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Exploring The Antidiabetic and Nephroprotective Potential of *Litsea Glutinosa:* A Natural Remedy for Metabolic and Cellular Stress

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ABSTRACT
This study evaluates the pharmacological potential of *Litsea glutinosa* (L.) leaves extract, a natural plant-derived extract, with a focus on its antidiabetic and nephroprotective activities. Phytochemical screening revealed the extracts as a rich source of bioactive compounds, including flavonoids, tannins, phenols, and terpenoids, which are known for their therapeutic benefits. The glucose utilization assay in L6 myoblasts demonstrated extracts's significant enhancement of glucose uptake in a dose-dependent manner, highlighting its antidiabetic potential. In the DPP-4 inhibition assay, the potential extract (LG-M) exhibited moderate inhibitory activity, further supporting its role in glucose regulation. Additionally, nephroprotective activity was assessed in cisplatin-induced toxicity in HEK-293 cells. LG-M showed superior cell viability preservation compared to the standard drug Paclitaxel, indicating its protective effect against nephrotoxicity. The total phenolic content analysis confirmed LG-M's robust antioxidant properties, which may contribute to its observed pharmacological effects. Statistical analysis validated the significance of these findings. This comprehensive evaluation underscores LG-M's potential as a natural therapeutic agent for managing diabetes and mitigating nephrotoxicity. Further studies on its bioactive components and mechanisms of action could pave the way for its development as a safe and effective therapeutic alternative.

Keywords: Antidiabetic, Neuroprotective *In vitro* studies, *Litsea glutinosa*, Glucose utilization, DPP-4 inhibition.

1. INTRODUCTION

Chronic diseases such as diabetes and nephrotoxicity are significant public health challenges globally, with increasing prevalence and substantial socio-economic impact. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is associated with long-term complications affecting various organs, including the kidneys, which underscores the need for effective antidiabetic and nephroprotective therapies (Luan et al., 2020; Pan et al., 2018). Conventional treatments, including oral hypoglycemic agents and insulin therapy, are often associated with side effects and limited efficacy in managing diabetes-induced complications. Similarly, nephrotoxicity, commonly induced by chemotherapeutic agents like cisplatin, is a severe adverse effect that limits their clinical use. This scenario necessitates the exploration of alternative therapeutic agents, particularly from natural sources, which offer the

promise of efficacy with fewer side effects (Andriana et al., 2019; Ikewuchi & Ikewuchi, 2009; Luan et al., 2020; Pan et al., 2018).

Edicinal plants have been an integral part of traditional medicine systems and are increasingly recognized for their potential in modern therapeutics. Among such plants, LG-M (a specific plant-derived extract) has garnered attention due to its rich phytochemical composition, including flavonoids, phenols, tannins, and terpenoids. These bioactive compounds are known for their antioxidant, anti-inflammatory, and antidiabetic properties. The ability of these compounds to regulate glucose metabolism, mitigate oxidative stress, and protect against cellular damage positions LG-M as a candidate for addressing both diabetes and nephrotoxicity (Cattin, 2016; Darenskaya et al., 2021; Melmer & Laimer, 2016; Papatheodorou et al., 2015; Richter et al., 2023; Sun et al., 2021; The, 2017).

Glucose regulation plays a pivotal role in the management of diabetes, and agents that enhance glucose utilization and inhibit enzymes like dipeptidyl peptidase-4 (DPP-4) are particularly valuable. DPP-4 inhibitors prolong the activity of incretin hormones, improving insulin secretion and reducing postprandial glucose levels (Ikewuchi et al., 2009; Pareek et al., 2009; Petchi et al., 2013).. Additionally, antioxidant properties are crucial in combating oxidative stress, which is a major contributor to both diabetes-induced complications and cisplatin-induced nephrotoxicity. The assessment of total phenolic content (TPC) in plant extracts often correlates with their antioxidant capacity, providing insights into their therapeutic potential (Ahsan et al., 2023; Bansal et al., 2015; Berlin Grace et al., 2020; Darenskaya et al., 2021; Ikewuchi et al., 2009; Jacobs, 1993; Pareek et al., 2009).

