RESEARCH PAPER

Protective Effect of Leea asiatica Against Oxidative Stress and Hyperglycemia in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, oxidative stress, and impaired insulin secretion, leading to severe systemic complications. The present study was designed to investigate the antioxidant and antidiabetic potential of different extracts of Leea asiatica through comprehensive in-vitro and in-vivo experimental models. Leaves and flowers of the plant were collected from Madhya Pradesh, India, and subjected to pharmacognostic, physicochemical, phytochemical, antioxidant, and antidiabetic evaluations. Successive extraction was carried out using petroleum ether, ethyl acetate, methanol, and aqueous solvents. Preliminary phytochemical screening revealed the presence of flavonoids, phenolics, tannins, alkaloids, glycosides, proteins, and triterpenoids, particularly in the methanol extract. The methanol extract exhibited the highest total phenolic content (60.71 ± 1.04 mg GAE/g dry extract) and total flavonoid content (47.20 ± 0.68 mg QE/g dry extract). In-vitro antioxidant studies demonstrated significant nitric oxide and superoxide radical scavenging activities with IC50 values of 48.48 µg/ml and 49.18 µg/ml, respectively. Acute toxicity studies confirmed the safety of extracts up to 2000 mg/kg body weight. In the oral glucose tolerance test, methanol extract significantly improved glucose utilization and reduced elevated blood glucose levels. Streptozotocin-induced diabetic rats treated with methanol and aqueous extracts of Leea asiatica for 28 days showed significant reductions in fasting blood glucose and glycosylated hemoglobin levels, along with improvement in body weight, pancreatic weight, and serum insulin levels. The methanol extract at 200 mg/kg exhibited maximum antidiabetic activity comparable to standard glibenclamide treatment. The findings suggest that Leea asiatica possesses potent antioxidant and antidiabetic activities, possibly due to its rich phenolic and flavonoid contents, and may serve as a promising natural therapeutic agent for the management of diabetes mellitus.

Keywords: Leea asiatica, antioxidant activity, antidiabetic activity, streptozotocin, flavonoids, phenolics.

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INTRODUCTION

Diabetes mellitus is a major global health problem characterized by chronic hyperglycemia and associated metabolic abnormalities. Persistent elevation of blood glucose levels causes oxidative stress and generation of reactive oxygen species, leading to pancreatic β-cell dysfunction and various diabetic complications [1]. Diabetes mellitus is one of the most prevalent chronic metabolic disorders worldwide and has emerged as a major public health challenge in India. It is characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Among the different forms of diabetes, Type 2 Diabetes Mellitus (T2DM) is the most common and accounts for nearly 90–95% of all diabetic cases. Rapid urbanization, sedentary lifestyle, unhealthy dietary habits, obesity, stress, and genetic predisposition have significantly contributed to the increasing burden of diabetes in the Indian population. India is often referred to as the "diabetes capital of the world" due to the rapidly growing number of diabetic patients [2-4].

The prevalence of diabetes in India has increased dramatically over the past few decades. According to recent epidemiological studies, millions of Indians are affected by diabetes, and the number is expected to rise further in the coming years. The disease not only affects the quality of life but also leads to severe complications such as cardiovascular disorders, nephropathy, neuropathy, retinopathy, and diabetic foot ulcers. These complications increase healthcare costs and contribute substantially to morbidity and mortality. In addition, limited healthcare access in rural regions and poor awareness regarding disease management further aggravate the diabetic burden in India [5-6].

Conventional antidiabetic drugs such as sulfonylureas, biguanides, thiazolidinediones, and insulin therapy are widely used for diabetes management. Although these therapies are effective in controlling blood glucose levels, prolonged use is often associated with adverse effects including hypoglycemia, gastrointestinal disturbances, weight gain, hepatic dysfunction, and drug resistance. Therefore, there is growing interest in the development of safer and more effective therapeutic alternatives derived from natural sources [7-10].

