Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

PHARMACOLOGICAL EVALUATION OF GUAVA TENDER LEAVES EXTRACT IN CONTROLLING DIABETES-RELATED HYPERGLYCEMIA AND DYSLIPIDEMIA

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Cite this paper as: Damayanthi Dalu, Rohini Karunakaran, Narinder Kaur, Konda V V S Krishna, Ashok Kumar BS, Anita Pari, Nilesh Pratapsing Babre, Gaurav (2024) Pharmacological Evaluation Of Guava Tender Leaves Extract In Controlling Diabetes-Related Hyperglycemia And Dyslipidemia. *Frontiers in Health Informatics, 13* (8),

ABSTRACT: The antidiabetic and antioxidant qualities of a methanolic extract from *Psidium guajava* tender leaves (PGTL-M) were examined in rats with diabetes caused by streptozotocin. PGTL-M at 100 mg/kg, PGTL-M at 200 mg/kg, normal control, diabetic control, and a group treated with Glibenclamide at 5 mg/kg were the five groups of adult male Wistar rats. A single 50 mg/kg dosage of streptozotocin was used to produce diabetes, and the patients were managed for 30 days. Blood glucose levels, lipid profiles, and oxidative stress indicators including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and thiobarbituric acid reactive substances (TBARS) were assessed. Pancreatic tissue was also subjected to histological examination. The results showed that the 200 mg/kg dosage of PGTL-M was more successful in reducing blood glucose levels than the 100 mg/kg dose. Lipid profiles improved as total cholesterol, low-density lipoprotein (LDL), and triglycerides (TG) dropped and high-density lipoprotein (HDL)

rose. Higher levels of CAT, SOD, and GSH, as well as lower levels of TBARS, which indicate less oxidative stress, demonstrated improved antioxidant defences. Pancreatic beta cells significantly recovered at the 200 mg/kg dose, according to histological examination. To sum up, PGTL-M showed dose-dependent antioxidant and antidiabetic effects, indicating that it may be used therapeutically to treat diabetes and its consequences.

Keywords: *Psidium guajava*, Diabetes, Acute toxicity, Oxidative stress, Antidiabetic, Antioxidant

INTRODUCTION:

Diabetes mellitus, a chronic metabolic disease marked by persistent hyperglycaemia, is one of the most common non-communicable diseases worldwide. Its occurrence is rapidly increasing due to sedentary behaviour, shifting lifestyles, and growing obesity rates. Blood glucose levels rise as a result of Type 1 diabetes's inadequate pancreatic synthesis of insulin and Type 2 diabetes's decreased insulin action. Diabetes-induced chronic hyperglycaemia damages the kidneys, heart, blood vessels, nerves, eyes, and kidneys over time. One of the main metabolic disorders associated with diabetes is hyperlipidaemia, a condition characterised by elevated blood lipid levels. Hyperlipidaemia, commonly referred to as dyslipidaemia, is common in people with type 2 diabetes. Because HDL is low in this disease, triglycerides (TG), low-density lipoprotein (LDL), total cholesterol (TC), LDL, and VLDL are all elevated. The primary cause of hyperlipidaemia in diabetics is insulin resistance, which leads to abnormal lipid metabolism. TG-rich lipoproteins accumulate because insulin resistance reduces the activity of lipoprotein lipase, an enzyme required for the breakdown of triglycerides. Moreover, hyperlipidaemia in diabetics is caused by both reduced hepatic lipid clearance and increased transfer of free fatty acids from adipose tissue (Abbate & Brunzell, 1990; O'Brien, Nguyen, & Zimmerman, 1998).

The risk of cardiovascular diseases (CVD), the primary cause of death for those with diabetes, is greatly increased when hyperlipidaemia and diabetes coexist. Atherosclerosis, a disorder in which lipid deposits create plaques on blood vessel walls, causes constriction or blockages that obstruct blood flow, is accelerated by hyperlipidaemia. Peripheral vascular disease, heart attacks, and strokes are all made more likely by this surgery. In actuality, those who have Type 2 diabetes are two to four times more likely to experience cardiovascular issues than those who do not (Anderson & Zochodne, 2016). For diabetic patients, controlling both hyperglycemia and hyperlipidaemia is essential to lowering their risk of complications. To treat hyperlipidaemia, pharmacological treatments like fibrates and statins are frequently employed. However, traditional therapies frequently have negative side effects, and because they may be able to manage both hyperglycaemia and hyperlipidaemia with fewer negative consequences, there is growing interest in alternative solutions such plant extracts. Natural compounds with lipid-lowering and hypoglycaemic qualities have been the subject of recent studies because they may help manage diabetes and related metabolic diseases (Menke, Casagrande, Geiss, & Cowie, 2015; Pop-Busui et al., 2016). To sum up, hyperlipidaemia is a serious side effect of diabetes, and controlling both diseases is essential to lowering cardiovascular risk and enhancing long-term results for diabetics. Investigating natural treatments that address cholesterol and blood glucose levels may offer a viable strategy for managing diabetes holistically (Chan, Gordon, Zochodne, & Power, 2014; Gore, Brandenburg, Hoffman, Tai, & Stacey, 2006).

