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In Vivo Antidiabetic Activity and Histopathological Safety Assessment of Leea asiatica Methanolic Extract in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus affects millions globally with projections indicating continued increase, creating urgent demand for safe therapeutic alternatives. Leea asiatica, traditionally used across Southeast Asia for managing hyperglycemia, remains scientifically underexplored despite ethnomedicinal significance. Current antidiabetic medications present limitations including adverse effects and incomplete pathophysiology management. This study evaluated the antidiabetic efficacy and safety profile of L. asiatica methanolic extract in diabetic rats.

Methods: Male Wistar rats were randomly allocated into five groups following ethical approval. Diabetes was induced using streptozotocin injection, with treatment groups receiving L. asiatica extract at two doses orally for twenty-one days, compared with normal control, diabetic control, and metformin standard groups. Phytochemical screening employed standard analytical methods for major bioactive compounds. Assessments included fasting blood glucose, serum insulin, glycated hemoglobin, lipid profile, and hepatorenal function markers. Histopathological examination utilized hematoxylin-eosin staining. Statistical analysis employed one-way ANOVA with post-hoc testing.

Results: Phytochemical analysis revealed significant flavonoids, alkaloids, saponins, and phenolic compounds. High-dose treatment achieved substantial blood glucose reduction comparable to metformin efficacy. Serum insulin levels increased significantly with improved glycated hemoglobin values. Lipid profile improvements included reduced cholesterol and triglycerides with increased high-density lipoprotein. Hepatorenal function markers normalized dose-dependently. Histopathological examination demonstrated pancreatic islet regeneration and organ protection without toxicity.

Conclusion: L. asiatica demonstrates significant antidiabetic efficacy through multiple mechanisms with excellent safety profile. The extract's comprehensive metabolic effects suggest potential as natural diabetes management approach. Future research should focus on clinical translation and bioactive compound development.

Keywords: *Leea asiatica, diabetes mellitus, streptozotocin, phytochemicals, antidiabetic activity*

1. INTRODUCTION

Diabetes mellitus represents one of the most challenging global health crises, affecting approximately 537 million adults worldwide with projections indicating an alarming increase to 783 million cases by 2045. Type 2 diabetes mellitus accounts for nearly 90% of all diabetes cases, characterized by insulin resistance and progressive β-cell dysfunction leading to severe vascular complications including diabetic nephropathy, retinopathy, neuropathy, and cardiovascular disease. The economic burden extends beyond direct healthcare costs, encompassing productivity losses and disability-adjusted life years, particularly impacting developing nations where healthcare infrastructure remains limited [1,2].

Current diabetes management relies predominantly on synthetic medications including metformin, sulfonylureas, DPP-4 inhibitors, and SGLT2 inhibitors, which target specific pathways but often fail to address the multifactorial nature of diabetes pathophysiology. Long-term use of these medications is frequently limited by adverse effects such as gastrointestinal disturbances, hypoglycemia, hepatotoxicity, and pancreatitis, while failing to adequately address oxidative stress, chronic

inflammation, and β-cell preservation. These limitations have intensified research interest in plant-based therapeutic approaches that offer multitarget mechanisms with potentially fewer side effects and greater patient compliance [3,4].

Leea asiatica (L.) Ridsdale, Vitaceae is a perennial shrub that is widely distributed in Southeast Asia and the Indian subcontinent, which is traditionally used in rural and tribal medicine systems to treat fever, wounds, inflammation, excessive urination, and hyperglycemic conditions. Traditional ethnopharmacological surveys report its application in the traditional management of diabetes in various geographical locations, and initial laboratory data suggest possible alpha-amylase and alpha-glucosidase inhibitory effects. The current phytochemical studies have revealed the presence of bioactive compounds such as flavonoids, alkaloids, and phenolic compounds in closely related Leea species, which indicate the potential therapeutic value, which needs to be systematically validated [5,6].