Herbal medicines have been used in traditional Indian systems of medicine such as Ayurveda, Siddha, and Unani for centuries in the treatment of diabetes. Medicinal plants contain various bioactive phytoconstituents including flavonoids, alkaloids, glycosides, terpenoids, tannins, and phenolic compounds that exhibit antidiabetic potential through different mechanisms such as enhancement of insulin secretion, improvement of insulin sensitivity, inhibition of carbohydrate metabolizing enzymes, reduction of oxidative stress, and regeneration of pancreatic β-cells. Herbal therapy is considered relatively safe, economical, and culturally acceptable, making it an attractive option for long-term diabetes management [11].

Several Indian medicinal plants have demonstrated significant antidiabetic activity in experimental and clinical studies. These plants are reported to possess antioxidant, anti-inflammatory, hypolipidemic, and glucose-lowering properties that may help in preventing diabetic complications. Scientific validation of traditional herbal medicines has therefore become an important area of pharmaceutical and biomedical research [12].

In recent years, extensive research has focused on the isolation of active phytoconstituents, formulation development, and evaluation of herbal products for antidiabetic activity. The integration of traditional herbal knowledge with modern scientific approaches may provide novel therapeutic agents for effective diabetes management. Hence, exploration of herbal medicines as alternative or complementary therapy for diabetes represents a promising and economically viable approach, particularly in developing countries like India where the disease burden is continuously increasing [13-14].

Plant-derived medicines are widely explored as alternative therapies for diabetes due to their effectiveness, safety, and reduced adverse effects. Leea asiatica is a traditionally important medicinal plant widely used in folk and Ayurvedic systems of medicine for the treatment of various ailments. *Leea asiatica* is a medicinal plant traditionally used for treatment of inflammation, wounds, metabolic disorders, and various systemic diseases. Different parts of the plant, including leaves, roots, and stems, are used as remedies for fever, skin diseases, wounds, diarrhea, dysentery, and inflammatory disorders. Tribal communities have also utilized the plant for its analgesic, antimicrobial, and antipyretic properties. Paste prepared from the leaves is commonly applied externally for cuts, ulcers, and swelling, while decoctions of roots and stems are administered internally to relieve digestive disorders and body pain. The plant is also believed to possess antioxidant and hepatoprotective activities, which support its traditional use in maintaining general health and treating chronic diseases. The plant contains several phytochemicals including flavonoids, phenolics, tannins, alkaloids, glycosides, and terpenoids that may contribute to its therapeutic effects [15-16]. Natural antioxidants protect biological systems from oxidative damage by neutralizing free radicals. Similarly, inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase is an important therapeutic approach for controlling postprandial hyperglycemia. Therefore, the present study aimed to evaluate the antioxidant and antidiabetic potential of *Leea asiatica* through detailed in-vitro and in-vivo investigations.

MATERIALS AND METHODS

Collection and Authentication

Fresh leaves and flowers of *Leea asiatica* were collected from Bhopal region, Madhya Pradesh, India and authenticated by a qualified taxonomist.

Pharmacognostic Studies

Morphological and microscopic characteristics of leaves were examined using standard pharmacognostic procedures. Powder microscopy and leaf surface analysis were also carried out.

Physicochemical Parameters

Total ash, acid insoluble ash, water soluble ash, extractive values, moisture content, and foreign organic matter were determined according to WHO guidelines.

Preparation of Extracts

Powdered plant material was successively extracted with petroleum ether, chloroform, methanol, and water. Extracts were concentrated and percentage yield was calculated.

Preliminary Phytochemical Screening

Qualitative tests were performed for detection of alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, proteins, carbohydrates, phytosterols, and triterpenoids.

Determination of total phenolic content

The Total Phenolic content of extract of *Gardenia Gumnifera and Leea asiatica* was determined by using Folin's-Ciocalteu reagent and Gallic acid as a calibration standard**.** The total phenolic content of the *Leea asiatica* extracts was determined by using Gallic acid equivalence (GAE).The dry extracts were diluted in methanol in the concentration of mg/ml of the samples and 1ml was transferred to a 10 ml volumetric flasks, to which 0.5ml Folin's- Ciocalteu reagent was added. After one minute, 1.5ml of 20% (w/v) Na2CO3 was added and the volume made up to 10 ml with distilled water. The reaction mixture incubated at 25˚C for one hour and the absorbance was measured at 760nm and compared with a prepared gallic acid calibration curve. Quantification was done on the basis of a standard curve of gallic acid. A standard curve of absorbance against gallic acid concentration was prepared.