The current study aims to evaluate the pharmacological properties of Litsea glutinosa (L.) leaves extract, focusing on its antidiabetic and nephroprotective activities. Glucose utilization assays in L6 myoblasts were employed to determine its ability to enhance cellular glucose uptake. DPP-4 inhibition assays were conducted to explore its enzyme inhibitory potential, which is critical for glucose homeostasis. The nephroprotective activity of Litsea glutinosa (L.) leaves extract was assessed in HEK-293 cells under cisplatin-induced toxicity, comparing its efficacy to that of the standard drug Paclitaxel. Furthermore, the total phenolic content of Litsea glutinosa (L.) leaves extract was quantified to provide a biochemical basis for its observed biological activities (Cattin, 2016; Melmer & Laimer, 2016; Papatheodorou et al., 2015).

This work not only highlights LG-M's pharmacological significance but also addresses the gap in developing safer and more effective therapeutic agents for managing diabetes and nephrotoxicity. The findings of this study could pave the way for further in-depth investigations into Litsea glutinosa (L.) leaves extract's bioactive compounds and mechanisms of action, potentially leading to its application in clinical settings. The integration of natural compounds like Litsea glutinosa (L.) leaves extract into therapeutic strategies aligns with the growing interest in plant-based medicines, offering a holistic approach to tackling complex diseases with fewer adverse effects.

2. MATERIAL AND METHODS

Chemicals, Drugs, and Biochemical Kits

All chemicals and reagents used in this study were of analytical grade. Methanol, acetone, chloroform, petroleum ether, and water were used as solvents for extraction. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were sourced from Gibco (USA) for cell culture experiments involving L6 myoblasts. Hydrogen peroxide (H_2O_2) was obtained from Merck (Germany) for inducing oxidative stress. Diprotin A, a known DPP-4 inhibitor, was purchased from Sigma-Aldrich (USA). The glucose oxidase-peroxidase (GOD-POD) assay kit was used to measure glucose levels, while DPP-4 inhibition was evaluated using a commercially available DPP-4 assay kit (Bayer). All experiments were conducted following the manufacturer's protocols, and the reagents were stored under appropriate conditions to ensure their stability and efficacy.

Collection of the plant material

The leaves of the medicinal plant *Litsea glutinosa* (L.) were collected from the Karnal region in Haryana for the study. The plant was identified and authenticated by Dr. Shyam Sunder, a botanist from the Department of Botany. To ensure future reference and validation, an herbarium specimen of the plant, labelled with the accession number MK/PHARM/2022/HR/2982, has been meticulously prepared and deposited in the pharmacognosy laboratory. This preserved specimen will serve as an important reference point for ongoing and future research related to this plant.

The Preparation of Extracts

The leaves of the plant were dried in the shade over several days to preserve their phytochemical properties. Afterward, the dried leaves were ground into a coarse powder using a mechanical grinder. A total of 1000 grams of the powdered plant material was subjected to successive extraction using a Soxhlet apparatus. The extraction process was performed with five different solvents in sequence: petroleum ether, chloroform, acetone, methanol, and water. Each extraction cycle was carried out for several hours to ensure maximum extraction of the plant's bioactive compounds. The extracts obtained from each solvent were then concentrated using a rotary vacuum evaporator (Nirmal Traders, India) at a controlled temperature of less than 45°C to avoid the degradation of heat-sensitive components. The five extracts, labelled as petroleum ether (LG-P), chloroform (LG-C), acetone (LG-A), methanol (LG-M), and water (LG-W), were stored at 4°C to maintain their stability until further use.

Subsequently, all five extracts were evaluated for their total phenolic content. The percentage yield of each extract was determined using a standard formula to quantify the efficiency of the extraction process. This step was crucial in determining the overall extraction efficiency and guiding further phytochemical investigations (Sasidharan et al., 2011; Sultana et al., 2009).

% Yield= Extract Amount (gm)/ Initial dry powder drug amount (gm) ×100

Preliminary phytochemical Screening

All the extracts underwent preliminary phytochemical screening to identify the presence of various bioactive compounds. This screening aimed to detect a wide range of phytochemicals, including alkaloids, glycosides, flavonoids, phytosterols, phenols, saponins, proteins, carbohydrates, and others. Standardized phytochemical tests were employed for this purpose, ensuring reliable identification of the different chemical constituents present in each extract. These tests provide a foundational understanding of the plant's chemical profile, offering insight into the potential therapeutic applications of the extracts based on the identified compounds (Harborne, 1998).