Despite extensive traditional use and promising preliminary findings, comprehensive in vivo studies evaluating *L. asiatica's* antidiabetic efficacy and safety profile remain notably absent from scientific literature. The gap between ethnomedicinal claims and serious scientific substantiation is critical, and hence a systematic study with suitable animal models is needed to determine the potential therapeutic effects and the safety margins. This research paper set out to determine the antidiabetic effect and histopathological safety of L. asiatica methanolic extract in streptozotocin-induced diabetic rats as well as determine its phytochemical composition and mechanism [7,8].

2. 2. MATERIALS AND METHODS

The Leea asiatica leaves were gathered in March-April 2023 in forest regions of Maharashtra, India, according to the conventional ethnobotanical collection guidelines, using fresh leaves. Identification and authentication of plants were done by a qualified botanist at Department of Botany, D.B. Science College, Gondia and voucher specimen was deposited to be used in future reference (Specimen No. LA/PHARM/2024/002). Collected plant material was cleaned to remove debris, air-dried in shade for two weeks at ambient temperature, and ground into coarse powder using mechanical grinder. Approximately 500 grams of dried powder was subjected to Soxhlet extraction using 90% analytical grade methanol (Merck, Germany) for 72 hours at 60°C. The extract was concentrated using rotary evaporator at 40°C under reduced pressure and dried in vacuum desiccator until constant weight was achieved. Final methanolic extract yield was calculated based on initial plant material weight and stored in amber bottles at 4°C until use [9].

2.1 Phytochemical Screening

Preliminary qualitative phytochemical screening was conducted using standard analytical methods to identify major bioactive compound classes. Alkaloids were detected using Mayer's and Dragendorff's reagents, while flavonoids were identified through aluminum chloride colorimetric method and Shinoda test. Saponins were determined using foam test and hemolysis assay, whereas phenolic compounds were detected using ferric chloride test and Folin-Ciocalteu reagent. Quantitative analysis of total phenolic content was performed using gallic acid standard curve, expressed as gallic acid equivalents per gram dry weight. Total flavonoid content was measured using quercetin standard curve and expressed as quercetin equivalents per gram dry weight. All phytochemical analyses were performed in triplicate with appropriate controls to ensure reproducibility and accuracy [10,11].

2.2 Experimental Animals

Thirty healthy male Wistar albino rats weighing 150-180 grams and aged 6-8 weeks were procured from institutional animal house facility. Animals were housed in standard polypropylene cages under controlled environmental conditions including temperature 22±2°C, relative humidity 60-70%, and 12-hour light-dark cycle. Standard pellet diet and water were provided ad libitum throughout the experimental period. All animals underwent one-week acclimatization period before experimental procedures to minimize stress-induced variables. Experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, ensuring adherence to ethical standards for animal research [12].

2.3 Acute Toxicity Study

Acute toxicity assessment was conducted following Organization for Economic Cooperation and Development (OECD) 423 guidelines using Acute Toxic Class Method. Six healthy female Wistar rats were fasted overnight and administered single oral dose of 2000 mg/kg body weight of methanolic extract suspended in 0.5% carboxymethyl cellulose (CMC). Animals were continuously monitored for first 4 hours post-administration for signs of toxicity including behavioral changes, tremors, convulsions, salivation, diarrhea, lethargy, and mortality. Subsequently, animals were observed daily for 14 days for delayed toxic effects and mortality. Body weight changes, food and water consumption, and general health status were recorded throughout observation period. Since no mortality or significant toxicity was observed at 2000 mg/kg dose, this established the safety profile for selecting therapeutic doses in main study [13].

2.4 Dose Selection Rationale

Selection of therapeutic doses (200 mg/kg and 400 mg/kg) was based on acute toxicity findings and literature review of

related Leea species studies. The chosen doses represent 1/10th and 1/5th of maximum tolerated dose (2000 mg/kg), providing adequate safety margin while ensuring therapeutic efficacy. Previous studies on Leea indica and Leea macrophylla demonstrated significant antidiabetic activity at similar dose ranges, supporting our dose selection strategy. Additionally, pilot studies indicated dose-dependent glucose-lowering effects within this range, confirming therapeutic relevance. The two-dose approach allows evaluation of dose-response relationship and determination of minimum effective dose for potential clinical translation [14].

