PANCREA TO PROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF *PTEROCORPUS MARSUPIUM* ON RAT PANCREATIC ISLETS IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETES MODEL

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ABSTRACT

Background: Type 2 Diabetic complications are one of the most common problems in the society. Increased glucose causes human pancreatic beta cells to produce cytokines, which impede insulin production and cause apoptosis and reduced cell proliferation. The secreted proinflammatory cytokine can cause local pancreatic-islet inflammation (insulitis) resulting in the gradual depletion of β cells that produce insulin. Here we evaluated the protective and anti-inflammatory effects of *Pterocarpus marsupium* on pancreatic islets in diabetes rats. Methods: Rats were given streptozotocin-nicotinamide (STZ-NA) intraperitoneally (i.p.) to induce diabetes. Five groups of animals were created, including normal control (NC), disease control (DC), two groups treated with 250 & 500 mg/kg of *P.marsupium* (PM250 & PM500) and a group by standard drug glibenclamide (500 µg/kg) (Glib500). After 120 days of treatment, the blood was collected from tail vain and estimation of insulin in plasma, IL-6, and IL-10 was ELISA-determined. HbA1c and fasting blood sugar levels were calculated by glucometer and nephelometry, respectively. Morphology of the rat pancreas and islet was evaluated by H&E staining. Results: Insulin levels and inflammatory cytokines DC had significantly greater levels of IL-6 and IL-10 ($p<0.004$).. The IL-6 levels in the PM250 & PM500 and Glib500 groups significantly decreased ($p<0.05$), but the alterations in insulin and IL-10 levels were negligible. When compared to NC, diabetic controls had as substantially higher glucose and HbA1c levels ($p < 0.05$). Test groups PM250 & PM500 and Glib500 showed significant decrease in glucose & HbA1c. Pancreatic acinar cell damage, cell atrophy and destruction of beta cells in DC was observed under microscope. There was a clear improvement in pancreatic beta cell regeneration, decreased congestion and edema in test groups. Conclusion: According to our research, P. marsupium may be a game-changer for reducing inflammation and islet damage in type 2 diabetes, as it exhibited anti-inflammatory activity and a significant improvement in beta cell regeneration of pancreatic islets in diabetes rats.

KEYWORDS: Pterocarpus marsupium, Diabetes mellitus, Inflammation, Pancreatic beta cells, Streptozotocin-Nicotinamide.

INTRODUCTION

A substantial rise in blood sugar levels brought on by a partial or whole lack of insulin action or secretion is the hallmark of diabetes mellitus, a chronic metabolic disease. According to the WHO Diabetes Global Report 2021, diabetes directly caused one point five million deaths in 2019 and affects 42. 2 crore persons globally, elevated blood glucose levels also contributed to 3.7 million deaths, which were caused by a variety of diseases and organ failures (1). From a pathogenic perspective, insulin resistance and the widespread death of beta cells in the pancreas (due to the production of autoantibodies) are the causes of diabetes mellitus of t1 and t2, respectively (2). Both t 1 and t 2 diabetes have been shown to have inflammation is probably a major factor in the destruction or functioning of the pancreatic islets. By improving glucose utilization and altering oxidative phosphorylation, increased free fatty acids (FFAs) and hyperglycemia may sustain inflammation. Studies reveal that the pro-inflammatory characteristics of macrophages found in or invading adipose tissues and

islets are influenced by these aberrant metabolic processes. Furthermore, by producing more interleukin-1 and interleukin-6, two active inflammatory cytokines, oxidative stress brought on by hyperglycemia and lipotoxicity might trigger an inflammatory response. By encouraging the overproduction of extra cytokines and chemokines, which in turn draw in more macrophages, interleukins exacerbate inflammation. Increased inflammation can cause insulin resistance, beta-cell malfunction, and eventually cell apoptosis (8).