Oxidative stress is one of the primary causes of diabetes and its associated comorbidities, including hyperlipidaemia. It occurs when the production of reactive oxygen species (ROS) is out of equilibrium with the body's ability to neutralise them through antioxidant defences. Chronic hyperglycaemia linked to diabetes increases the generation of reactive oxygen species (ROS),

which can oxidatively damage proteins, lipids, DNA, and other components of cells. Beta-cell dysfunction, insulin resistance, and the emergence of diabetes sequelae, such as retinopathy, neuropathy, nephropathy, and cardiovascular disease, are all impacted by this oxidative damage.
Diabetes and hyperlipidaemia frequently coexist. Hyperlipidaemia intensifies oxidative stress by encouraging lipid peroxidation, a process in which reactive oxygen species (ROS) target lipids in cell membranes and generate toxic byproducts such malondialdehyde (MDA). These byproducts harm cellular structures, impair insulin signalling and endothelial function, and exacerbate the metabolic issues associated with diabetes. Together, hyperglycemia, hyperlipidaemia, and oxidative stress create a vicious cycle that speeds up the onset of cardiovascular diseases and other diabetes-related side effects (Arfan, Amin, Kosiska, Karama, & Amarowicz, 2008; Poonia, Sasmal, & Mazumdar, 2007). One potential approach to managing diabetes is to use antioxidant therapy to target oxidative stress in addition to regulating blood glucose and lipid levels. By scavenging ROS, lowering oxidative damage, and enhancing general metabolic health in diabetes patients, natural antioxidants—especially those included in plant extracts—offer significant advantages (Arfan et al., 2008; Poonia et al., 2007).
Psidium guajava tender leaves extract (PGTL-M) is being tested on the basis of the hypothesis that young, tender leaves may contain even more bioactive molecules than mature leaves, enhancing their therapeutic potential in treating diabetes and its related effects. Numerous phytochemicals found in tender leaves, including as flavonoids, saponins, tannins, and polyphenols, have been connected to antidiabetic, antioxidant, and lipid-lowering properties. These bioactive components are essential for enhancing insulin sensitivity, regulating glucose metabolism, and providing defence against oxidative damage. Although juvenile leaves in many plants generally contain more strong concentrations of active chemicals due to their function in early plant defence systems, research on *Psidium guajava's* delicate leaves is still very restricted when compared to mature leaves. Because they may provide more protection against oxidative stress, a primary cause of diabetes and its related effects, such as hyperlipidaemia and cardiovascular disease, tender leaves have particularly noteworthy antioxidant qualities. Therefore, testing the methanolic extract of Psidium guajava tender leaves presents a fresh and maybe more effective method of managing diabetes. It provides a holistic and natural treatment approach for enhancing metabolic health in diabetics by addressing not just hyperglycaemia but also the lipid abnormalities and oxidative stress that accompany diabetes (CSIR, 1985; Marchete et al., 2021; Pereira et al., 2007).
Rats with diabetes caused by streptozotocin (STZ) were used to examine the antidiabetic, antihyperlipidemic, and antioxidant properties of the methanolic extract of *Psidium guajava* tender leaves (PGTL-M). The main goal was to evaluate how well PGTL-M worked to improve lipid profiles and reduce blood glucose levels in diabetics. Additionally, this study looked at how PGTL-M might improve antioxidant defences and lower lipid peroxidation to lessen oxidative stress. Investigating the protective effects of PGTL-M on pancreatic beta cells and evaluating its capacity to repair or maintain pancreatic tissue in order to enhance insulin production and glucose homeostasis was one of the main goals. In a nutshell, the study aimed to scientifically confirm the traditional use of *Psidium guajava* tender leaves as a natural remedy for diabetes and its aftereffects.

EXPERIMENTAL:

Plant material collection, authentication, and extraction procedure:

Psidium guajava (Myrtaceae) tender leaves were gathered in the Mandi District of Himachal Pradesh, India, in November 2023. A botanist recognised and verified the plant material, and a voucher specimen (BKS/MD/PG/2023/122) was submitted for use in the future. A mechanical grinder was used to crush the collected leaves into a powder after they had been shade-dried.

Following a comprehensive extraction process, the methanolic extract was produced, concentrated at lowered pressure, and yielded 0.85% of the initial material's dry weight. The finished extract, known as PGTL-M (methanolic extract), was kept for later use at 4°C. To guarantee total removal of fatty components, one kilogramme of the powdered material was defatted by macerating it in petroleum ether for 72 hours at room temperature. This procedure was carried out four times.

Animals:

The study included mature male Wistar rats that weighed between 160 and 230 grammes. These animals were housed at 25 ± 2°C in polypropylene cages with a 12-hour light/dark cycle. Before the trials started, the animals, who were acquired from a reliable animal facility, were allowed to acclimatise to the lab for a week. The rats were given a regular pellet meal and unrestricted access to water during the study. Following the rules established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, the Institutional Animal Ethics Committee (IAEC) examined and approved the experimental procedures.

Material: Reagents, chemicals, and drugs:

Glibenclamide and streptozotocin (STZ), the two main medications employed in this investigation, were acquired from Sigma–Aldrich, Mumbai, India, a reputable provider of premium biochemical goods. Because of their unique pharmacological characteristics, these substances were essential to the experimental processes. To guarantee the highest degree of purity and dependability, all additional chemicals and reagents utilised in the investigation were of analytical grade. These supplies were obtained from reputable suppliers of high-quality lab chemicals, including SRL Mumbai, Loba Chem, and E. Merck India. To preserve the accuracy and repeatability of the test findings, these materials must be carefully chosen and acquired.