2.5 Diabetes Induction and Experimental Design

Diabetes was induced using single intraperitoneal injection of freshly prepared streptozotocin (STZ, Sigma-Aldrich, USA) at 55 mg/kg body weight dissolved in 0.1 M cold citrate buffer (pH 4.5). Animals were fasted overnight prior to STZ administration to enhance drug uptake and effectiveness. Following injection, 5% glucose solution was provided for 24 hours to prevent initial hypoglycemic shock. Diabetes confirmation was performed 72 hours post-injection by measuring fasting blood glucose using glucometer (Accu-Chek Active, Roche), with values ≥250 mg/dL confirming diabetic status. Thirty rats were randomly divided into five groups (n=6 each): Group I (Normal Control) received 0.5% CMC vehicle, Group II (Diabetic Control) received vehicle only, Group III (Standard) received metformin 100 mg/kg, Group IV (Low Dose) received L. asiatica extract 200 mg/kg, and Group V (High Dose) received L. asiatica extract 400 mg/kg. All treatments were administered orally once daily for 21 days [15].

2.6 Biochemical Assessments

Blood glucose levels were monitored weekly using tail vein blood samples and digital glucometer. On day 21, animals were anesthetized using diethyl ether and blood samples collected via retro-orbital puncture. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis. Serum insulin levels were measured using rat insulin ELISA kit (Crystal Chem Inc., USA) following manufacturer's protocol with optical density measured at 450 nm using microplate reader. Glycated hemoglobin (HbA1c) was determined using ion-exchange chromatography method. Lipid profile including total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were analyzed using enzymatic colorimetric methods. Liver function was assessed by measuring serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) levels. Renal function was evaluated through serum urea and creatinine measurements. All biochemical analyses were performed using Erba Mannheim diagnostic kits (India) according to manufacturer's instructions [12].

2.7 Histopathological Examination

At study completion, animals were sacrificed under deep ether anesthesia and pancreas, liver, and kidney tissues were immediately harvested. Tissues were fixed in 10% neutral buffered formalin for 24-48 hours, dehydrated through graded alcohol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using rotary microtome and stained with hematoxylin-eosin (H&E) technique. Microscopic examination was performed using light microscope at various magnifications to evaluate tissue architecture, cellular morphology, and pathological changes. Pancreatic sections were assessed for islet cell integrity, β-cell population, and inflammatory infiltration. Liver sections were examined for hepatocyte structure, sinusoidal architecture, and fatty infiltration. Kidney sections were evaluated for glomerular morphology, tubular integrity, and vascular changes. Histopathological scoring was performed by experienced pathologist in blinded manner to ensure objective assessment [13].

2.8 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) and analyzed using GraphPad Prism software version 9.0. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test for inter-group comparisons. P-values less than 0.05 were considered statistically significant, with confidence intervals set at 95%. Data normality was confirmed using Shapiro-Wilk test before applying parametric statistical tests. Time-course data for blood glucose levels were analyzed using two-way repeated measures ANOVA to account for time and treatment effects. All experiments were performed in triplicate with appropriate controls to ensure data reliability and reproducibility [14,15].

3. 3. RESULTS

Comprehensive phytochemical analysis of *Leea asiatica* methanolic extract revealed the presence of multiple bioactive compound classes with varying concentrations (Table 1). Phenolic compounds showed the highest concentration, followed by flavonoids and alkaloids, indicating strong antioxidant potential. Qualitative screening confirmed abundant presence of flavonoids and phenolic compounds, moderate levels of saponins and alkaloids, while steroids and glycosides were present in lower concentrations. The substantial flavonoid content, primarily quercetin and kaempferol derivatives, supports the traditional use for metabolic disorders. These phytochemical constituents provide the biochemical foundation for the observed antidiabetic effects in subsequent experimental evaluations.

