



Therapeutic potential of *Peganum harmala* in Pancreatic regeneration in Streptozotocin-induced diabetic albino rats

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Abstract

The pancreas is a vital and complex organ that plays a key role in regulating digestion and nutrient metabolism. Conventional treatments for various pancreatic diseases often involve drug therapies, which, while effective, can lead to adverse side effects and further complications in the body. Diabetes, a chronic condition associated with the pancreas, is characterized by a loss of control over blood glucose levels due to impaired beta-cell function and insufficient insulin production. This study aims to investigate the protective effects of *Peganum harmala* leaf powder on streptozotocin-induced diabetes in albino rats, utilizing a nutraceutical approach.

In this experiment, 35 albino rats (180-200g) were randomly assigned to seven groups: After 14 days, blood samples and pancreatic tissue were collected, and biochemical assays (blood glucose, amylase, and lipase) along with PCR gene expression analysis were conducted.

The results showed a significant reduction in blood glucose levels in the *Peganum harmala*-treated groups compared to the diabetic control group. Amylase and lipase levels also showed marked improvement, indicating enhanced pancreatic function. Gene expression analysis revealed a significant increase in IGF, GPx, and Ki67 levels in the treatment groups, while Caspase 3 expression decreased significantly, suggesting improved cell proliferation and reduced apoptosis in pancreatic cells. Histopathological findings corroborated these results, with *Peganum harmala*-treated groups showing better preservation of pancreatic tissue structure. *Peganum harmala* leaf powder effectively improves pancreatic function and lowers blood glucose in diabetic rats for diabetes treatment.

Introduction

Diabetes, a widespread endocrine disorder, has reached epidemic proportions globally. It is characterized by either insufficient insulin production by the pancreas or the body's inability to use insulin effectively. The pancreas is divided into two main components: the exocrine and endocrine systems. The exocrine system is primarily composed of acinar cells, which secrete digestive enzymes (Kumar et al., 2017). These enzymes, along with electrolytes and bicarbonate ions, are transported through a complex network of branched tubules

lined with epithelial cells into the pancreatic ducts and then into the duodenum to aid in digestion (Damasceno et al., 2014, Gorelick et al., 2018). The endocrine portion, which constitutes approximately 2% of the pancreas, is made up of clusters of cells known as pancreatic islets (Enge et al., 2017). These islets consist of five types of cells: α -cells (15-20%) that release glucagon, β -cells (65-80%) that produce insulin, γ -cells (3-10%) that secrete somatostatin, PP cells (1%) that contain pancreatic polypeptides, and ϵ -cells that produce ghrelin. In rats, mice, and other rodents, the β -cells are centrally located within the islets, surrounded by α - and γ -cells forming a

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mantle. The β -cells play a crucial role in maintaining blood glucose homeostasis by regulating insulin release (Baig and Panchal, 2020). There is essential communication between these hormone-secreting cells in a normal physiological state. For instance, glucagon promotes the secretion of insulin, while somatostatin inhibits the release of both insulin and glucagon. This hormonal interplay is vital for maintaining the body's metabolic balance (Lee et al., 2003).

The global prevalence of diabetes is increasing at an alarming rate, and without proper intervention, the number of individuals affected may double by 2030. In developed nations, diabetes is among the most widespread diseases. The incidence of type 1 diabetes differs across regions, ranging from 0.14 to 10 per 100,000 annually in Asia, 1.8 to 7.0 per 100,000 in Africa, 3.4 to 36 per 100,000 in Europe, 7.6 to 25.6 per 100,000 in North America, and 2.61 to 20.18 per 100,000 in the Middle East. Type 2 diabetes is even more prevalent, with rates between 0.7% and 11.6% in Europe, 4% and 17.9% in Africa, 5.6% and 40% in the Middle East, 1.2% and 14.6% in Asia, and 7% to 28.2% in North America (Surya et al., 2014).

Current treatments for diabetes involve a multifaceted approach, including controlling high blood pressure, promoting regular physical activity, and implementing therapeutic strategies to manage cardiovascular risk. However, chemical and synthetic medications may cause various adverse effects. As a result, plant-based remedies have been widely used in herbal medicine for their accessibility and affordability, particularly in lower-income countries (Ahmed et al., 2020). In recent years, there has been growing interest in exploring natural alternatives for diabetes management with fewer side effects. Ethnobotanical or nutraceutical treatments derived from plants are gaining recognition due to their efficacy, minimal side effects, and cost-effectiveness (Gad-Elkareem et al., 2019). *Peganum harmala*, a plant from the Zygophyllaceae family, is also known as wild rue or Syrian rue (Javadian et al., 2016). It is referred to as Harmal in North Africa and Espand in Iran (Asgarpanah and Ramezanloo, 2012). Different parts of this plant, such as its seeds, leaves, fruits, roots, and bark, have been utilized in traditional medicine for centuries in Iran and other regions. The key alkaloids found in *Peganum harmala*—harmaline, harmine, harmalol, and harman—are known for their therapeutic applications, including potential anticancer properties (Moloudizargari et al., 2013). *Peganum harmala* has been shown to have hypoglycemic and cytoprotective effects, though some studies suggest that its hypoglycemic activity may diminish at higher doses (Singh et al., 2008).