Study of acute toxicity:

The Organisation for Economic Co-operation and Development (OECD) guideline 423 was used to assess the extracts' acute toxicity (OECD, 2008). The assessment of acute toxicity was carried out in compliance with the chemical testing guidelines established by the Organisation for Economic Cooperation and Development (OECD). Specifically, OECD guideline 423—the Acute Oral Toxicity-Acute Toxic Class Method—was used. Sixteen treatment groups were randomly allocated to adult female Wistar rats weighing between 100 and 60 grammes. The animals were fed just water at will for a full twelve hours prior to the test chemical being administered, during which time they were permitted to fast. The test substance was administered orally in a single dosage, first at a lower level, and over the next 24 hours, any signs of toxicity were tracked. The dosage was gradually increased if there was no sign of toxicity. Over the course of 14 days, the animals were observed for any indications of sickness or death, behavioural abnormalities, and changes in body weight that would indicate delayed toxicity.

Diabetes induction:

In this study, a single intraperitoneal (i.p.) injection of 50 mg/kg of streptozotocin (STZ) dissolved in 0.1 M citrate buffer at pH 4.5 was given. Diabetes occurred in wistar rats after they were starved all night (Ozay, Ozek, Yildirim, & Yildirim, 2020). Adult male Wistar rats that had fasted all night were given a single intraperitoneal injection of newly made streptozotocin (STZ) at a dose of 50 mg/kg body weight in order to induce diabetes. To guarantee the stability and effectiveness of the STZ, it was dissolved in cold citrate buffer (pH 4.5). A glucometer was used to evaluate blood glucose levels 72 hours after injection; rats with values more than 250 mg/dL were diagnosed with diabetes. After being introduced, the animals were given regular pellet food and water while being watched for any indications of discomfort. Before starting therapy, the diabetic state was given

seven days to stabilise (Kohzaki, Vingrys, & Bui, 2008).

Experimental design:

Assessing the impact of a test chemical on rats suffering from streptozotocin-induced diabetes (STZ) was the aim of the study. Thirty adult male Wistar rats, each weighing between 160 and 230 grammes, were randomly assigned to five groups of six (Reda, Zaitone, & Moustafa, 2016):

- Group 1 (Normal Control): Rats were not given diabetes induction; instead, they were given citrate buffer as a vehicle.
- Group 2 (Diabetic Control): Without any further therapy, an intraperitoneal injection of STZ at a dose of 50 mg/kg body weight caused diabetes.
- Group 3 (Standard Treatment Group): The well-known antidiabetic medication Glibenclamide was given orally to diabetic rats at a dose of 10 mg/kg body weight.
- Group 4 (Low Dose Test Group): The test extract was administered orally to diabetic rats at a dosage of 100 mg/kg body weight.
- Group 5 (High Dose Test Group): The test extract was administered orally to diabetic rats at a dosage of 200 mg/kg body weight.

For 28 days, treatments were given once daily. All research animals were fed a regular pellet meal and given unrestricted access to water. Weekly measurements were made of body weight and fasting blood glucose levels. Blood samples were taken for biochemical studies of oxidative stress indicators, insulin levels, and lipid profiles after the rats were put to sleep on the last day.

General Parameters:

To evaluate the physiological and metabolic alterations in the animals as well as the impact of the therapies given on diabetes and general health, a number of general parameters were tracked during the study. These metrics were essential for assessing the test substance's therapeutic potential and offered a thorough grasp of the treatment outcomes.

Body Weight: At the beginning of the trial (the baseline), and then weekly for the next 28 days of the treatment phase, the body weight of each rat was measured. Monitoring body weight was essential since diabetes often causes significant weight loss due to changes in the metabolism of fat, protein, and carbs. A drop in body weight, which indicates the animals' catabolic state, is a common symptom of untreated diabetes. The way the test chemical and reference medication (glibenclamide) changed body weight demonstrated their ability to restore metabolic balance and overall health in diabetic rats.

Fasting Blood Glucose (FBG): A glucometer was used to assess FBG levels at baseline, three days after the streptozotocin (STZ) injection to confirm the onset of diabetes, and then once weekly during the duration of treatment. A 12-hour fast was followed by the collection of blood samples from the tail vein. The main technique for determining the animals' hyperglycaemic condition and the test substance's ability to reduce glucose levels was to monitor FBG levels. The test extract may have antidiabetic effects if the treated groups' FBG levels were noticeably lower than those of the diabetic control group.

Food and Water Intake: Throughout the trial, daily food and drink intake was tracked. Polyphagia, or increased food intake, and polydipsia, or increased water intake, are commonly linked to diabetes and are signs of poor glycaemic control and metabolic dysregulation. The number of metabolic abnormalities in diabetic rats and the test substance's capacity to restore normal eating and drinking behaviour were both better understood by measuring these measures. Following therapy, a decrease in excessive food and drink consumption may serve as a proxy for better glycaemic control.

Mortality and Clinical Signs: Throughout the trial, the animals were constantly observed for any indications of disease, unusual behaviour, or death. Clinical symptoms that are frequently indicative of diabetes complications were noted, including lethargy, dehydration, poor grooming, and untidy

fur. Particular focus was placed on identifying any negative therapy side effects, such toxicity or intolerance. To guarantee the safety of the test material and to identify any undesirable side effects early on, it was crucial to observe the animals' overall behaviour and look.

Biochemical Parameters:

At the conclusion of the treatment session, the animals were administered a mild anaesthesia to facilitate the collection of a blood sample by heart puncture after an overnight fast. To determine how diabetes and therapy impact lipid metabolism, the researchers examined the lipid profile, which includes total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Dyslipidaemia, a disorder marked by high triglyceride and cholesterol levels that raises the risk of cardiovascular disease, is frequently brought on by diabetes. A return to normal cholesterol levels following treatment would indicate that lipid metabolism has improved.