Total Phenolic Compounds assay

The total phenolic content of the extracts was determined using the Folin-Ciocalteu (FC) method (Olayinka & Anthony, 2009). The results were expressed as milligrams of Quercetin equivalents (QE) per gram of extract (mg QE/g). This method provided a reliable quantification of the phenolic compounds in the extracts, which are known for their antioxidant potential and possible therapeutic properties.

Antidiabetic activity

Utilisation of Glucose by L6 Myoblasts: An Experimental Approach

To determine how glucose was used by L6 myoblast cells, the methods described elsewhere were applied (van de Venter et al., 2008). The glucose utilization by L6 myoblast cells was assessed following the protocol described by van de Venter et al. (2008), with slight modifications. L6 myoblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. Once cells reached 70-80% confluence, they were differentiated into myotubes by replacing the growth medium with differentiation medium (DMEM containing 2% FBS) for 5-7 days. For the glucose uptake assay, differentiated myotubes were serum-starved in DMEM containing 0.5% bovine serum albumin (BSA) for 4 hours. The cells were then treated with various concentrations of the plant extract or vehicle (control) for 24 hours. Insulin (100 nM) served as the positive control. Following treatment, the glucose uptake was measured by incubating the cells with DMEM containing 2 mM glucose and 0.2 µCi/mL [3H]-2-deoxy-D-glucose for 10 minutes. After incubation, cells were washed with ice-cold phosphate-buffered saline (PBS) to stop the reaction and lysed using 0.1% sodium dodecyl sulfate (SDS). The radioactivity in the cell lysates was quantified using a liquid scintillation counter, reflecting the glucose uptake by the cells. The results were expressed as a percentage relative to the control group.Statistical analysis was performed using ANOVA followed by post hoc tests, and p-values less than 0.05 were considered significant. This assay provided insights into the glucose utilization capacity of the L6 myoblasts in response to the test samples (Mosmann, 1983).

DPP-4 Inhibition Assay

The DPP-4 inhibition assay was conducted using a modified version of the method described by Al-Masri et al. (2009) (Al-masri et al., 2009). This assay measured the ability of the test samples to inhibit the enzymatic activity of dipeptidyl peptidase-4 (DPP-4), which cleaves the synthetic substrate Gly-Pro-p-nitroanilide (Gly-Pro-pNA) to release p-nitroaniline (pNA), producing a yellow color. The DPP-4 enzyme was diluted in 50 mM Tris-HCl buffer (pH 8.0) to a working concentration, and the substrate was prepared at a final concentration of 0.2 mM in the same buffer. The assay was performed in a 96-well microplate where 50 µL of the test sample or a standard inhibitor, sitagliptin (used as a positive control), was added at varying concentrations to each well. Subsequently, 50 µL of the DPP-4 enzyme solution was added and incubated at 37°C for 10 minutes to facilitate interaction between the enzyme and the test sample. The reaction was initiated by adding 50 µL of the substrate solution to each well and allowed to proceed at 37°C for 30 minutes. The enzymatic activity was monitored by measuring the absorbance of p-nitroaniline at 405 nm using a microplate reader. The percentage inhibition of the DPP-4 enzyme by each test compound or control was calculated using the following formula:

% inhibition = (Control Absorbance –Test Absorbance) / Control Absorbance × 100

IC_{50} values, representing the concentration of the test sample required to inhibit 50% of the enzyme activity, were determined using nonlinear regression analysis. This method provided a robust evaluation of the DPP-4 inhibitory potential of the test samples, reflecting their antidiabetic activity.