This study aimed to investigate the impact of *Peganum harmala* leaf powder on the functional recovery of the pancreas in streptozotocin-induced diabetic albino rats.

Materials and Methods

Experiment design

Thirty-five adult albino rats (weighing 180-200g) were obtained from the Animal House Care at the Institute of Molecular Biology and Biotechnology, University of Lahore. The rats were divided into seven groups and housed in polyacrylic cages, with five rats per cage Table 1.

Table 1. Albino rats were divided into control and treatment groups

Sr.	Group type	Treatment
1.	Normal control	albino rats were used as normal
2.	Diabetic control	albino rats had diabetes, without any treatment
3.	Standard drug group	Gliclazide drug 80mg/kg of body weight to diabetic rat
4.	Placebo 1	Rice powder 1g/kg of BW
5.	Placebo 2	Rice powder 2g/kg of BW
6.	Treatment 1	<i>Peganum harmala</i> leaves powder 1g/kg of body weight
7.	Treatment 2	<i>Peganum harmala</i> leaves powder 2g/kg of body weight

Before the experiments began, the animals were acclimatized to standard laboratory conditions for one week. Diabetes was induced in the rats using Streptozotocin through the following method (Fig. 1), (Rajasekaran et al., 2006).



Fig 1: Induction of diabetes in albino rats

Mature, healthy leaves of *Peganum harmala* were collected, washed thoroughly with water, dried, and ground into powder.

Sample collection

After 14 days, the rats were anesthetized using chloroform, and blood and pancreatic tissue samples were collected (Fig. 2). Blood was drawn directly from the heart's left ventricle and stored in EDTA vacutainers. Serum was separated via centrifugation at 2200-2500 rpm for biochemical analysis. The pancreas was surgically removed; half of the tissue was preserved in formalin for histopathological examination, while the remaining portion was stored in Trizol for gene expression analysis.



Fig 2: Dissection of Rat

Biochemical profile

Blood glucose levels were measured using a glucometer. Pancreatic enzyme activity for amylase and lipase was determined with a fully automated analyzer (Cobas 6000) using assay kits from Roche to assess the treatment effects.

RNA Extraction and Molecular Analysis

RNA was extracted from pancreatic tissue using the standard Trizol method (Shabbir et al., 2014). cDNA synthesis was performed using M-MLV reverse transcriptase (Invitrogen, USA) following the manufacturer's protocol. Gene expression for ki67, Caspase 3, GPx, and IGF was analyzed using Real-Time PCR (Applied Bio System CFX Connect), while GAPDH was used as the internal control.

Histopathology analysis

Pancreatic tissue was preserved in 10% formalin and processed for histopathology using Hematoxylin and Eosin staining, followed by examination under a high-efficiency microscope.

Statistical analysis

Quantitative data from various groups were statistically compared to evaluate treatment effects using One-Way ANOVA (GraphPad Prism 7.04; La Jolla, CA, USA).

Results were expressed as mean $\pm$ SD, with a p-value ≤ 0.05 considered statistically significant.

Results

Growth

At the start of the experiment, all rats weighed between 180-200g. By the end, the body weight of the experimental groups showed significant differences compared to the control group (Fig. 3).

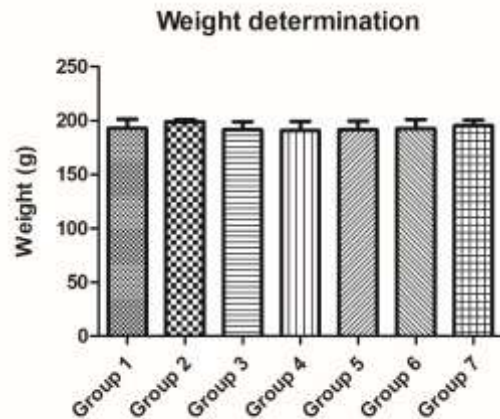


Fig 3: Mean body weight determination in control and treated groups.