Determination of total flavonoid content
The total flavonoid content was determined by aluminum chloride method. Quercetin was used as standard and calibration standard. Ten milligram of Quercetin was dissolved in 100 ml of methanol (80%) (100 µg/ml) and then further diluted to 10, 20, 30, 40 or 50 µg/ml. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of methanol (95%), 0.1 ml of aluminium chloride (10%), 0.1 ml of 1 M potassium acetate and 2.8 ml of diluted water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with UV/VIS spectrophotometer. A standard curve of absorbance against rutin concentration was prepared. Results were expressed as percentage w/w.

Flavonoids content (% w/w) = QE×V×D×10–6×100/W,

In-vitro Antioxidant Assays
Nitric oxide radical scavenging assay and superoxide radical scavenging assay were carried out using standard procedures.

Sample code for Leea asiatica Flower extract
PLA: Petroleum ether extract of Leea asiatica

CLA: ethyl acetate extract of Leea asiatica

MLA: Methanol extract of Leea asiatica

AGG: Aqueous extract of Leea asiatica

Oral glucose tolerance test: OGTT was evaluated in all the animals after an overnight fast. Briefly, the animals were dosed with 2 g/kg of glucose solution and blood samples were collected by tail pricking method at five time point (0, 30, 60, 90 and 120 mins) after glucose load for estimation of blood glucose level. Glucose is the main source of energy in our body; oral glucose tolerance test measures the body’s ability to use glucose effectively. Plant extract was suspended in CMC, were administered orally using an intragastric tube to different groups.

Experimental design for Oral glucose tolerance test of *Leea asiatica*

Normal Control	Normal saline
Standard treated	Glibenclamide5 mg/kg b.w
MLA 100 mg/kg	Methanol extract of *Leea asiatica* 100 mg/kg
MLA 200 mg/kg	Methanol extract of *Leea asiatica* 200 mg/kg
ALA 100 mg/kg	Aqueous extract of *Leea asiatica* 100 mg/kg
ALA 200 mg/kg	Aqueous extract of *Leea asiatica* 200 mg/kg

In-vitro Antidiabetic Activity
α-Amylase inhibitory assay and α-glucosidase inhibitory assay were performed using acarbose as standard drug.

Acute Toxicity Studies
Acute oral toxicity studies were conducted according to OECD guideline 423.

In-vivo Antidiabetic Study
Streptozotocin-induced diabetic rats were treated with methanol and aqueous extracts of *Leea asiatica* for 28 days. Blood glucose levels, lipid profile, HbA1c, renal biomarkers, pancreatic weight, insulin levels, and antioxidant enzyme activities were evaluated.

Experimental design for anti-diabetic study of *Leea asiatica*

Group	**Treatment**
Normal Control	Control rats gavaged with normal saline
Diabetic Control	Diabetic rats + normal saline.
Standard treated	Diabetic rat + Glibenclamide 5mg/kg b.w
MLA 100 mg/kg	Diabetic rat + methanol extract of *Leea asiatica* 100 mg/kg
MLA 200 mg/kg	Diabetic rat + methanol extract of *Leea asiatica* 200 mg/kg
ALA 100 mg/kg	Diabetic rat + aqueous extract of *Leea asiatica* 100 mg/kg

ALA 200 mg/kg	Diabetic rat + aqueous extract of *Leea asiatica* 200 mg/kg

RESULTS AND DISCUSSION

Pharmacognostic Evaluation

Leea asiatica was identified as a rigid shrub or small tree. Microscopic analysis showed anisocytic stomata, wavy epidermal cells, cluster crystals, lignified fibers, rosette crystals, tannin-containing cells, starch grains, and raphides.

