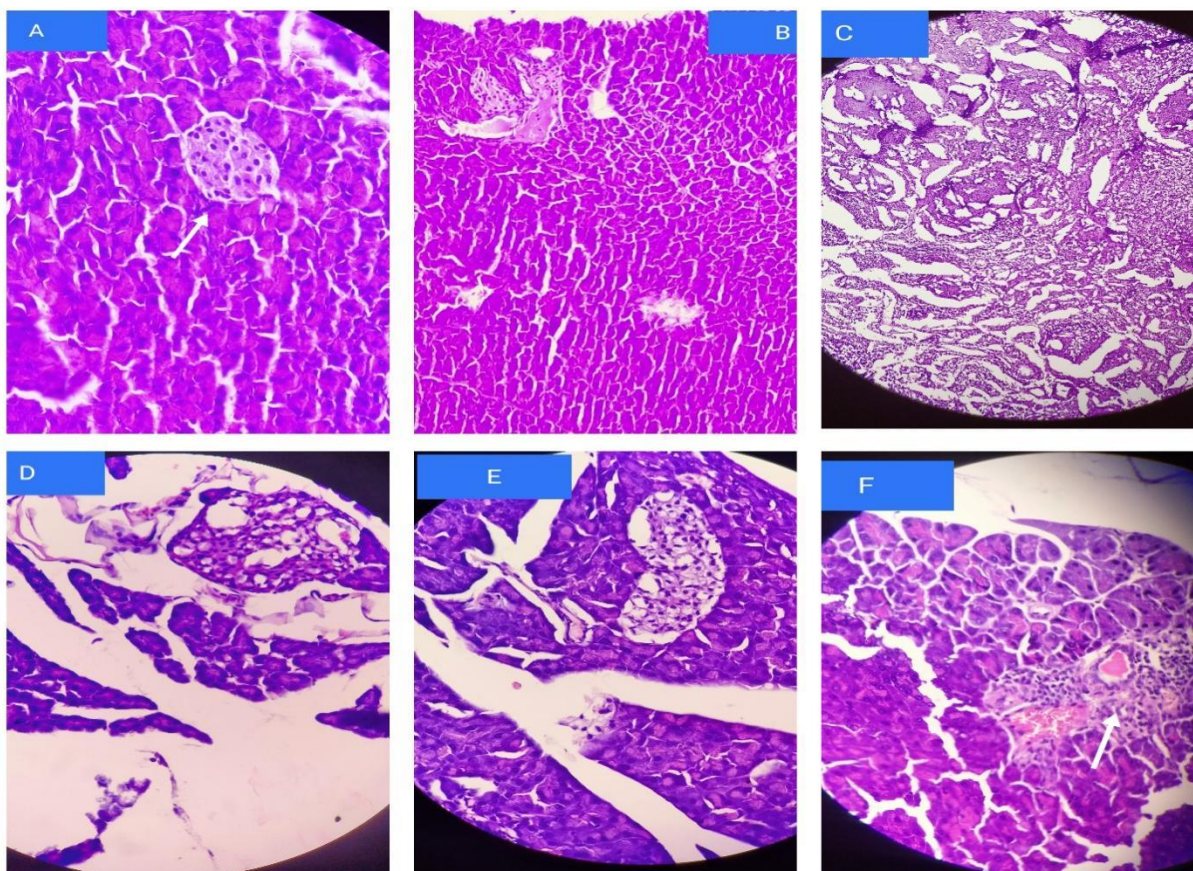


Figure 5: In the study of pancreatic histopathology, the normal control group (A) displayed well-organized and intact pancreatic acini, with Langerhans islets that maintained their typical size and architecture, showing no pathological changes in either the exocrine or endocrine components. In contrast, the STZ-induced diabetic control group (B) exhibited significant damage to the pancreatic tissue, with a complete absence of beta cells. The group treated with the conventional drug (C) showed notable improvement in pancreatic morphology compared to the diabetic control, with larger Langerhans islets suggesting some regeneration of beta cells. In the group receiving 100 mg/kg of *Pleurospermum candollei* extract (D), signs of islet regeneration were evident, although the protective effects of the extract appeared to be only moderate. When the dose was increased to 200 mg/kg (E), there was a slight increase in islet regeneration, indicating a more favorable therapeutic effect. Finally, in the group treated with 400 mg/kg of the extract (F), some regeneration of Langerhans islets was observed, although this effect was not as pronounced as in the group receiving the standard drug. Overall, the findings suggest a dose-dependent response to *Pleurospermum candollei* extract in promoting pancreatic tissue regeneration.