Biochemical analysis

Blood glucose, amylase, and lipase levels were measured after 14 days in both control and treated groups. Significant differences in blood glucose levels were observed across all groups compared to the normal control. *Peganum harmala* leaf powder had a notable effect on amylase levels in streptozotocin-induced diabetic rats (Fig. 4).

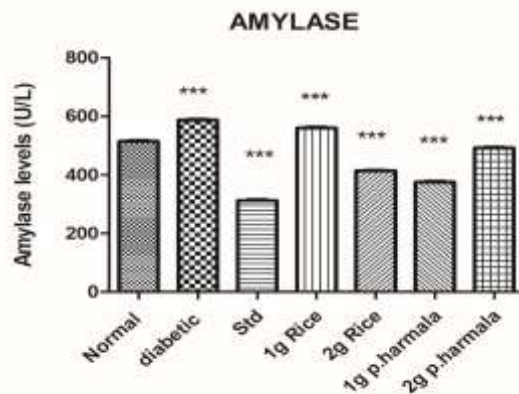


Fig 4: Amylase levels in diseased and treated rats with selected doses of *Peganum harmala* and standard drug.

Similarly, lipase levels in rats treated with different doses of *Peganum harmala* leaf powder or the standard drug showed statistically significant differences compared to the normal group ($P < 0.0001$) (Fig. 5).

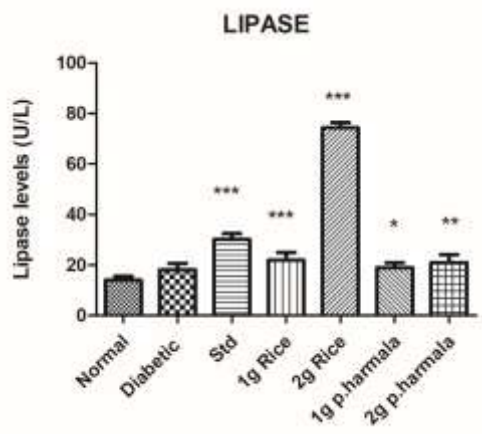


Fig 5: Lipase level in different groups of rats.

Gene expression profile of mRNA

The relative expression of *IGF*, *GPx*, *Caspase 3* and *ki67* genes was analyzed in pancreas tissue of control and treated groups.

IGF: Diabetic rats showed decreased IGF expression compared to normal control. The 2g *Peganum harmala* treated group exhibited increased IGF expression compared to the diabetic group, while the 1g and 2g rice groups showed significantly lower expression levels (Fig. 6).

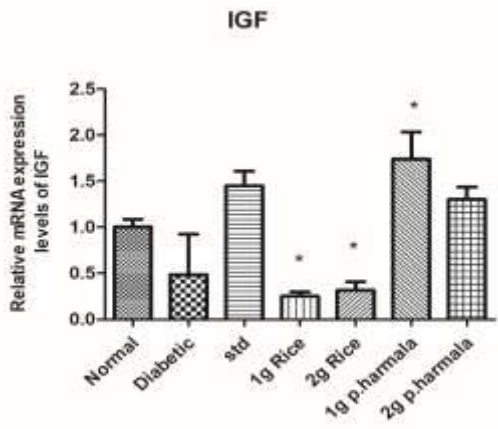


Fig 6: Expression levels of *IGF* in the pancreas of all rats

GPx: Diabetic rats exhibited lower GPx expression than the normal group. GPx expression was significantly increased in the 1g and moderately increased in the 2g *Peganum harmala* group compared to the diabetic group. In contrast, the standard drug group had decreased GPx expression compared to normal, with a marked reduction in the placebo groups (Fig. 7).

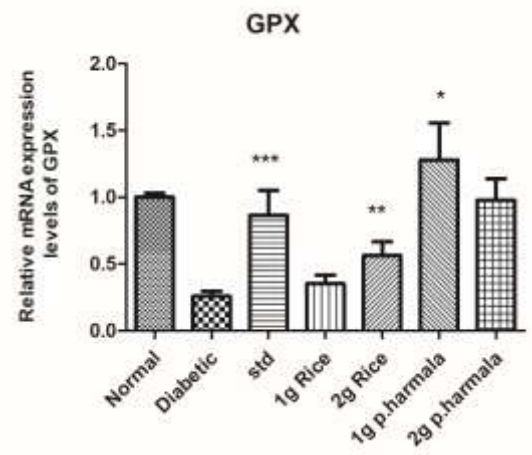


Fig 7: *GPx* mRNA expression and activity in the pancreas of all groups.