Physicochemical Evaluation

Physicochemical analysis revealed total ash value of 7.9%, water soluble ash of 4.72%, acid insoluble ash of 2.08%, alcohol soluble extractive value of 11.2%, and water soluble extractive value of 15.3%. The lower foreign organic matter content confirmed purity of plant material.

Phytochemical Screening

Methanol extract showed the presence of maximum phytoconstituents including flavonoids, phenolics, alkaloids, tannins, glycosides, proteins, and triterpenoids.

Total Phenolic and Flavonoid Content

The methanol extract exhibited the highest total phenolic content (60.71 ± 1.04 mg GAE/g dry extract) and total flavonoid content (47.20 ± 0.68 mg QE/g dry extract). The high concentration of phenolic compounds indicates strong antioxidant potential.

Nitric Oxide Radical Scavenging Activity

Methanol extract exhibited potent nitric oxide radical scavenging activity ranging from 51.87% to 77.05% inhibition at concentrations of 50–250 µg/ml. The IC50 value was 48.48 µg/ml. The concentration-dependent inhibition confirmed effective free radical scavenging ability.

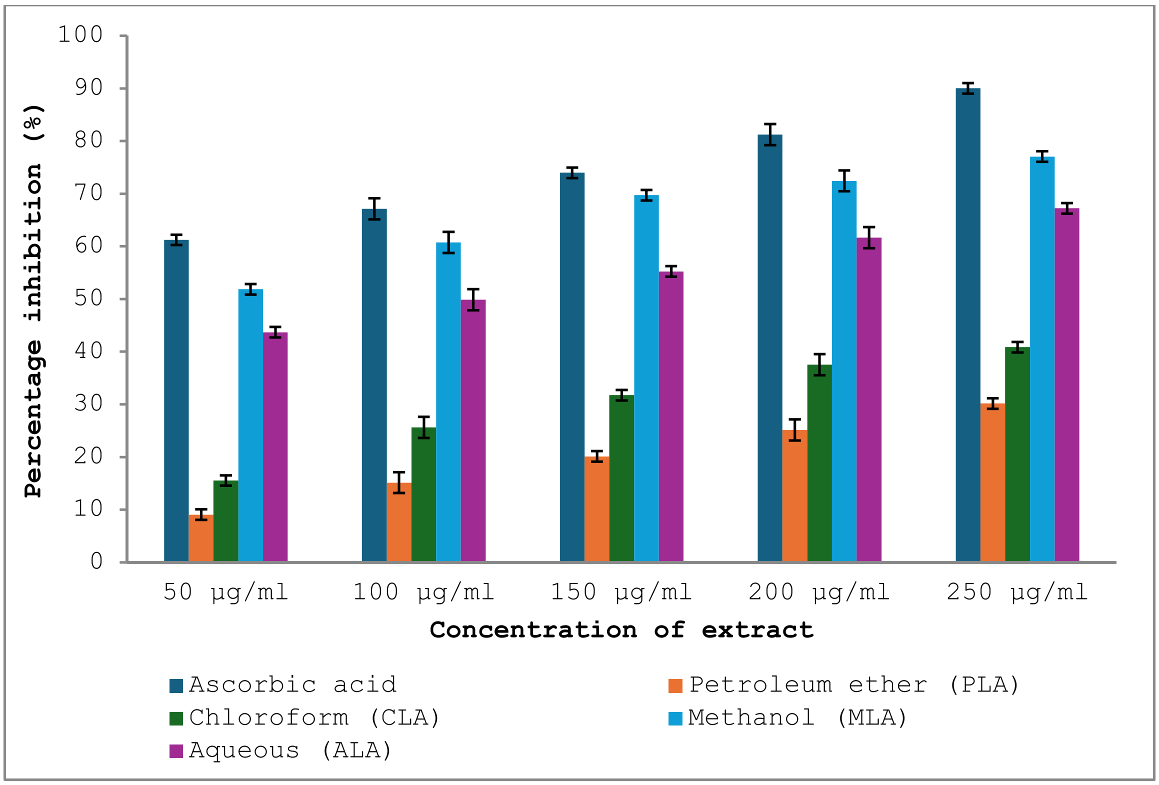

Figure 5.13: Results of Leea asiatica extracts on Nitric oxide radical scavenging model

Superoxide Radical Scavenging Activity

The methanol extract demonstrated maximum superoxide scavenging activity with inhibition values ranging from 28.14% to 62.87%. The IC50 value was 49.18 µg/ml, indicating excellent antioxidant activity.