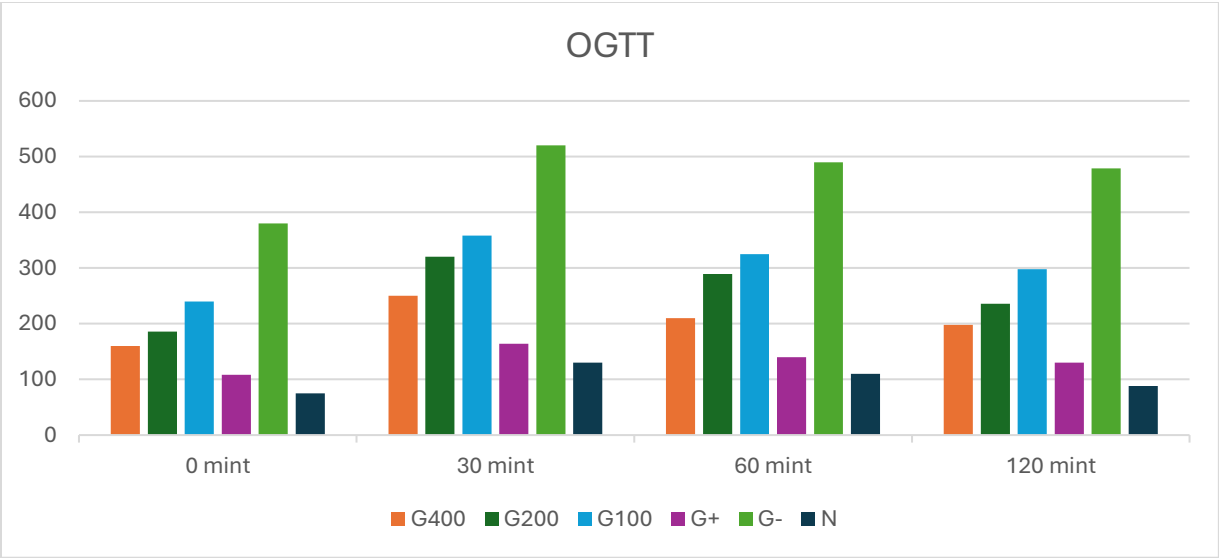


Figure 1: Comparison of OGTT of different groups of mice. Y axis represents the blood glucose levels and X-axis represents the groups having blood glucose levels measured at 0,30,60,120 mint.