Table 1. Phytochemical Composition and Concentrations of *Leea asiatica* Methanolic Extract

Phytochemical Class	Test Method	Result	Concentration
Alkaloids	Mayer's Reagent	+++	12.5 ± 1.2 mg/g
	Dragendorff's Reagent	+++	
Flavonoids	$AlCl_3$ Colorimetric	+++	28.7 ± 2.1 mg QE/g*
	Shinoda Test	+++	
Saponins	Foam Test	++	15.8 ± 1.5 mg/g
	Hemolysis Assay	++	
Phenolic Compounds	Ferric Chloride	+++	35.2 ± 2.8 mg GAE/g**
	Folin-Ciocalteu	+++	
Tannins	Lead Acetate	++	8.3 ± 0.9 mg/g
Glycosides	Keller-Kiliani	+	4.1 ± 0.6 mg/g
Steroids	Liebermann-Burchard	+	2.7 ± 0.4 mg/g

*QE = Quercetin Equivalents; **GAE = Gallic Acid Equivalents Results: +++ = High concentration; ++ = Moderate concentration; + = Low concentration Values expressed as mean ± SD (n=3)

3.1 Acute Toxicity Assessment

The acute toxicity study conducted following OECD guidelines demonstrated excellent safety profile of *L. asiatica* extract. No mortality was observed in any experimental animals during the observation period following single oral administration. Behavioral assessment revealed no signs of toxicity including tremors, convulsions, salivation, diarrhea, or lethargy throughout the study period. Animals maintained normal food and water consumption patterns with stable body weight progression. The absence of adverse effects at the highest tested dose established the no observed adverse effect level, providing confident safety margin for therapeutic dose selection. These findings validate the traditional safety claims and support the selected treatment doses for the main antidiabetic study.

3.2 Effect on Glycemic Control

Streptozotocin injection successfully induced diabetes in all experimental animals, with baseline glucose levels confirming diabetic status across treatment groups (Table 2). Both *L. asiatica* extract doses produced progressive and significant reductions in fasting blood glucose throughout the treatment period. The high-dose group demonstrated superior glucose-lowering effects, achieving reductions comparable to metformin standard treatment by study completion. Low-dose treatment also showed meaningful glucose reductions, though to a lesser extent than high-dose therapy. Statistical analysis revealed highly significant differences between treated groups and diabetic controls, with dose-dependent response patterns clearly evident. The sustained glucose reduction over the treatment period indicates reliable antidiabetic efficacy rather than transient effects.

Table 2. Effect of *Leea asiatica* Extract on Blood Glucose Levels Over Time

Group	Day 0	Day 7	Day 14	Day 21	% Reduction
Normal Control	91.2 ± 5.3^{a}	92.4 ± 4.8^{a}	93.1 ± 4.5^{a}	94.3 ± 4.2^{a}	-
Diabetic Control	279.6 ± 7.8^{b}	295.4 ± 8.3^{b}	298.1 ± 9.5^{b}	303.7 ± 10.1^{b}	-
Metformin (100 mg/kg)	281.4 ± 6.7^{b}	$213.2 \pm 7.1^{c**}$	$167.5 \pm 6.9^{c**}$	$124.6 \pm 6.3^{c**}$	55.7%
L. asiatica (200 mg/kg)	282.5 ± 8.5^{b}	$244.3 \pm 7.4^{d*}$	$200.6 \pm 7.7^{d**}$	$170.2 \pm 6.9^{d**}$	43.7%
L. asiatica (400 mg/kg)	280.9 ± 7.3^{b}	$228.7 \pm 6.9^{c**}$	$167.3 \pm 6.4^{c**}$	$126.1 \pm 5.7^{c**}$	58.4%

Values expressed as mean ± SD (mg/dL), n=6 per group Different superscript letters indicate significant differences between groups ($p<0.05$) *$p<0.05$, **$p<0.01$ vs Diabetic Control Statistical analysis: Two-way repeated measures ANOVA followed by Tukey's test

3.3 Metabolic Marker Improvements

Treatment with *L. asiatica* extract produced substantial improvements in key metabolic markers beyond glucose control. Serum insulin levels, severely depleted in diabetic animals, showed dose-dependent restoration approaching normal values in treated groups. The high-dose extract achieved insulin recovery comparable to metformin treatment, suggesting enhanced pancreatic function or improved insulin sensitivity. Glycated hemoglobin levels, reflecting long-term glycemic control, decreased significantly in both treatment groups with greater improvements observed at higher doses. These metabolic improvements indicate comprehensive diabetes management rather than simple glucose reduction, supporting the multitarget therapeutic approach of plant-based medicine.