The antioxidant activity may be attributed to flavonoids and phenolic compounds capable of donating electrons to neutralize superoxide radicals.

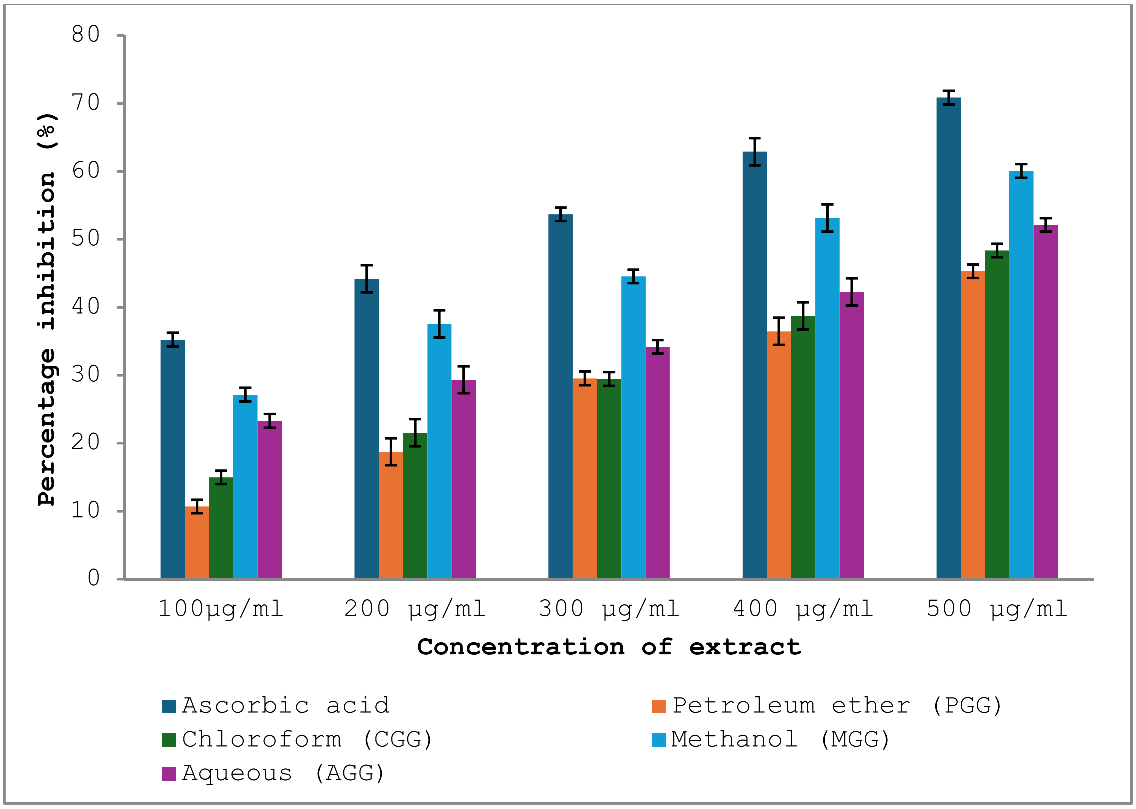

Figure 5.16: Effect of Gardenia gummifera extracts on Super oxide radical scavenging activity

Acute Toxicity Study

No mortality or toxic manifestations were observed at doses up to 2000 mg/kg, confirming safety of extracts.

Oral glucose tolerance test: OGTT was evaluated in all the animals after an overnight fast. Briefly, the animals were dosed with 2 g/kg of glucose solution and blood samples were collected by tail pricking method at five time point (0, 30, 60, 90 and 120 mins) after glucose load for estimation of blood glucose level. Glucose is the main source of energy in our body; oral glucose tolerance test measures the body's ability to use glucose effectively.