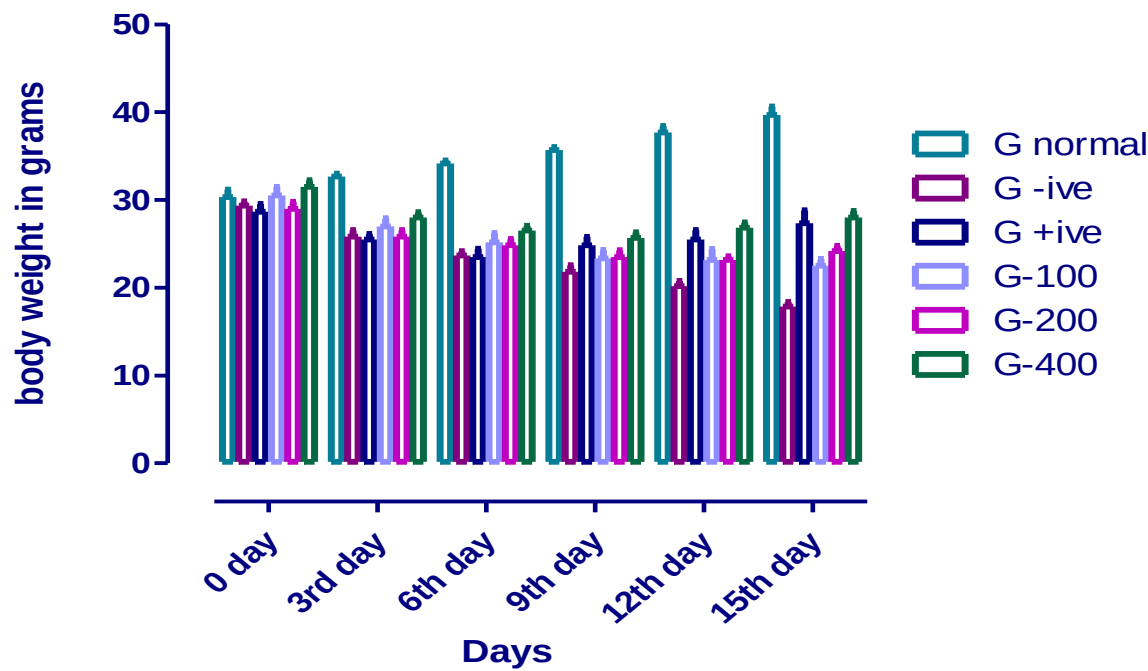


Figure 2: Comparison of body weights of mice of experimental groups

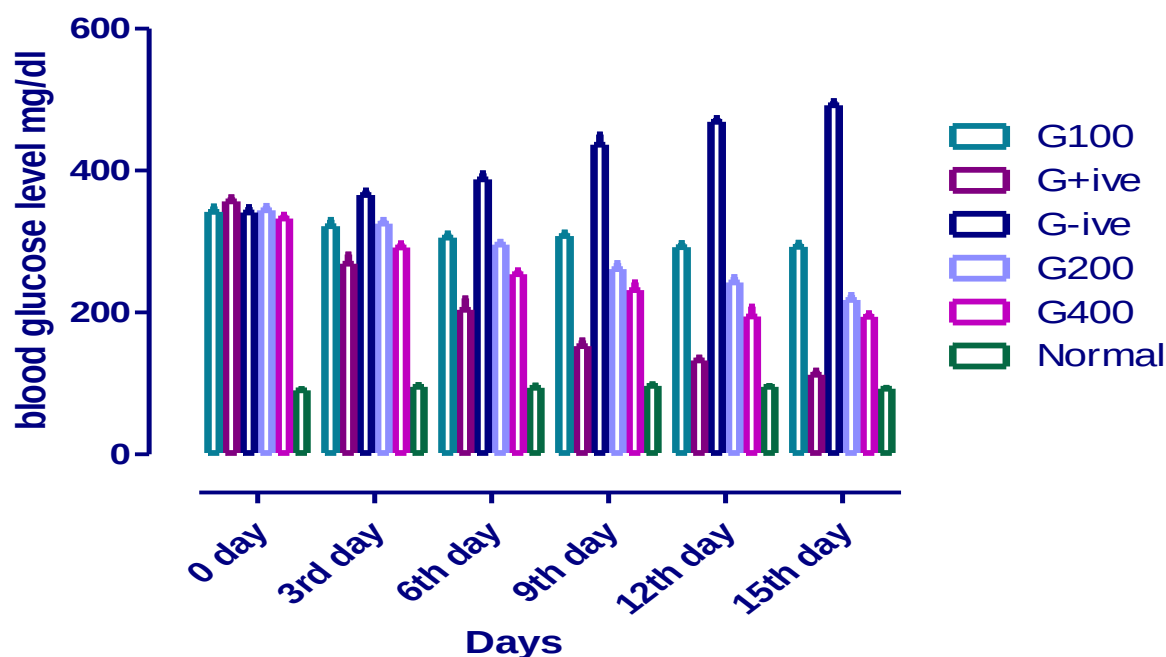


Figure 3: Comparison of blood glucose levels mg/dl of mice of experimental groups

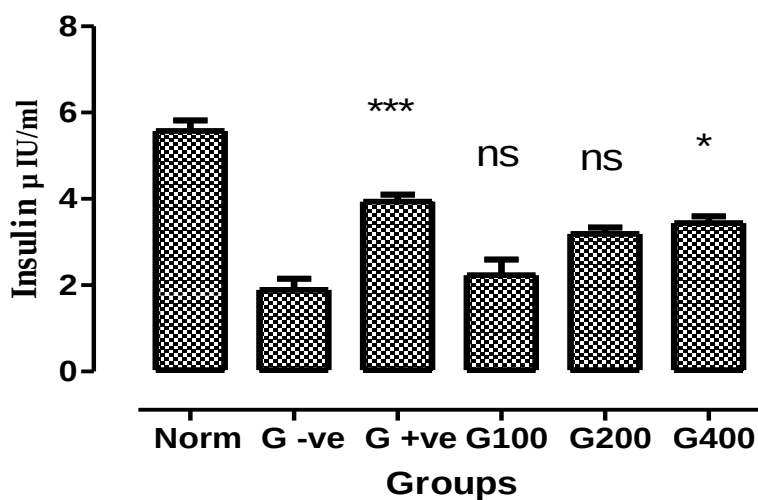


Fig 4: Effect of PC extract and standard drug on serum Insulin (μ IU/ml). All groups except normal were injected STZ (180 mg/kg, once), i.p. Treatment with glimepiride or PC extract started three days after STZ injection. Each value represents the mean $\pm$ SEM (n=6). * $p < 0.1$, *** $p < 0.001$ (One-way ANOVA followed by Dunnet's test, Graph pad prism software) -ive control was compared with all other groups.