Insulin replacement treatment and oral hypoglycemic medications, such as inhibitors of sodium-glucose co-transporter type 2 (SGLT-2), sulfonylureas, metformin, acarbose, and inhibitors of Both t1 and t2 diabetes are treated with dipeptidyl peptidase 4 (DPP-4). Even though there are several anti-diabetic drugs available, their use is frequently restricted because of their high cost and potential for long-term negative effects. As a result, research is still being done to find safe and effective therapy alternatives.

Numerous medicinal plant species contain alkaloids, which have been investigated as possible alternatives to medications that reduce inflammation and diabetes. Numerous previous studies have shown the advantages Using plant extracts and their byproducts in diabetes care. The Leguminosae family includes the tall tree Pterocarpus marsupium Roxb, also called Bijasar or Vijayasar. It is frequently seen in Sri Lanka western, eastern, and southern regions and in India. For many years, Ayurveda has utilized the bark, heartwood, leaves, and flowers of the P. marsupium tree for therapeutic reasons. In the past, studies have shown that P. marsupium heartwood has positive benefits on inflammatory diseases and diabetes. Additionally, research has demonstrated that the tree's flavonoid and phenolic component concentration confers antihyperlipidemic and antioxidant qualities. Furthermore, P. marsupium heartwood is used for its astringent and antihelmintic qualities as well as to treat leprosy, bronchitis, asthma, diarrhea, and skin conditions. Therefore, in albino Wistar rats that had been given STZ-NA diabetes, We sought to evaluate the pancreatic regeneration and anti-inflammatory qualities of P. marsupium, a natural extract.

MATERIALS AND METHODS

Animals: A total of 100±5g male albino Wistar rats were supplied by the Central Animal House of Mangaluru's Kasturba Medical College. The rats were kept in a controlled environment with a 12-hour light and dark cycle (16) and were given full access to water and normal rat food.

Plant collection: Dr. Nagalakshamma from the Botany Department at Santosius College in Mangaluru, Karnataka, confirmed the identity of the P. marsupium heartwood, which was obtained from Alva's herbal pharmacy in Moodbidri, Karnataka (voucher number: The Wood/2006/745/62).

To prepare the plant extract, 30g of powdered P. marsupium heartwood, dry and coarse was cooked for 15 minutes at 50˚C in 16 parts (480ml) of water. A rotary vacuum flash evaporator was used to evaporate the filtrate for seven hours at 75˚C after the resultant mixture had been filtered through muslin cloth. A semi-solid extract was obtained by collecting the leftover residue from the round-bottom flask and drying it with a heating mantle for three hours. After that, this extract was kept for later research at -4˚C in a refrigerator.

Chemicals: Cipla Pvt Ltd in Mumbai, India, provided the glibenclamide, while Himedia Drug Company in India provided the nicotinamide and streptozotocin.

Ethical approval: Prior to commencement, the study required approval from the Institutional Animal Ethics Committee (IAEC) of Kasturba Medical College, Manipal University, Karnataka, India (Certification No. 14062013).

Diabetes induction: 15 minutes prior to the administration of STZ at a dosage of 50 mg/kg dissolved in 0.1M citrate buffer (pH 4.5), the rats were given intraperitoneal injections of 25 mg/kg nicotinamide dissolved in normal saline (17). Rats were chosen from the general population, and only after their blood glucose levels exceeded 250 mg/dL were they placed in the appropriate groups.

Experimental Design:

The study included 30 rats in total, 24 of which had diabetes and 6 of which were normal. Five groups of six rats each were created from the rats. On the seventh day following the STZ-NA injection, oral therapy with plant extract and Glibenclamide started. This course of therapy lasted for sixteen weeks.

Group I: Saline-treated normal controls (Control)

Group II: Diabetic untreated group (Model)

Group III: Glibenclamide 500µg/kg BW (Glib500)-treated diabetic rats

Group IV: P. marsupium 250 mg/kg BW (PM250)-treated diabetic rats

Group V: P. marsupium 500 mg/kg BW (PM500)-treated diabetic rats

Estimation of Glycemic profile: A glucometer was used to measure blood glucose, and nephelometry was used to measure HbA1c. Insulin was quantitated in

serum collected at the end of the study period, by Sandwich ELISA technique using anti-rat insulin antibodies from Genexbio pvt.ltd. Delhi, India as per the instructions given by the manufacturer.