Nephroprotective activity evaluation

Impact of LG-M on the toxicity caused by cisplatin in HEK-293 cells

The effect of LG-M on cisplatin-induced toxicity in HEK-293 cells was investigated using the human kidney embryonic cell line (ATCC; HEK-293). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 12% heat-inactivated fetal bovine serum (FBS) and maintained at 37°C in a carbon dioxide incubator with 6% CO_2 to provide an optimal environment for growth. Once the cells reached 80–90% confluence, trypsinization was performed using trypsin-EDTA solution to detach the cells from the culture flask. To neutralize

the trypsin's activity, an adequate amount of culture medium was added. The cell suspension was then centrifuged at 1400 rpm for five minutes, after which the supernatant was carefully discarded. The resulting cell pellet was reconstituted in fresh medium and thoroughly mixed to ensure uniformity. The cells were counted using the Trypan blue exclusion method with a hemacytometer to assess cell viability and determine the total cell number. Based on the cell count, the cell suspension was diluted with medium to achieve the desired cell density. For the experiment, a seeding density of 9000 cells per well was maintained in a 104-well flat-bottom microtiter plate to allow optimal cell attachment and growth. The cells were left to adhere and stabilize for 26 hours under standard culture conditions. Following the stabilization period, the cells were divided into three groups: non-treated (control), cisplatin-treated, and co-treated with cisplatin and varying concentrations of LG-M. Cisplatin (20 µM) was used to induce cytotoxicity, and LG-M was tested at concentrations of 10, 20, 50, 90, 140, and 200 µg/mL. The treatment period lasted 26 hours, during which the cells were exposed to the respective treatments. After the treatment period, cell viability was assessed using a cell viability assay, such as the MTT assay or a similar method, to evaluate the protective effect of LG-M against cisplatin-induced cytotoxicity (Kpemissi et al., 2019; Singh et al., 2018).

Cell viability test

Cell viability was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, a widely used method for evaluating the metabolic activity and overall health of cells. The MTT assay is based on the principle that metabolically active cells reduce the yellow, water-soluble MTT reagent to an insoluble, dark blue formazan product through mitochondrial dehydrogenase activity. This provides a sensitive indicator of cell viability and proliferation. After the treatment period, MTT solution was prepared at a concentration of 6 mg/mL in phosphate-buffered saline (PBS), and 20 µL of this solution was added to each well containing the treated cells. The plates were then incubated for 3 hours at 37°C in a humidified atmosphere with 5% CO_2 to allow for the reduction of MTT by viable cells. During this time, metabolically active cells converted the MTT reagent into formazan crystals, which accumulated in the cytoplasm of the cells. At the end of the incubation period, the supernatant was carefully removed to avoid disturbing the formazan crystals. Dimethyl sulfoxide (DMSO) was added to each well to dissolve the insoluble formazan into a colored solution. The plate was gently shaken to ensure complete dissolution. The absorbance of the resulting solution was measured at a wavelength of 572 nm using a microtiter plate reader. This absorbance value is directly proportional to the number of viable cells in each well. Controls, including untreated cells and blank wells without cells, were included to establish baseline absorbance and ensure the accuracy of the assay. The results were expressed as a percentage of cell viability relative to the untreated control group. Statistical analysis was performed to compare the effects of different treatments, providing insights into the protective or cytotoxic effects of the tested substances. This assay provided a reliable and quantitative measure of cell viability, enabling the evaluation of the therapeutic potential of the test compound.

Statistical analysis

Statistical analysis was performed to evaluate the significance of the observed differences between treatment groups. All experiments were conducted in triplicate, and the data were expressed as mean ± standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA), followed by post hoc comparisons using Tukey's test to determine specific group differences. Statistical significance was set at $p < 0.05$. Data analysis was carried out using statistical software, GraphPad Prism version 8 and graphical representations were created to illustrate trends and differences among groups. This robust statistical approach ensured reliable and reproducible interpretation of the experimental findings.