4. Discussion

Diabetes is a significant challenge for the healthcare system today. Managing primary diabetes mellitus and its complications can be quite expensive, even with modern medications. Over the years, extensive research has shown that natural products could be a promising source for discovering new anti-diabetic treatments. This exploration into natural remedies offers hope for more effective and potentially less costly solutions to this pressing health issue (Jugran et al., 2021). A toxicity test was performed to assess the safety of extracts on body metabolism, focusing on two types: acute and subacute toxicity tests. The acute toxicity test is essential for measuring the immediate toxic effects of a chemical substance. It provides valuable insights into the potential risks to human health from short-term oral exposure to these substances. The results of the acute toxicity test showed no mortality up to the dose of 2000mg/kg in mice. This is supported by the work of George, who noted that medicinal plants offer a unique advantage when it comes to assessing toxicity. Their long history of use by humans suggests that the bioactive compounds found in these plants are likely to have lower toxicity for both animals and people (George, 2011). The study employed the STZ-induced diabetes model because STZ causes the pancreas to enlarge and ultimately results in Langerhans islet beta cell degeneration, which produces hyperglycemia, hypoinsulinemia, and hyperlipidemic conditions. The production of free radicals and nitric oxide, which hinder beta cell mitochondrial activity, is one of the ways that streptozotocin causes its diabetic condition (Rakieten, 1963). In diabetic animal models, plant extracts such as *Cymbopogon citratus* and *J. schimperiana* significantly reduce blood glucose levels and improve lipid profiles and body weight, like conventional antidiabetic drugs (Nnam et al., 2024; Feleke et al., 2024). *Pleurospermum candollei* also demonstrates dose-dependent improvements in glucose tolerance and metabolic regulation. Additionally, these extracts have shown to be safe at high doses in acute toxicity tests. The conventional criteria for interpreting oral glucose tolerance test (OGTT) results are as follows: a 2-hour plasma glucose level under 140 mg/dL is normal, indicating proper glucose control. Levels between 140 and 199 mg/dL indicate impaired glucose tolerance and a higher diabetes risk. Levels of 200 mg/dL or above signify diabetes, reflecting poor glucose metabolism and clearance (Eyth et al., 2023). The OGTT results showed efficient glucose homeostasis in the Normal Control group and severe impairment in the Diabetic Control group. The Positive Control group, treated with glimepiride, had improved glucose response. *Pleurospermum candollei* extract-treated groups demonstrated dose-dependent improvements, with the 400 mg/kg and 200 mg/kg groups showing better glucose control than the 100 mg/kg group, indicating higher doses may enhance glucose homeostasis and metabolic regulation. Examining pancreatic histopathology in in vivo diabetes research using plant extracts offers important insights into the potential healing properties of these natural substances. In diabetic models, particularly those induced by STZ, we often see significant damage to the pancreatic tissue, especially in the Langerhans islets, where beta cells play a vital role in insulin production. Research has indicated that certain plant extracts, like *Vinca rosea*, can aid in regenerating these damaged islets, leading to improved pancreatic structure and function. For example, treatments with these extracts have shown increases in both the size and number of islets, suggesting they may help protect against the tissue degeneration typically associated with diabetes. Overall, the findings suggest a dose-dependent response to *Pleurospermum candollei* extract in promoting pancreatic tissue regeneration (Ahmed et al., 2011).

5. Conclusion

This study highlights the potential of *Pleurospermum candollei* extract as a promising treatment for diabetes. The acute toxicity test showed that the extract is safe to use, and it was effective in lowering blood sugar levels and boosting insulin production. Importantly, histopathological analysis revealed encouraging signs of pancreatic tissue regeneration, especially in the Langerhans islets. These results suggest that *Pleurospermum candollei* extract not only helps manage high blood sugar and low insulin levels but also supports the healing of pancreatic function. This positions it as a valuable natural remedy for diabetes. However, more research is needed to fully understand how it works and to explore its therapeutic potential further.

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