Markers of Oxidative Stress:

The development and course of diabetes problems are significantly influenced by oxidative stress. The study examined a number of important variables to assess oxidative damage and the antioxidant defence system. Because catalase (CAT) converts hydrogen peroxide into oxygen and water, it lowers dangerous reactive oxygen species. As a result, its activity, measured in units per minute, was evaluated. The quantity of superoxide dismutase (SOD), expressed in units per milligramme of protein, was assessed to ascertain its role in transforming superoxide radicals into less hazardous compounds. An improved antioxidant response would be shown by an increase in SOD activity following therapy.

Additionally, the amount of reduced glutathione (GSH), measured in micromoles per gramme of tissue, was determined. GSH is a potent antioxidant that fights free radicals and assists in detoxification; greater GSH levels indicate better cellular defence against oxidative stress. Lipid peroxidation was evaluated using thiobarbituric acid reactive substances (TBARS), which are quantified in nanomoles per minute per milligramme of protein. Elevated levels of TBARS indicate greater oxidative damage to lipids. Following therapy, a drop in TBARS would suggest less oxidative damage and lipid peroxidation. A consequence of lipid peroxidation, malondialdehyde (MDA), was also assessed. The test extract's antioxidative activity and potential to lessen oxidative stress in diabetic rats would be demonstrated by a decrease in MDA and TBARS levels and an increase in antioxidant enzymes like SOD and CAT (Finley & Tietz, 1996; King, 2012).

Histopathological assessments:

To evaluate the prophylactic effects of the test extract and the structural alterations brought on by diabetes, a histological analysis of the pancreas was conducted. All experimental groups, including the test extract group, diabetic control, normal control, and regular therapy group, had their pancreatic tissues taken at the end of the research. Following a 24-hour preservation period in 10% neutral buffered formalin, the tissues underwent xylene washing, graded alcohol dehydration, and paraffin wax embedding. For microscopic examination, thin slices that were around 5 µm thick were cut using a microtome and stained with haematoxylin and eosin (H&E). The work focused on the beta cells that create insulin in the islets of Langerhans. It was anticipated that the diabetes control group would have considerable degeneration since streptozotocin (STZ) selectively destroys these cells. We looked for indications of beta-cell regeneration or preservation in the therapy groups. The test extract's restorative or protective properties would be demonstrated by the observation of healthy islets, improved cell density, or decreased beta-cell mortality. It was anticipated that the conventional treatment group, which was given the beta-cell-protecting medication Glibenclamide, would exhibit better pancreatic architecture. A comparison of the

several groups would demonstrate how well the test extract mitigates pancreatic damage brought on by diabetes (Culling, 2013; I. Tasci & M. Bozdayi, 2007).

Statistical Treatment:

The mean ± standard deviation was used to express the results. One-way analysis of variance (ANOVA) was used to analyse statistical differences between groups at a significance level of $p < 0.05$. Tukey's post hoc test was used to identify particular differences between groups. All analyses were performed using the GraphPad Prism software (Version 8.01). By ensuring a comprehensive and trustworthy analysis of the experimental data, this statistical method made it possible to precisely evaluate the treatment results.

RESULTS:

Over the course of 30 days, the study assessed the impact of a methanolic extract from Psidium guajava tender leaves (PGTL-M) on blood glucose levels in rats suffering from streptozotocin-induced diabetes (STZ). Five groups—normal control, diabetic control, regular Glibenclamide medication, and two test groups that received PGTL-M at dosages of 100 mg/kg and 200 mg/kg—were observed at predetermined intervals. On day 0, 48 hours later, and on days 8, 16, 24, and 30, blood glucose levels were assessed.

Normal glucose homeostasis was demonstrated by the normal control group's consistent glucose levels during the course of the investigation. Following STZ induction, the diabetic control group's blood glucose levels significantly increased and continued to be hyperglycaemic without therapy. Beginning on day 8, the glucose levels in the conventional treatment group significantly decreased, indicating that Glibenclamide is effective in lowering hyperglycaemia.

When compared to the diabetic control group, rats given 100 mg/kg of PGTL-M shown a little but significant decrease in blood glucose levels, indicating a possible antidiabetic activity. A more noticeable drop was seen in the group that received 200 mg/kg of PGTL-M, suggesting that the greater dosage had a more potent antidiabetic effect. However, Glibenclamide was more effective than PGTL-M in lowering blood glucose levels. The dose-dependent antidiabetic effects of PGTL-M are likely attributed to its bioactive compounds—flavonoids, tannins, and polyphenols—which may enhance insulin sensitivity, promote glucose uptake in peripheral tissues, or protect pancreatic beta cells from STZ-induced oxidative damage. All things considered, the results indicate that PGTL-M may be able to lower blood glucose levels in diabetes settings; nevertheless, more investigation is required to fully comprehend its exact processes and long-term impacts.

Table 1. PGTL-M's impact on blood glucose levels

Animal groups	Glucose levels in Blood (mg/dl)					
	Day					
	1	2 (48 hours)	8	16	24	30

		)				
Normal control	87.52+1.51	84.20+1.87	86.31+2.68	88.30+1.87	91.50+2.47	96.57±2.31
Diabetic control	84.13+1.45	[a]310.21+2.311***	[a]354.50+2.82***	[a]248.45±3.75***	[a]258.67±3.41***	[a]267.79±2.94***
Standard treatment	86.46+1.42	314.83+2.19	246.84+2.91	198.42±3.28	178.54+2.54	179.50±3.15
PGTL-M100 mg/kg	84.70+1.61	205.54±2.72	23.18±2.39	[b]226.15±3.73*	[b]232.46±3.11*	[b]239.68±3.04*
PGTL-M	86.25	199.2	214.2	[b]218.	[b]224.	[b]228.