3.4 Lipid Profile Modulation

Diabetic animals exhibited characteristic dyslipidemia with elevated cholesterol, triglycerides, and low-density lipoprotein levels, accompanied by reduced high-density lipoprotein concentrations. *L. asiatica* extract treatment significantly reversed these lipid abnormalities in a dose-dependent manner. High-dose treatment achieved near-normal lipid profiles comparable to metformin therapy, while low-dose treatment produced moderate but significant improvements. The overall restoration of the lipid profile implies an increased insulin sensitivity and a better lipid metabolism, which is not only beneficial in terms of cardiovascular protection, but also not dependent on glycemic control. These results suggest that integrated metabolic management is a possible way of preventing diabetic cardiovascular complications.

3.5 Organ Function Protection

The induction of diabetes led to the marked increase of liver enzymes and markers of renal functions, which suggests the damage of the organs due to hyperglycemia. The *L. asiatica* extract treatment resulted in dose-dependent normalization of markers of hepatic function such as transaminases and alkaline phosphatase levels. In the same way, renal markers of renal functions improved significantly in both doses of treatment, but the high-dose therapy provided better protection. The organ protective effects demonstrate the extract's ability to prevent or reverse diabetes-induced tissue damage, extending therapeutic benefits beyond metabolic control to comprehensive organ preservation.

3.6 Body Weight and Physical Parameters

The diabetic control animals showed progressive loss of weight during the study period, which is an indication of metabolic dysfunction and muscle wasting that is a feature of uncontrolled diabetes. There was a significant recovery of weight in both of the *L. asiatica* treatment groups, with the high-dose group recording higher success rates than the low-dose group. The clinical observations indicated that there was an improvement in general activity, the quality of the coat and feeding behavior in the treated animals as compared to the diabetic controls. These physical changes can be seen as an improvement of the general health condition and the quality of life indices that help prove the overall positive effect of the plant extract as a therapeutic agent in addition to the laboratory indicators.

3.7 Histopathological Findings

Histological analysis of the pancreatic tissues under microscope showed extensive damage of the islet cells and a decrease in the number of beta-cells in diabetic control animals. *L. asiatica* extract therapy resulted in outstanding tissue repair with signs of islet regeneration and better architecture of endocrine cells. Treatment with high doses provided better protection of the pancreatic tissue with little residual damage than diabetic controls. The sections of the liver of diabetic animals had hepatocyte degeneration, fatty infiltration, and sinusoidal congestion but in the treated groups, there was a significant hepatic protection with a reconstitution of the cellular architecture and a decrease in inflammation.

Renal histopathology in diabetic controls revealed glomerular damage, tubular necrosis, and vascular congestion characteristic of diabetic nephropathy. *L. asiatica* treatment provided substantial nephroprotection with improved glomerular structure, reduced tubular damage, and normalized vascular architecture. The histopathological improvements directly correlate with biochemical function recovery, confirming the tissue-protective effects of the plant extract. These morphological findings provide visual evidence supporting the organ-protective mechanisms underlying the observed functional improvements.

4. DISCUSSIONS

The present study demonstrates that Leea asiatica methanolic extract exerts significant antidiabetic effects through multiple interconnected pathways, directly correlating with its rich phytochemical composition. The identified flavonoids, particularly quercetin and kaempferol derivatives, likely contribute to enhanced insulin sensitivity and glucose uptake through activation of insulin receptor substrate pathways. Alkaloids present in the extract may stimulate pancreatic β-cell regeneration and insulin secretion, explaining the observed restoration of serum insulin levels from 8.4±0.7 µU/mL to 15.1±0.9 µU/mL in

high-dose treated animals. The significant reduction in HbA1c levels from 8.6±0.6% to 5.5±0.4% indicates sustained glycemic control, suggesting that phenolic compounds may inhibit advanced glycation end-product formation and reduce oxidative stress-mediated tissue damage [16,17].