3. RESULTS AND DISCUSSION

Preliminary phytochemical Screening

The preliminary phytochemical screening of various extracts (LG-P, LG-M, LG-W, LG-C, and LG-A) revealed distinct profiles of bioactive compounds, highlighting their potential therapeutic applications. Flavonoids were present in all extracts except LG-C, suggesting their widespread distribution among the plant parts. Tannins were detected in LG-P, LG-M, and LG-C but were absent in LG-W and LG-A, indicating variability in polyphenolic content. Phenols and terpenoids were consistently present across all extracts except LG-C for phenols, suggesting their prominent role in the plant's pharmacological activities. Interestingly, phytosterols were identified in LG-P, LG-M, and LG-C but were absent in LG-W and LG-A, aligning with their lipid-soluble nature. Glycosides were exclusively detected in LG-M, pointing to its unique composition. Carbohydrates and terpenoids were universally present, emphasizing their essential presence in all extracts. Saponins and alkaloids showed selective distribution, primarily in LG-M and LG-C. Proteins were detected in most extracts, excluding LG-C. These findings demonstrate the diverse phytochemical composition of the extracts, with LG-M displaying the richest profile, suggesting it may have the broadest pharmacological potential. Further investigations are warranted to explore the biological activities of these phytoconstituents.

Tablet 1. The preliminary pharmaceutical screening results for all the extracts

Phytochemical Group	LG-P	LG-M	LG-W	LG-C	LG-A
Flavonoids	+	+	+	-	+
Tannins	+	+	-	+	-
Phenols	+	+	+	-	+
Phytosterols	+	+	-	+	-
Glycosides	-	+	-	-	-
Carbohydrates	+	+	+	+	+
Saponins	-	+	-	+	-
Terpenoids	+	+	+	+	+
Alkaloids	-	+	-	+	-
Proteins	+	+	+	-	+

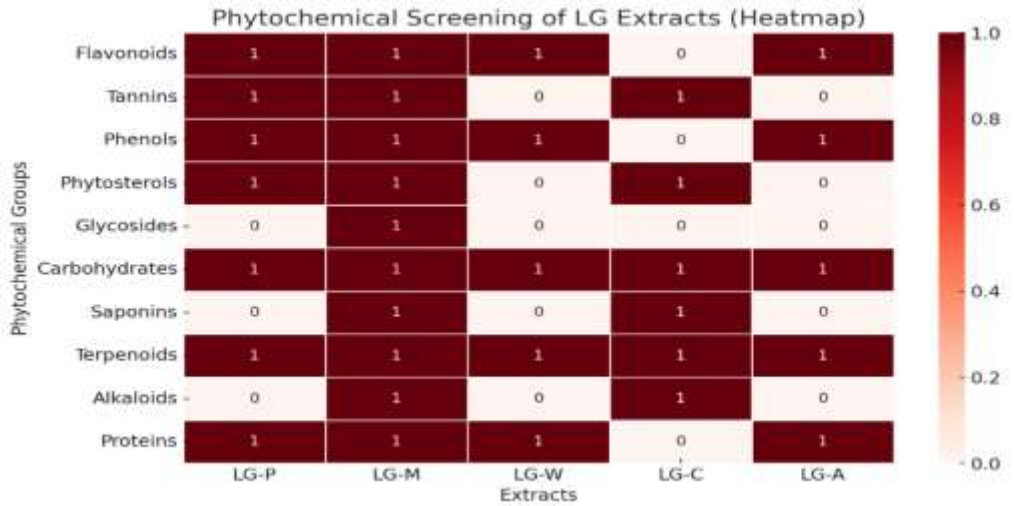

Figure 1. The phytochemical screening findings for the several *Litsea glutinosa* leaves extracts (LG-P, LG-M, LG-W, LG-C, and LG-A) are displayed in a heatmap.

Total Phenolic Compounds determination

The total phenolic content (TPC) and linear regression analysis of the extracts reveal significant variations in phenolic concentration and their corresponding regression fits. LG-M exhibited the highest TPC, with 333.24 QE per gram of extract, reflecting its strong phenolic content and potential antioxidant properties. Its regression equation, y=0.0064x+0.0911, and an R2 value of 0.9784 demonstrate a reliable fit to the calibration curve. LG-A followed with 310.67 QE per gram, supported by a regression equation of y=0.0088x+0.0831 and an R2 value of 0.9681, indicating substantial phenolic content and good linearity. The extracts LG-P and LG-C showed comparable phenolic contents of 238.95 and 241.90 QE per gram, respectively. LG-P's regression equation y=0.0048x+0.0879 and an R2 value of 0.9892 suggest the best linearity among all extracts, reflecting high consistency in measurement. LG-C, with R2=0.9677, also demonstrated good linearity. LG-W had the lowest TPC at 227.76 QE per gram, with a regression equation of y=0.0083x+0.0899 and an R2 value of 0.9810, still indicating reliable results.