Estimation of Interleukins: Il-6 and Il-10 of serum and kidney were estimated using Rat IL-6 & IL-10 ELISA kits which were bought from RayBiotech pvt ltd. USA. by using ELX 800 ELISA reader.

Histological assessment of pancreas: Following the procedure outlined by Feldman et al., 2014, the pancreas was prepared for histological analysis after being removed from anesthetized rats (18). In a nutshell, tissue was placed in paraffin wax after being treated with 10% formalin. The blocks were divided into 4µm thick slices using a rotary microtome. Hematoxylin and Eosin was used to stain these sections after they had been deparaffinized. To evaluate and rank the histo-architectural distortions for each experimental group, sections were examined under a microscope. Histomicrographs of typical sections were produced.

Statistical analysis:

For every batch of six animals, the data was shown as Mean ± S.D. The means of the groups were compared using Tukey's post hoc test following a one-way ANOVA. The statistical software SPSS version 16 was used for the analysis. A p-value was considered significant if it fell below 0.05.

RESULTS

Group	RBG (mg/dL)	HbA1C (%)	Insulin (ng/ml)
Control	119.17±8.65	4.11±0.26	12.75 ± 2.65
Model	469.67±20.04a	7.33±0.25a	26.82 ± 2.24a
Glibenclamide 500µg/kg (Glib500)	148.41±8.74b	4.73±0.36b	15.97 ± 1.38b
P.Marsupium 250mg/kg (PM250)	171.17±18.69 b	5.24±0.12b	16.6 ± 4.82b
P.Marsupium 500mg/kg (PM500)	169.83±15.43 b	5.50±0.48b	20.28 ± 3.37b

Table 1: Random Blood Glucose, HbA1c and Insulin in various groups

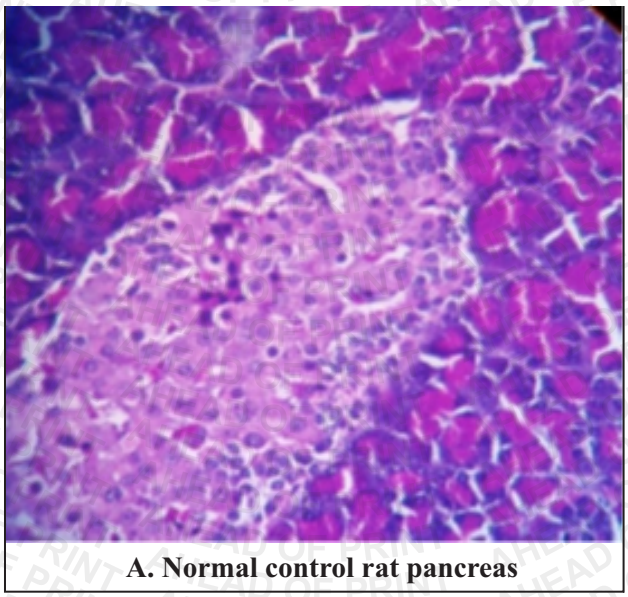
Mean ± SD is employed to denote values (n = 6 per group). P < 0.01 represents a significant difference compared to the sickness control group. P < 0.05 denotes the comparison between Control and Model.b- P < 0.05 (Model vs. PM or Glibenclamide).