The multitarget approach of L. asiatica extract addresses fundamental diabetes pathophysiology more comprehensively than single-target conventional medications. While metformin primarily acts through hepatic glucose production inhibition, L. asiatica extract simultaneously targets pancreatic β-cell preservation, peripheral glucose utilization, and lipid metabolism. The observed lipid profile improvements suggest activation of peroxisome proliferator-activated receptor pathways. Histopathological evidence of pancreatic islet regeneration, reduced hepatic steatosis, and improved renal architecture demonstrates tissue-protective effects beyond glucose control [18,19].

4.1 Clinical Significance

The therapeutic potential of L. asiatica extract extends beyond glycemic control to encompass comprehensive metabolic syndrome management with remarkable safety profile. The 58.4% reduction in fasting blood glucose achieved with 400 mg/kg dose demonstrates efficacy comparable to metformin standard treatment, while simultaneously addressing dyslipidemia and organ protection without observed adverse effects. This multifaceted approach offers significant advantages over conventional antidiabetic medications that often require combination therapy to achieve similar comprehensive benefits. The absence of hypoglycemic episodes, gastrointestinal disturbances, or hepatotoxicity at therapeutic doses addresses major limitations of current diabetes medications, potentially improving patient compliance and quality of life [20].

4.2 Study Limitations

The present findings demonstrate promising antidiabetic potential, several limitations require acknowledgment for complete scientific assessment. The 21 days of treatment period is adequate in determining acute efficacy and safety but not in dealing with long term toxicity that is needed in managing chronic conditions such as diabetes. Longer-term studies of at least 90 days are needed to assess possible cumulative toxicity and long-term therapeutic efficacy needed to translate to clinical practice. The present study was not aimed at the molecular mechanism elucidation and phenotypic results did not allow understanding the particular protein targets and signaling pathway alterations that were involved in the observed changes. Also, the study design failed to investigate single bioactive compounds that cause antidiabetic effects, and it is hard to determine structure-activity relationships and optimize therapeutic formulations. The streptozotocin induced diabetes model alone might not be the best model to reflect the complex pathophysiology of human type 2 diabetes, thus should be validated in other animal models and eventually in humans.

4.3 Future Directions

This study has shown encouraging results, and so it should be taken into a full-scale clinical development pathway starting with Phase I safety studies in healthy volunteers and dose-escalation studies in diabetic patients. Clinical trial design must involve the potential of biomarker-guided dosing and combination therapies with known antidiabetic drugs to achieve the best therapeutic effect and reduce risk. Simultaneous studies should be aimed at the isolation and characterization of bioactive compounds by means of modern chromatographic methods to find the lead compounds in the field of pharmaceutical development.

L. asiatica extract standardization is essential requirement of clinical translation, and quality control parameters, such as marker compound quantification and extraction protocols optimization, have to be developed. The exploration of the potential of combination therapy with conventional antidiabetic drugs may offer synergistic effects and allow a decrease in the amount of individual drugs and side effects. Advanced mechanistic studies using genomics and proteomics approaches will provide comprehensive understanding of L. asiatica's molecular targets for personalized medicine applications.

5. CONCLUSION

This comprehensive investigation provides robust scientific evidence supporting the traditional use of Leea asiatica for diabetes management through demonstration of significant antidiabetic efficacy, excellent safety profile, and multitarget therapeutic mechanisms. The methanolic extract was found to produce clinically significant decreases in blood glucose, HbA1c and diabetic complications and at the same time improving lipid profiles and preventing damage to vital organs due to diabetes. *L. asiatica* has a high potential of developing natural antidiabetic drugs due to its non-toxicity at therapeutic doses and its multifactorial effect on diabetes pathophysiology. The clinical implications are not limited to mere glucose control, but a full-fledged management of metabolic syndrome with the possibility of preventing diabetic complications by tissue-protective actions. The study has added value to the ethnopharmacological validation and forms a basis to further research of plant-based diabetes medicines. Such results justify the need to combine the knowledge of traditional medicine with modern scientific practices in order to come up with safe, effective, and affordable treatment to manage diabetes in the world.

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