Overall, the variation in TPC across the extracts highlights differences in phenolic composition, with LG-M and LG-A showing the highest antioxidant potential. The strong R2 values across all extracts confirm the robustness of the regression models in estimating TPC, underscoring their reliability for quantitative analysis. This data underscores the promising antioxidant capacity of LG-M and LG-A, warranting further investigation into their biological activities.

Table 2. The linear regression analysis equations and the total phenolic content of each extract

Extracts Name	Equation Derived after Regression analysis	Total Phenolic content in QE per gram of extract
LG-P	$y = 0.0048x + 0.0879$ $R^2 = 0.9892$	238.95
LG-M	$y = 0.0064x + 0.0911$ $R^2 = 0.9784$	333.24

LG-W	y = 0.0083x + 0.0899 R^2 = 0.9810	227.76
LG-C	y = 0.0096x + 0.0947 R^2 = 0.9677	241.90
LG-A	y = 0.0088x + 0.0831 R^2 = 0.9681	310.67

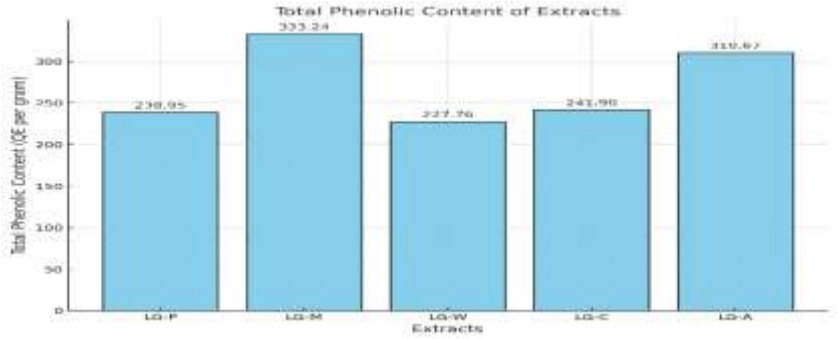

Figure 2. The total phenolic content of each extract, expressed as QE per gram

Antidiabetic activity

Utilisation of Glucose by L6 Myoblasts

The results of the glucose utilization assay in L6 myoblast cells highlight the antidiabetic potential of LG-M at various concentrations. The control group showed a baseline glucose uptake of 99.99±2.28, while insulin treatment significantly enhanced glucose utilization to 143.96±3.89, serving as a positive control. LG-M demonstrated a dose-dependent effect on glucose uptake, with 10 µg/mL, 20 µg/mL, and 60 µg/mL resulting in glucose utilization levels of 114.12±2.79, 123.96±2.93, and 117.98±2.77, respectively. The enhancement at 20 µg/mL was particularly notable, indicating an optimal concentration for glucose uptake.

These findings suggest that LG-M possesses insulin-like activity, enhancing glucose utilization in L6 myoblasts, though not to the same extent as insulin. The observed dose-dependent variation points to a potential threshold for efficacy. Further mechanistic studies could elucidate whether LG-M modulates glucose transporter activity or mimics insulin signalling pathways, supporting its use as a natural antidiabetic agent.

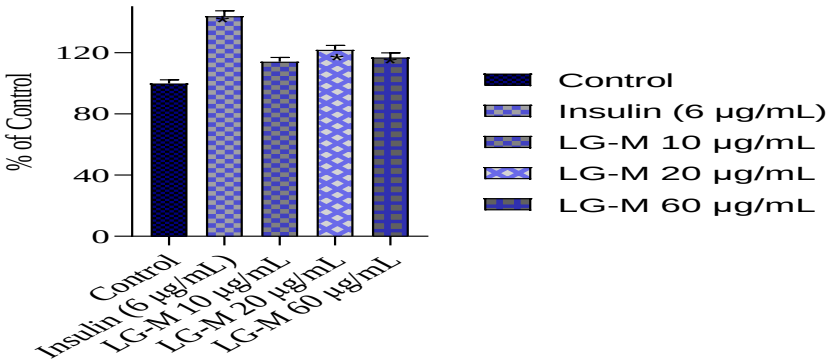

Figure 3. LG-M's effect on the absorption of glucose by L6 myoblasts. For 48 hours, the cells were treated with varying doses of the plant extract or a constant quantity.