Group	In Serum		In Kidney	
	IL-6 (pg/ml)	**IL-10 (pg/ml)**	**IL-6 (pg/ml)**	**IL-10 (pg/ml)**
Control	**20.43 ± 5.83**	**20.97 ± 8.62**	**74.70±33.60**	**1981.44±84.4**
Model	**115.70 ±13.65[a]**	**111.61 ± 31.43[a]**	**149.16±21.06[a]**	**3182.1±60.73[a]**
Glibenclamide 500µg/kg (Glib500)	**47.98 ± 7.29[b]**	**189.81 ± 21.29[b]**	**65.90±13.9[b]**	**1791.6±41.9[b]**
P.Marsupium 250mg/kg (PM250)	**54.58 ± 7.06[b]**	**101.11 ± 28.78**	**89.01±3.26[b]**	**2805.5±59**
P.Marsupium 500mg/kg (PM500)	**49.18 ± 9.02[b]**	**96.50 ± 10.43**	**117.87±25.3[b]**	**3017.0±65.2**

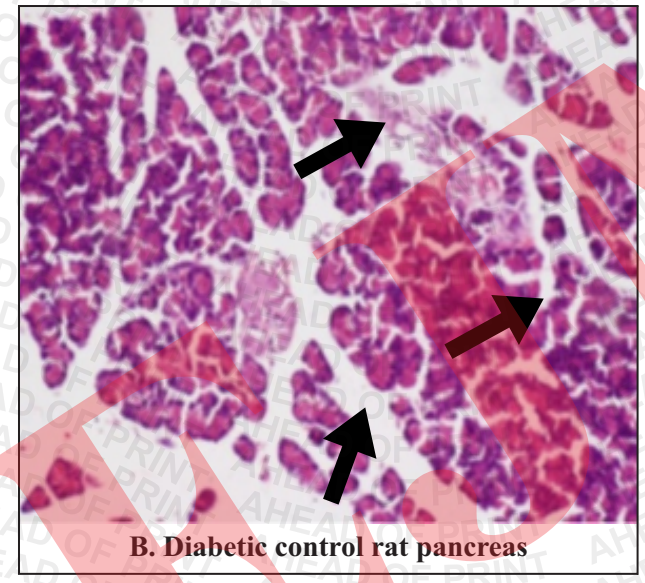
Table 2: IL-6 and IL-10 levels in Serum and Kidney in various groups

Values are expressed as mean ± SD (n = 6 per group). A significant difference from the ill control group is shown by a P < 0.01 value. P (Control vs. Model) < 0.05.

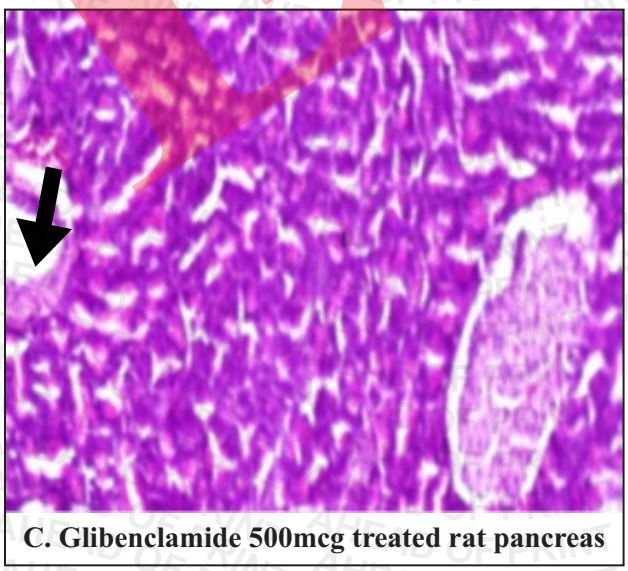
b- P < 0.05 (Model vs. PM or Glibenclamide).