Caspase 3: Diabetic and placebo groups had significantly higher expression compared to the normal group. The expression was reduced in the standard drug, 1g, and 2g *Peganum harmala* treated groups compared to the diabetic group (Fig. 8).

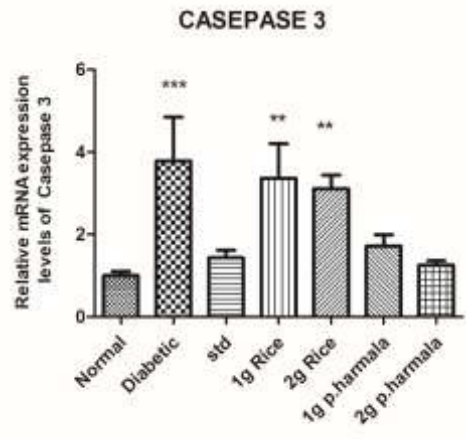


Fig 8: The expression level of *caspase3* in various groups

ki67: The expression was greatly reduced in the diabetic and placebo groups compared to the normal group. The 1g and 2g *Peganum harmala* treated groups showed significantly increased expression compared to the diabetic group, with

the standard drug group showing similar improvements (Fig. 9).

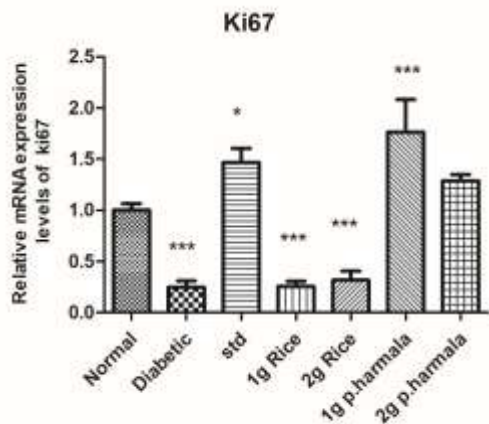


Fig 9: *ki67* mRNA expression and activity in the pancreas of all groups.

Histopathology

The normal control group, both beta and acinar cells appeared healthy and exhibited no signs of inflammation, degradation, calcification, or granuloma (Fig. 10a). In the diabetic control group, damage to beta cells was evident, though there were no signs of calcification, inflammation, granuloma, or distortion (Fig. 10b). The standard drug group (Gliclazide) showed mostly normal acinar and beta cells, with only minor degeneration of beta cells and no evidence of calcification, inflammation, or granuloma (Fig. 10c). In Placebo Group 1, there were fewer beta and acinar cells, but no signs of inflammation, calcification, granuloma, or malignancy were observed (Fig. 10d). Placebo Group 2 showed a reduction in both beta and acinar cells, along with slight beta cell destruction, though no signs of inflammation or calcification were present (Fig. 10e). The 1g *Peganum harmala* group displayed relatively normal beta and acinar cells, with no evidence of degeneration, inflammation, or granuloma (Fig. 10f). Lastly, in the 2g *Peganum harmala* group, the arrangement of beta cells was normal, and acinar cells were regular, with no signs of inflammation, degeneration, or calcification (Fig. 10g).

Discussion

Diabetes is a metabolic disorder that affects the metabolism of fats, proteins, and carbohydrates, leading to elevated blood glucose levels after consuming any type of diet. This condition is primarily caused by insulin deficiency and can result in serious complications such as heart disease, blindness, renal failure, and even death (Kooti et al., 2016). Globally, approximately 25% of the population is affected

by diabetes (Kavishankar et al., 2011). Type 1 diabetes is caused by the destruction of insulin-producing pancreatic beta cells, while type 2 diabetes is characterized by insulin resistance. Although modern diabetes treatments are available, they often have adverse side effects. As a result, the use of alternative medicine has gained significant attention as researchers explore safer and more effective options for managing diabetes (Al-Rowais, 2002). Medicinal plants play a key role in diabetes treatment (Preethi, 2013).

In vivo antidiabetic activity was conducted on STZ induced diabetic rats in which *Peganum harmala* leaf powder was given selected doses orally to two groups and two groups were given rice powder as a placebo groups excluding the diabetic and normal rats and one group was given gliclazide as a standard drug. During 14 days *Peganum harmala* leaf powder showed improvement in weight gained rats indicating the elevation in metabolic actions of body, signifying amplification of glucose metabolism by test.