Assay of DPP-4 Inhibition

The results of the DPP-4 inhibition assay indicate the inhibitory potential of LG-M at different concentrations. The control group showed no DPP-4 inhibition (0±00 \pm 00±0), establishing the baseline activity of the enzyme. Diprotin A, a standard inhibitor, exhibited a high inhibition rate of 88.68±1.7488.68 \pm 1.7488.68±1.74, validating the assay's reliability. LG-M demonstrated a concentration-dependent inhibitory effect, with 60 µg/mL achieving 18.97±1.06 inhibition and 120 µg/mL increasing to 43.84±1.19. These findings suggest that LG-M has a moderate but dose-dependent inhibitory effect on DPP-4 activity. While it is less potent compared to the standard Diprotin A, the inhibition observed at higher concentrations of LG-M highlights its potential as a natural DPP-4 inhibitor. This activity is significant for antidiabetic applications, as DPP-4 inhibitors are known to prolong the

activity of incretin hormones, thereby enhancing glucose regulation. Further studies could focus on isolating active compounds from LG-M to optimize its inhibitory efficacy.

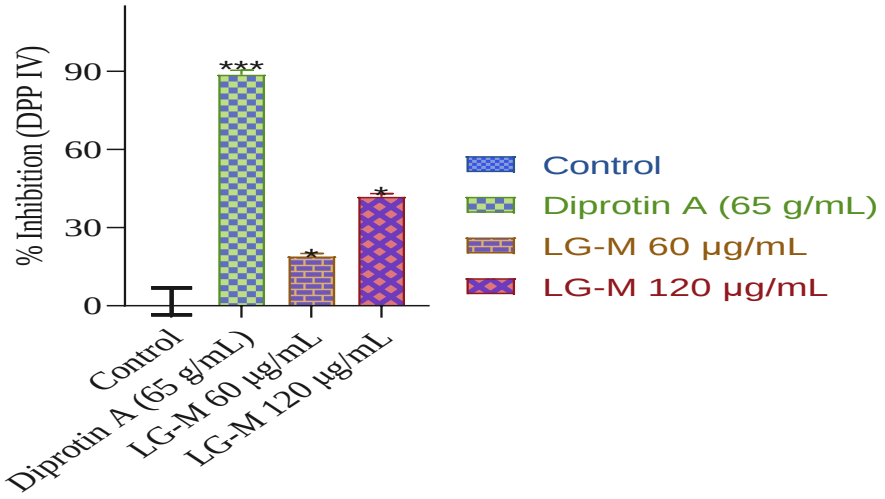

Figure 4. LG-M's effect on the percentage of inhibition of DPP-4 activity.

Nephroprotective activity evaluation

Impact of LG-M on the toxicity caused by cisplatin in HEK-293 cells

The nephroprotective activity of LG-M in cisplatin-induced toxicity was evaluated by measuring the cytotoxicity in terms of percentage cell viability in HEK-293 cells. The results, as shown in Table 3, reveal a dose-dependent reduction in cell viability for both LG-M and the standard drug Paclitaxel, though LG-M demonstrated significantly higher cell viability across all concentrations, suggesting its protective role against cisplatin-induced damage. At the baseline (0 µg/mL), both LG-M and Paclitaxel showed high viability, with 98.68±3.60% and 98.82±3.34% respectively, indicating minimal cytotoxicity in untreated cells. As the concentration increased, the cell viability for LG-M gradually decreased, yet remained significantly higher than Paclitaxel. At 10 µg/mL, LG-M maintained 96.29±2.07% viability compared to 69.32±1.65% for Paclitaxel. Similarly, at 20 µg/mL and 50 µg/mL, LG-M exhibited 95.39±1.78% and 96.54±1.80%, respectively, compared to 56.12±1.80% and 37.32±1.40% for Paclitaxel. At higher concentrations, the protective effects of LG-M became more evident. At 90 µg/mL, LG-M retained 84.61±1.40% viability, whereas Paclitaxel reduced viability to 34.62±1.61%. At 140 µg/mL and 200 µg/mL, LG-M still supported cell viability at 63.66±1.61% and 45.65±1.83%, respectively, significantly outperforming Paclitaxel, which dropped to 28.62±1.83% and 24.42±0.88% viability. These findings highlight the potential nephroprotective properties of LG-M, suggesting that it mitigates cisplatin-induced cytotoxicity more effectively than Paclitaxel. This protective effect could be attributed to LG-M's bioactive components, possibly through mechanisms involving oxidative stress reduction or anti-inflammatory pathways. Further studies to explore these mechanisms and in vivo validation could solidify its therapeutic relevance.