200 mg/kg	+1.63	0±3.33	4±2.87	02±3.86*	63±3.91*	76±3.41*

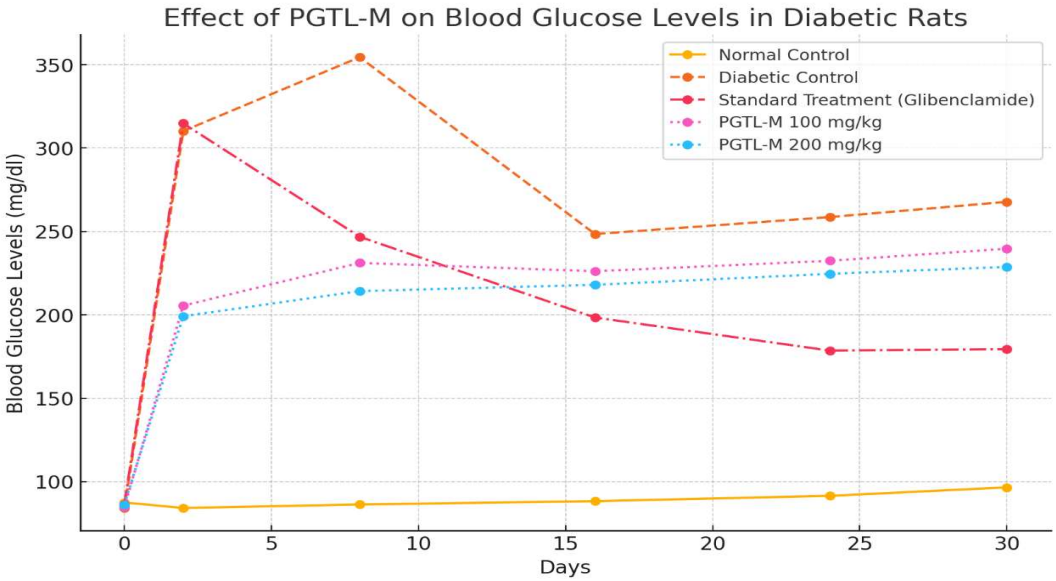

Figure 1. Impact of PGTL-M on blood glucose levels

PGTL-M's impact on the lipid profile:

This study investigated the lipid profile of diabetic rats in relation to a methanolic extract from Psidium guajava tender leaves (PGTL-M). Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were among the lipid parameters that were examined. For each batch of six animals, the data were shown as mean ± standard deviation.

The normal control group's typical lipid measurements demonstrated a normal metabolism. The development of diabetes-related dyslipidaemia was suggested by the diabetic control group's substantial increases in TC, TG, LDL, and VLDL levels and little reduction in HDL. Lipid profiles in the group receiving Glibenclamide, a common antidiabetic medication, were nearly normal, indicating that it was successful in restoring normal lipid metabolism.

Rats given 100 mg/kg of PGTL-M had lipid profiles that were significantly better than those of the diabetic control group. HDL levels marginally increased while TC, TG, LDL, and VLDL levels decreased. Even more pronounced alterations were indicated by the lipid readings of the group that got 200 mg/kg of PGTL-M, which were comparable to those of the group that took Glibenclamide. Our results show that, similarly to Glibenclamide, PGTL-M dramatically improves lipid profiles in diabetic rats, particularly at the higher dosage of 200 mg/kg. One common side effect of diabetes that increases the risk of cardiovascular disease is dyslipidaemia. The enhancements imply that PGTL-M could aid in the control of dyslipidaemia and hyperglycaemia, which could lessen cardiovascular problems in diabetic patients. The bioactive substances in PGTL-M, including polyphenols, tannins, and flavonoids, are probably what improve lipid metabolism. To clarify the

processes by which PGTL-M produces these beneficial benefits, more investigation is required.

Table 2: Lipid profile: the effect of PGTL-M.

Treatment Groups	**TC**	**LDL**	**VLDL**	**HDL**	**TG**
	(U/l)	**mg/dl**			
NC	65.62 ±1.77	59.43 ±1.78	10.45 ±0.67	17.78 ±1.87	36.42 ±1.88
DC	[a]75.72 ±1.80 **	[a]72.73 ±1.79 **	[a]12.85 ±0.95 **	[a]16.34 ±1.56 **	[a]45.32 ±1.92 **
ST	66.42 ±2.45	62.84 ±2.09	10.98 ±0.97	18.23 ±1.71	37.63 ±2.09
PGTL-M100mg/kg	[b]68.92± 2.67*	[b]63.73± 1.98*	10.89 ±0.98	[b]17.94± 1.78*	[b]38.92± 1.99*
PGTL-M200	[b]69.53± 2.81*	[b]62.97± 2.41**	[b]10.58± 1.01*	[b]18.26± 1.88*	[b]38.32± 2.05*

mg/kg					

*The values are shown as Mean ±SD for each of the six values. **p<0.01, ***p<0.001 probability level. Low density lipoprotein is referred to as LDL, very lowdensity lipoprotein as VLDL, total cholesterol as TC, triglycerides as TG, and high density lipoprotein as HDL. NC-* Normal control, *DC-Diabetic control, ST -* Standard treatment.