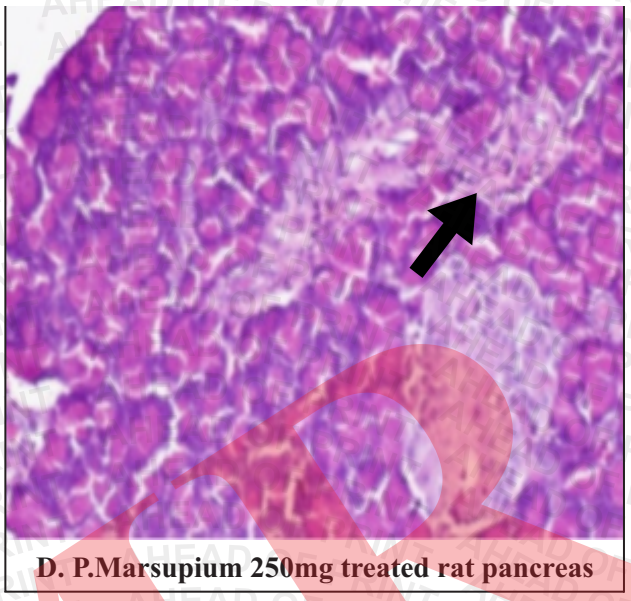
A. Normal control rat pancreas



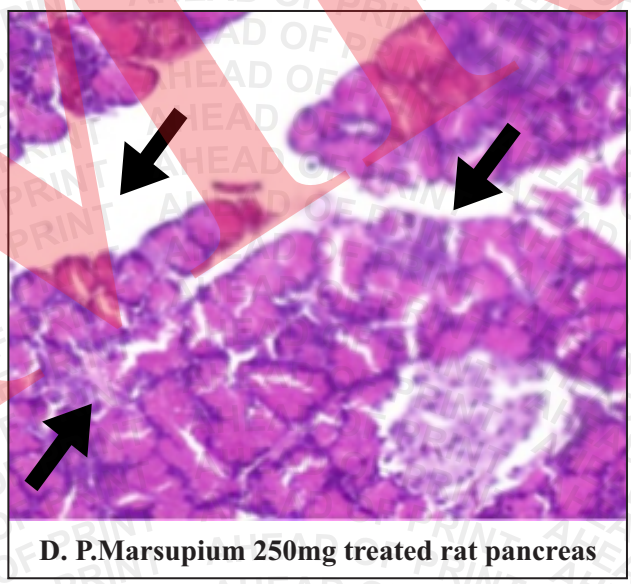
B. Diabetic control rat pancreas



C. Glibenclamide 500mcg treated rat pancreas



D. P.Marsupium 250mg treated rat pancreas



D. P.Marsupium 250mg treated rat pancreas

Fig. 1: Histopathology of Pancreas of various groups

Histopathology pictures of Pancreas of various groups:

The images (magnification ×200) show six animals each experimental group. a. Pancreatic lobules were arranged into tiny lobules, and islet cells were seen strewn among acinar cells. The control group had normal pancreatic anatomy, including normal exocrine gland and acinar cells. Compared to the nearby acinar cells, the islets appeared to be less defined. b. The exocrine and endocrine glands of diabetic rats showed pathological alterations. Small vacuoles and swollen acinar cells were observed in almost all the parts of pancreas and the epithelium was compressed [indicated by black arrow]. Model group rats showed almost complete destruction of Islet β-cells. c. Diabetic

rats treated with Glibenclamide exhibited some changes in the general architecture. Acinar damage, cell atrophy and vacuolation was observed in most of the exocrine part [indicated by green arrow]. Additionally, wider intralobular (shown by the blue arrow) and interlobular (shown by the red arrow) ducts were seen. d. After receiving PM250, diabetic rats showed almost normal islet cell structure. A distinct demarcation between the exocrine and endocrine sections was seen, and there was acinar cell atrophy and destruction that was somewhat less severe. g. PM500-treated diabetic rats showed that their islets cells had recovered. It was also discovered that the vacuoles in the acinar cells' basal region were smaller.