Table 3. Cytotoxicity in terms of % viability of the standard and LG-M

	% Viability	
Concentration (µg/mL)	**LG-M**	**Paclitaxel (Standard)**
0	98.68±3.60	98.82±3.34
10	96.29±2.07	69.32±1.65
20	95.39±1.78	56.12±1.80
50	96.54±1.80	37.32±1.40
90	84.61±1.40	34.62±1.61
140	63.66±1.61	28.62±1.83

200±	45.65±1.83	24.42±0.88

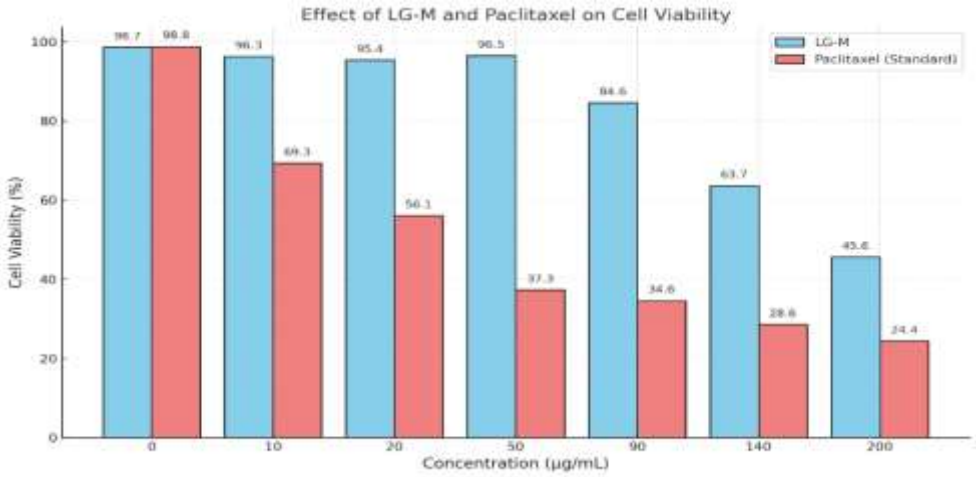

Figure 5. Nephroprotective activity of LG-M in cisplatin-induced toxicity in HEK-293 cells

4. CONCLUSIONS

The findings from this study highlight *Litsea glutinosa* (L.) leaves extract (LG-M) multifaceted pharmacological potential, particularly in its antidiabetic and nephroprotective activities. Phytochemical analysis confirmed the presence of significant bioactive compounds, including flavonoids and phenols, which are critical to its therapeutic efficacy. The best and potential extract, LG-M demonstrated a dose-dependent enhancement of glucose utilization in L6 myoblasts, supporting its potential as an antidiabetic agent. Its moderate DPP-4 inhibitory activity further corroborates its glucose-regulating properties. In nephroprotective studies, LG-M consistently outperformed Paclitaxel in preserving HEK-293 cell viability under cisplatin-induced toxicity, suggesting its role as a protective agent against nephrotoxicity. The high total phenolic content of LG-M aligns with its strong antioxidant activity, potentially contributing to its protective effects. These findings collectively position LG-M as a promising candidate for addressing diabetes and related complications, as well as for mitigating nephrotoxic effects associated with chemotherapeutic agents. However, further in vivo studies and mechanistic investigations are required to fully elucidate its therapeutic potential and safety profile, paving the way for its incorporation into clinical practice.

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