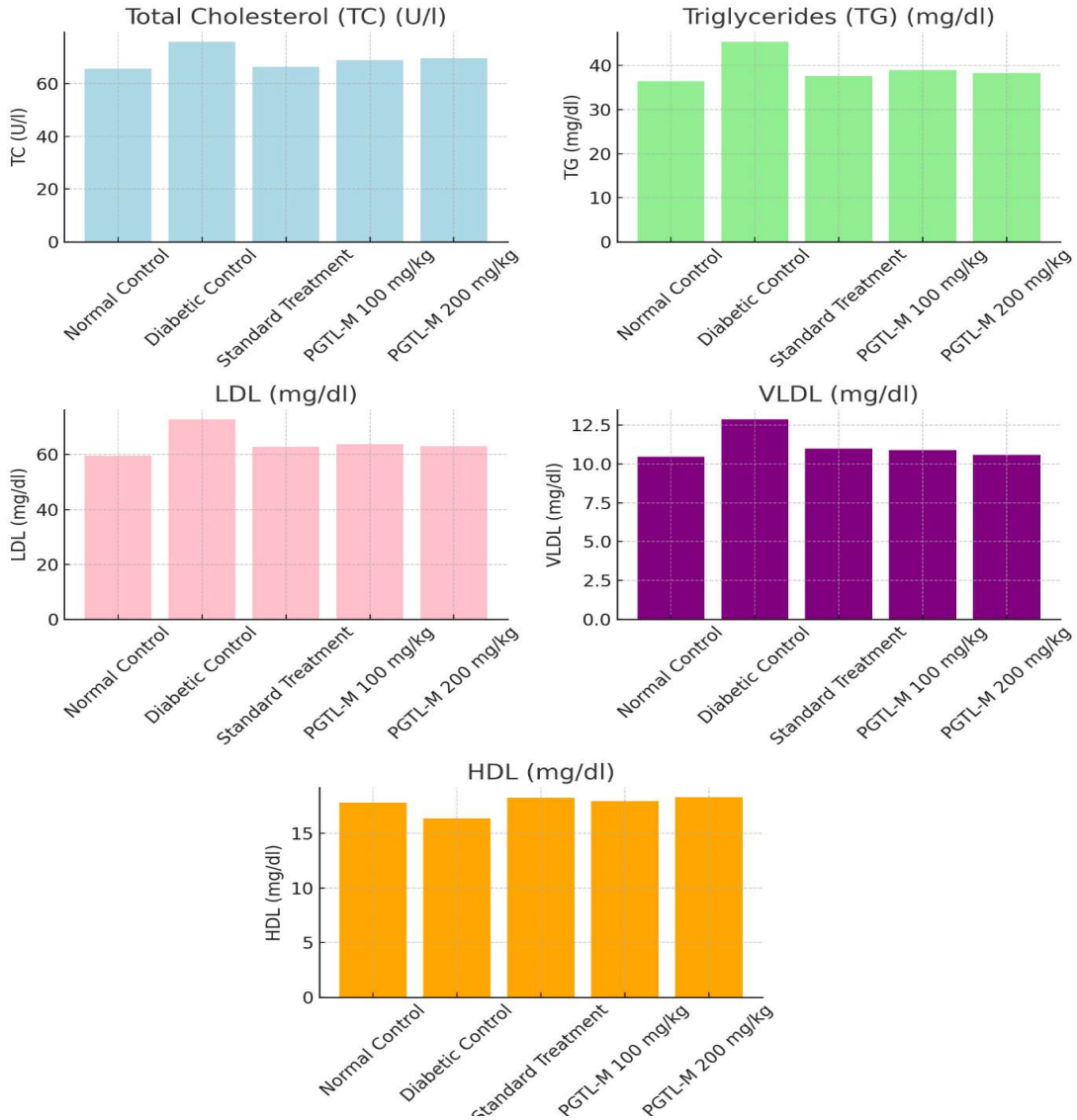

Figure 2. PGTL-M's impact on the lipid profile

Assessment of oxidative stress markers:

The antioxidant effects of a methanolic extract from Psidium guajava tender leaves (PGTL-M) on oxidative stress markers were evaluated in diabetic rats. Thiobarbituric acid reactive substances (TBARS), which show lipid peroxidation, reduced glutathione (GSH) levels, catalase (CAT) activity, and superoxide dismutase (SOD) activity, were the main indications that were assessed. Values were shown as mean ± standard deviation for groups of six animals.

The normal control group had significant GSH and antioxidant enzyme activity together with low

TBARS levels, indicating a balanced oxidative state. Significant reductions in CAT and SOD activity, GSH levels, and elevated TBARS levels were seen in the diabetic control group, suggesting elevated oxidative stress brought on by diabetes. Significant improvements were seen in rats receiving Glibenclamide (usual therapy), including higher levels of GSH, CAT, and SOD activity and lower levels of TBARS, which may indicate less oxidative damage. These indicators significantly improved in the group that had 100 mg/kg of PGTL-M, suggesting some sort of antioxidant activity. The group that received 200 mg/kg of PGTL-M shown even greater benefits than the group that received conventional treatment, with significant increases in GSH and antioxidant enzyme activity and a noteworthy drop in TBARS levels. According to these results, PGTL-M exhibits dose-dependent antioxidant qualities in diabetic rats, most likely as a result of bioactive substances such tannins, polyphenols, and flavonoids. PGTL-M may lessen the oxidative stress linked to diabetes by strengthening antioxidant defences and lowering oxidative damage. To elucidate the underlying processes and evaluate long-term effectiveness, more study is required.