DISCUSSION

In this investigation, diabetic rats treated with PM showed a distinct glucose response suggestive of normal glycemic kinetics, as indicated by RBG levels (below 175 mg/dL) and HbA1c levels (below 5.5%), in contrast to disease control, which showed noticeably higher values under comparable conditions. The substantial increase in insulin and decrease in blood glucose levels relative to healthy individuals were suggestive of PM's promotion of insulin release and glucose utilization. Epicatechin, an isolated component from PM, has been shown in previous studies to encourage the pancreatic beta-cells' production of insulin and to produce a dose-dependent rise in cAMP levels. Additionally, it has been proposed that PM converts pro-insulin into insulin while increasing the concentration of cAMP in the Langerhans islets in vitro. The standard approach to diabetes mellitus management and treatment with P. marsupium wood is somewhat supported by these preliminary findings, in addition to supporting a previous report on the insulin secretogague impact of P. marsupium (20). Histopathology of model group pancreas showed inflammation and reduction in endocrine part and treatment with PM increased the β-cells density and reduced the lymphocyte infiltration. This impressive finding shows that, in addition to the antidiabetic effect, β-cell regeneration is also possible with PM extract. Recent animal studies suggested that β-cell regeneration (Neogenesis) is possible with certain hormones, including as gastrin and glucagon-like peptide (GLP-1), prevent cell death and encourage the growth and development of β-cells. Many companies are now developing and testing the GLP-1 analogues in this regard in T1DM and T2DM patients (21). Hence plausible mechanism for β-cell neogenesis in the present study could be due to upholding the actions of above hormones by the plant extract.

Although hyperglycemia and insulin resistance/ insensitivity are the central pathologies in diabetes, it is now widely accepted that almost all the complications of diabetes share an inflammatory basis. This has been particularly appreciated in coronary artery disease and nephropathy. It is specified that pro-inflammatory cytokine, IL-6 is one of the earliest cytokines which is upregulated in many infectious conditions. TNF-α and IL-6 in diabetes, increases ROS production and causes activation of Ikkβ. Insulin function is compromised by the phosphorylation of Ser307 IRS-1, which results from Ikkβ activation (22). Furthermore, IL-6 overexpression increased β-cell inflammation (23). In a similar vein, the illness model group in this study had higher serum and kidney levels of the cytokine IL-6. In a number of renal illness models, mesangial proliferation, tubular atrophy, and interstitial infiltration are all directly correlated with kidney IL-6 expression, which further advances the course of the disease (24, 25). In comparison to the model group, the PM therapy group's serum and kidney IL-6 levels were noticeably lower. Our results are corroborated by earlier research showing that anti-IL-6 treatment or the usage of plants with anti-inflammatory qualities lowers blood levels of pro-inflammatory cytokines, improving the sensitivity of insulin and glucose metabolism (26). On the other hand, the model and PM groups' blood and kidneys had IL-10, an anti-inflammatory cytokine, was present in much greater amounts than in the control groups. The rise in IL-10 levels in the model group might be due to T helper cells' higher transforming growth factor-beta (TGF-β) production in reaction to IL-6 (27). Interestingly, IL-10 is believed to inhibit IL-6 production; earlier research has shown that IL-10 therapy can completely cure insulin resistance caused by IL-6. (28). The PM and Glib500 treated groups showed significantly higher levels of IL-10 in their blood and kidney homogenate, which is in line with previous research. IL-10's protective actions might be linked to the restoration of insulin signaling and muscle fatty acyl-CoA levels (29). Liver and skeletal muscle insulin resistance was caused by acute IL-6 injection in vivo; however, IL-10 co-treatment prevented both lipid-induced and IL-6-induced insulin resistance (28). Thus, the inhibitory activity of IL-10 is acknowledged as a possible explanation for the reduced IL-6 levels seen in the treated groups.

CONCLUSION

Using a diabetic model made using STZ-NA, the current study measured blood and kidney levels of IL-6 and IL-10 and looked at pancreatic histology to investigate the anti-inflammatory and pancreatic healing effects of the aqueous extract of PM.

Following PM treatment, beta cell regeneration increased, congestion, edema, and necrosis decreased, and the tissues' levels of IL-6 and IL-10 decreased. The herbal extract's therapeutic and supplemental potential in the treatment of diabetes and its associated conditions is supported by these findings.

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