Biochemical parameters, including amylase and lipase levels, were measured in all groups. The treated groups exhibited decreased amylase and lipase levels compared to the diabetic control group, suggesting improved pancreatic function. Previous studies also reported elevated amylase and lipase activity in diabetic conditions (Steinberg et al., 2017). Several studies have explored similar effects in diabetic models and found that pancreatic islets prevent lymphocytic infiltration and protect mice from type 1 diabetes, showing a significant decrease in IGF expression (Casellas et al., 2006). In 2009 Zeng *et al* investigate the effects of selenium on pancreatic pro-inflammatory in streptozotocin induced diabetic rats. The mRNA expression level of GPx was found to be decreased in selenite-treated diabetic rats (Zeng et al., 2009). Similarly, a significant decrease in Ki67 expression was observed in streptozotocin-induced diabetic rats treated with chlorella, indicating a protective effect on the pancreas (Amin et al., 2011). In another study, the expression level of Caspase 3 increased in diabetic rats treated with Ginsenoside Rg3 (Liu et al., 2015).

This study showed that the expression levels of *IGF*, *GPx*, and *Ki67* in *Peganum harmala*-treated albino rats were significantly elevated compared to diabetic rats. Moreover, the expression of Caspase 3 was significantly reduced in *Peganum harmala*-treated rats, indicating improved pancreatic function in the treated groups.

Conclusion

The present study demonstrates that *Peganum harmala* leaf powder has significant antidiabetic effects in streptozotocin-induced diabetic rats. The administration of *Peganum harmala* improved both biochemical and histopathological

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parameters, such as blood glucose, amylase, and lipase levels, as well as gene expression in the pancreas. These findings suggest that *Peganum harmala* can enhance pancreatic function and may serve as a promising natural therapy for diabetes management. Further studies are warranted to explore its therapeutic potential and underlying mechanisms in greater detail.

Contributions

SMA conceptualized and designed the study. GN, and SHK were responsible for sample collection and conducted the laboratory experiments. GN analyzed the data and drafted the manuscript.

Source of funding

N/A

Conflict of interest

The authors affirm that they have no conflicts of interest.

Ethical statement

The Institute of Molecular biology and Biotechnology Ethics Committee, University of Lahore, Punjab, Pakistan approved all animal handling procedures.

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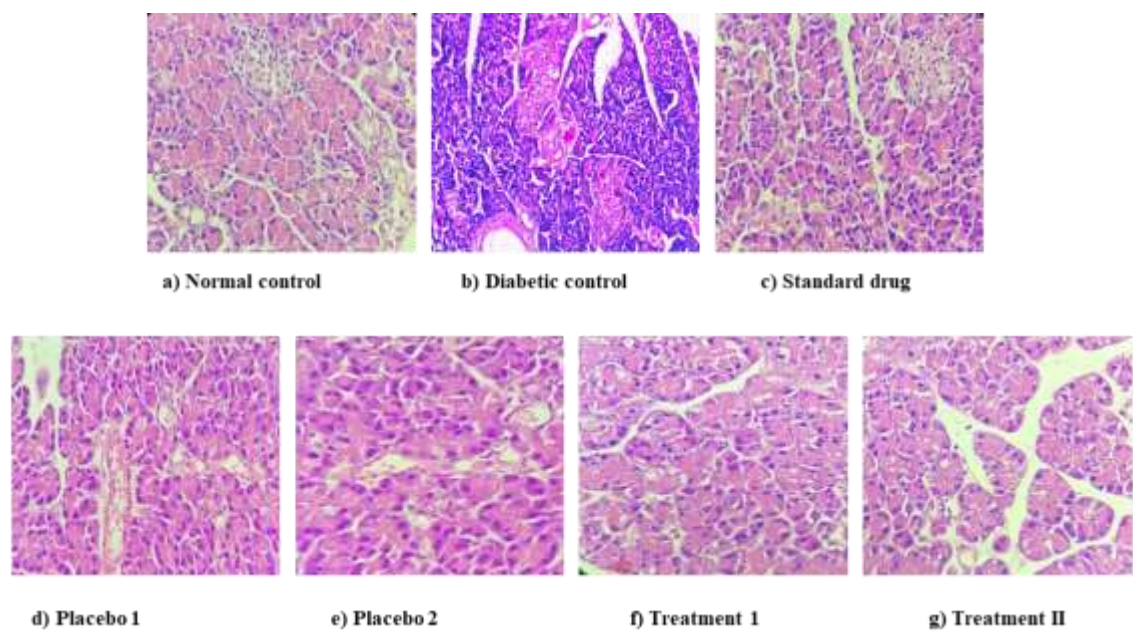


Fig 10: Histological examination of Pancreas tissue in control and treated groups