Table 3: Impact of PGTL-M on oxidative stress indicators

	CAT (U/min)	**TBARS (nM /min/ mg protein)**	**SOD (U/mg protein)**	**GSH (µM /g tissue)**
Normal Control	5.28± 0.97	33.30± 1.01	24.53 ±0.99	6.73± 0.97
Diabetes Control	[a] 3.73± 0.94***	[a] 57.28± 1.00***	[a] 13.91 ±1.00***	[a]2.11 ±0.99***
Standard treatment	[b] 5.81± 1.00**	[b]31.88 ±1.01**	[b] 22.88 ±1.02***	[b]7.62 ±0.97***
PGTL-M 100 mg/kg	[b] 5.45± 0.96*	[b]44.83 ±1.01**	[b] 18.52 ±0.98**	[b]5.81 ±0.99**
PGTL-M 200 mg/kg	[b] 5.65± 0.99**	[b]36.28 ±1.02***	[b] 21.61 ±1.01***	[b]6.56 ±1.02***

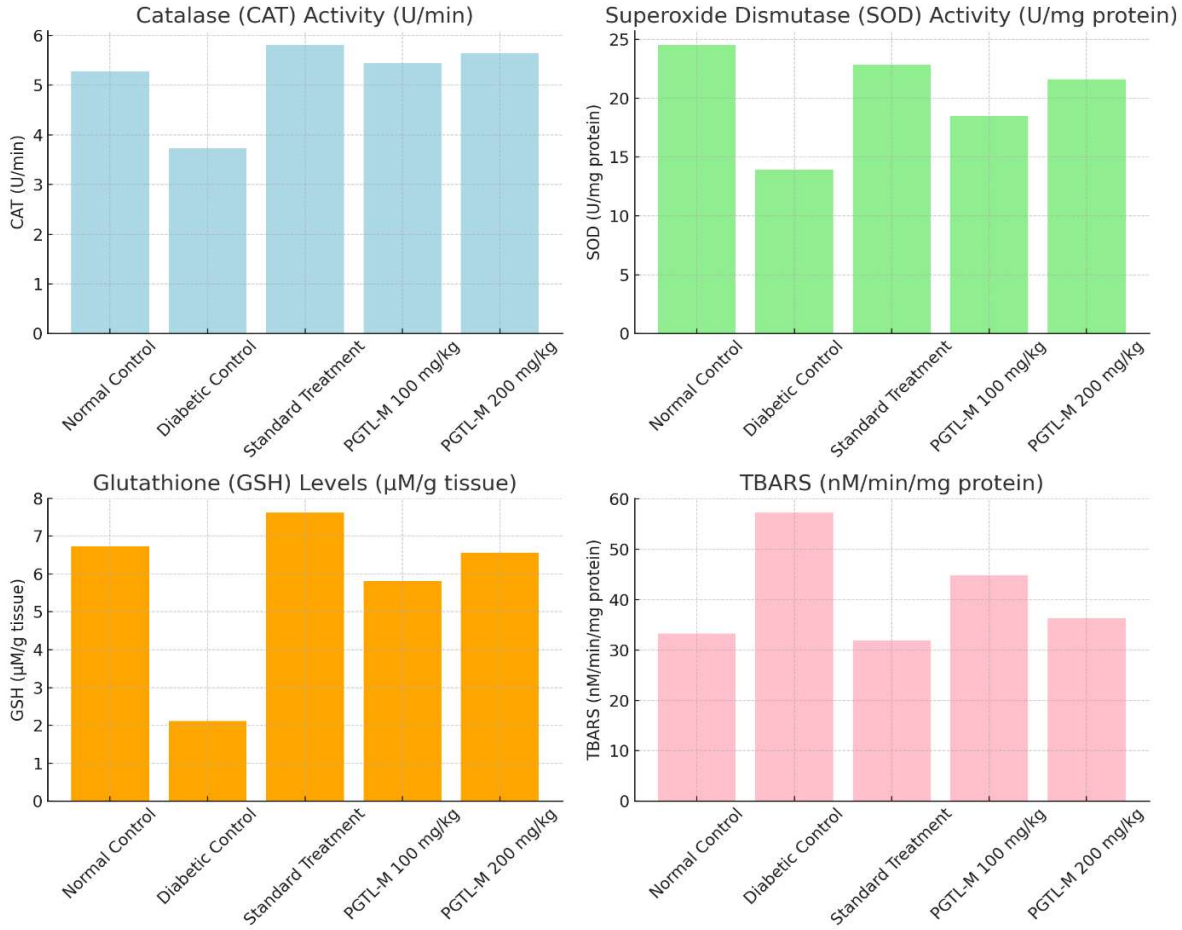

Figure 3. Impact of PGTL-M on oxidative stress indicators

Histopathological examination

Significant variations between the experimental groups were shown by histological examination of pancreatic tissues. The islets of Langerhans were undamaged and had healthy beta cells in the normal control group. The diabetic control group exhibited extensive necrosis, beta-cell degeneration, and considerable islet destruction due to streptozotocin exposure. The standard treatment group, which received Glibenclamide, demonstrated partial restoration of pancreatic architecture, including preserved islets and increased beta-cell regeneration. Rats treated with 100 mg/kg of PGTL-M showed slight improvements, with some beta-cell regeneration and reduced necrosis, although islet damage remained. The group receiving 200 mg/kg of PGTL-M experienced significant increases in beta-cell regeneration and islet structural integrity, suggesting a more complete recovery. At this higher dose, PGTL-M's impact was comparable to the standard treatment, indicating its potential to preserve and repair pancreatic function by reducing diabetes-induced beta-cell damage.

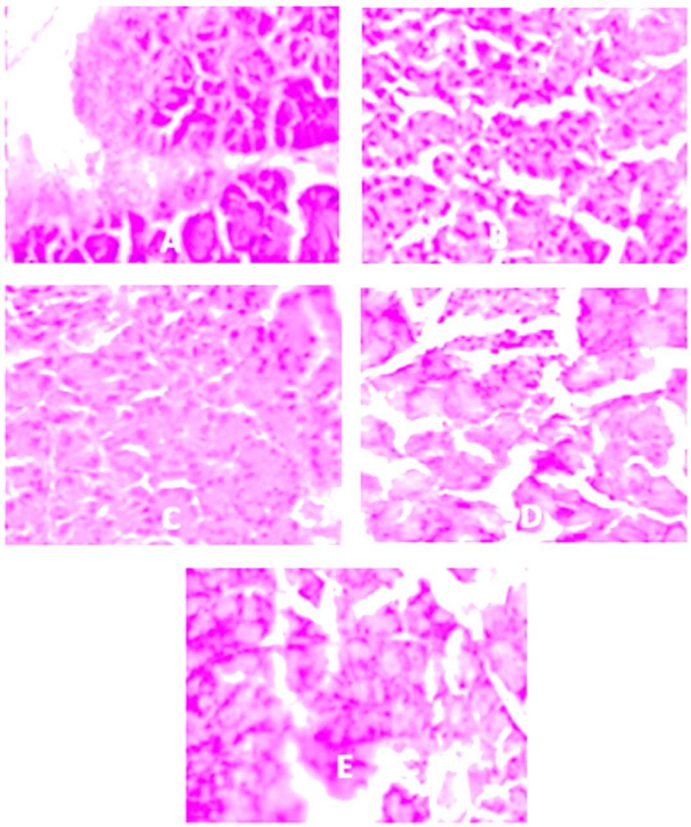

Figure 4. Photomicrographs of the pancreatic tissue from each group of rats that have been stained with H&E and magnified by 200x. A. represents the normal control group; B. represents the disease control or diabetes control group; C. represents the standard treatment group that received 10 mg/kg of Glibenclamide; D. represents the PGTL-M 100 mg/kg test group; and E. represents the test group that received a higher dosage of PGTL-M 200 mg/kg.

DISCUSSION:

Significant hypoglycaemic effects were observed with both the 100 mg/kg and 200 mg/kg doses of PGTL-M; however, the larger dose was more effective and comparable to the standard antidiabetic drug Glibenclamide. The study's findings indicate that PGTL-M, a methanolic extract made from the tender leaves of *Psidium guajava*, has therapeutic promise for the treatment of diabetes and its complications. One notable finding was the dose-dependent decrease in blood glucose levels across treatment groups. This glucose-lowering effect is likely due to bioactive compounds like flavonoids and polyphenols, which enhance insulin sensitivity and glucose uptake (Balaha, Kandeel, & Kabel, 2018; Cherng & Shih, 2006; Deeds et al., 2011; Wei et al., 2003). Additional evidence for PGTL-M's potential as a therapeutic drug came from lipid profile results. In diabetic rats given PGTL-M, triglycerides (TG), total cholesterol (TC), and low-density lipoproteins (LDL) significantly decreased while high-density lipoproteins (HDL) increased. Since dyslipidaemia and diabetes frequently combine and raise the risk of cardiovascular illnesses, it is imperative to return these lipid levels to normal. In addition to regulating hyperglycaemia, PGTL-M's lipid-lowering qualities, particularly at larger dosages, imply that it may lessen cardiovascular problems in diabetic patients. The antioxidant potential of PGTL-M was also shown in the study. Antioxidant enzyme levels,

including reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), sharply rose, suggesting a more robust antioxidant defence system. Furthermore, the extract improved the overall state of oxidative stress and lowered cellular damage by reducing lipid peroxidation, as seen by a drop in thiobarbituric acid reactive substances (TBARS) (Dewanjee et al., 2018; Lipinski, 2001; Liu et al., 2019; Petchi, Parasuraman, & Vijaya, 2013; Piconi, Quagliaro, & Ceriello, 2003; Pitocco, Tesauro, Alessandro, Ghirlanda, & Cardillo, 2013; Yang, Jin, Lam, & Yan, 2011). According to histopathological investigation, PGTL-M therapy, especially at the higher dose, resulted in beta-cell regeneration and the restoration of the pancreatic islet architecture. According to this pancreatic recovery, PGTL-M aids in the preservation or restoration of pancreatic function, which is crucial for long-term diabetic treatment. In conclusion, PGTL-M shows promise as a natural treatment for diabetes, providing several advantages, including decreasing blood sugar, enhancing lipid profiles, lowering oxidative stress, and safeguarding pancreatic tissue. Future studies should concentrate on determining which particular bioactive compounds are in charge of these advantages as well as assessing the long-term safety and effectiveness of PGTL-M in clinical settings.

CONCLUSION:

In rats with streptozotocin-induced diabetes, the study showed that a methanolic extract from the tender leaves of *Psidium guajava* (PGTL-M) has potent antioxidative and antidiabetic properties. Although the greater dose demonstrated superior effectiveness, both the 100 mg/kg and 200 mg/kg doses significantly decreased blood glucose levels and improved lipid profiles. By raising GSH (reduced glutathione) levels and triggering vital enzymes like CAT (catalase) and SOD (superoxide dismutase), PGTL-M improved antioxidant defences. Additionally, it minimised oxidative damage, as seen by lower levels of thiobarbituric acid reactive substances (TBARS) and lipid peroxidation. According to histopathological investigation, PGTL-M shielded pancreatic beta cells from harm brought on by the diabetes-inducing STZ, particularly when 200 mg/kg dose was administered. The notable alterations in pancreatic structure imply that PGTL-M may increase insulin synthesis and reduce the symptoms of diabetes. Overall, the results point to the probable and likely use of PGTL-M as an accepted and natural remedy to lower hyperglycaemia and oxidative stress in diabetic circumstances, providing pancreas protection, especially at larger dosages.

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