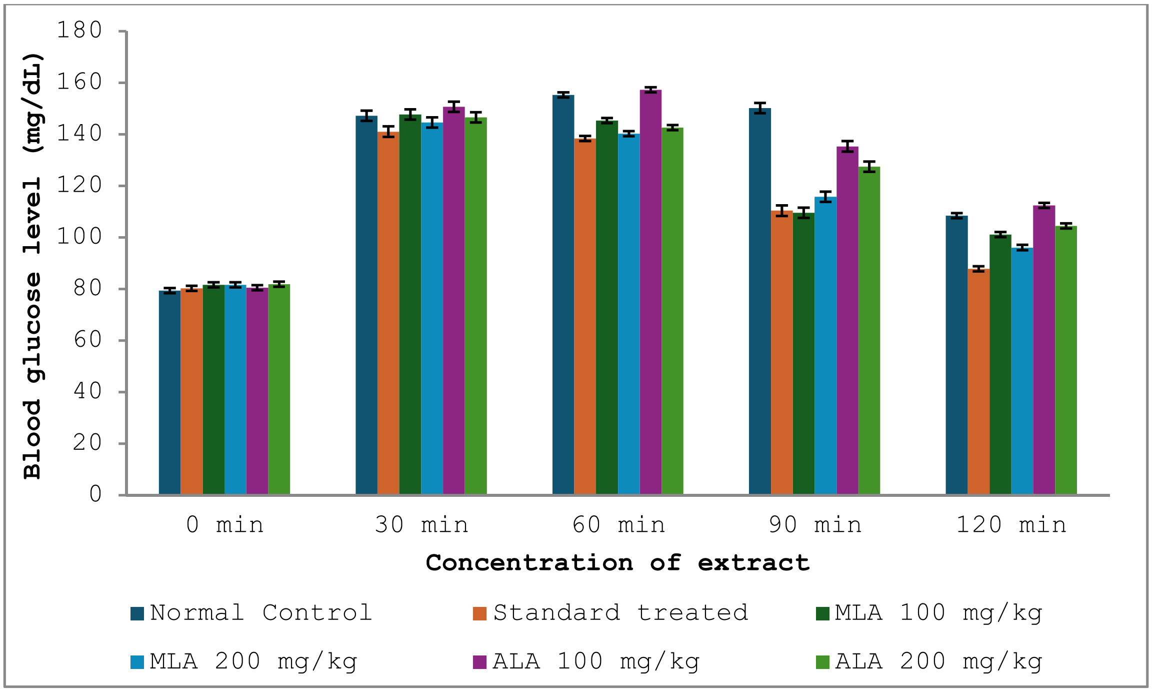

Figure 5.25: Effect of Leea asiatica On Oral glucose tolerance test

Since OGTT is performed to estimate the status of glucose intolerance and glucose transfer performance in animals. It is seen from the blood glucose profile that upon glucose challenge the levels were greatly increased in STZ animals compared to control rats. It was observed that in normal control group average blood glucose was found to be 80 mg/dL. Leea asiatica aqueous extract at dose 100mg/kg have minimum effect. High dose of MGG 100mg/kg has normalized the glucose intolerance close to normal control animals. Similar kind of improved glucose tolerance was found in glibencamide treated groups. It was found that Leea asiatica methanol extract cause significant reduction of the elevated blood glucose levels.

Antidiabetic activity

Rats with a fasting glucose level >250 mg dL−1 were classified as diabetic Animals with fasting blood glucose (FBG) level above 250 mg/dL with typical diabetic symptoms of polyuria, polydipsia and polyphagia were selected for the study. Treatment with methanol extract of Leea asiatica at different doses (100mg/kg, and 200 mg/kg) to STZ-induced diabetic rats caused significant reduction of blood glucose levels. The Antidiabetic activity of Leea asiatica depends upon the dose and duration of the treatment. The maximum reduction of blood glucose in rats was observed on 28th day.

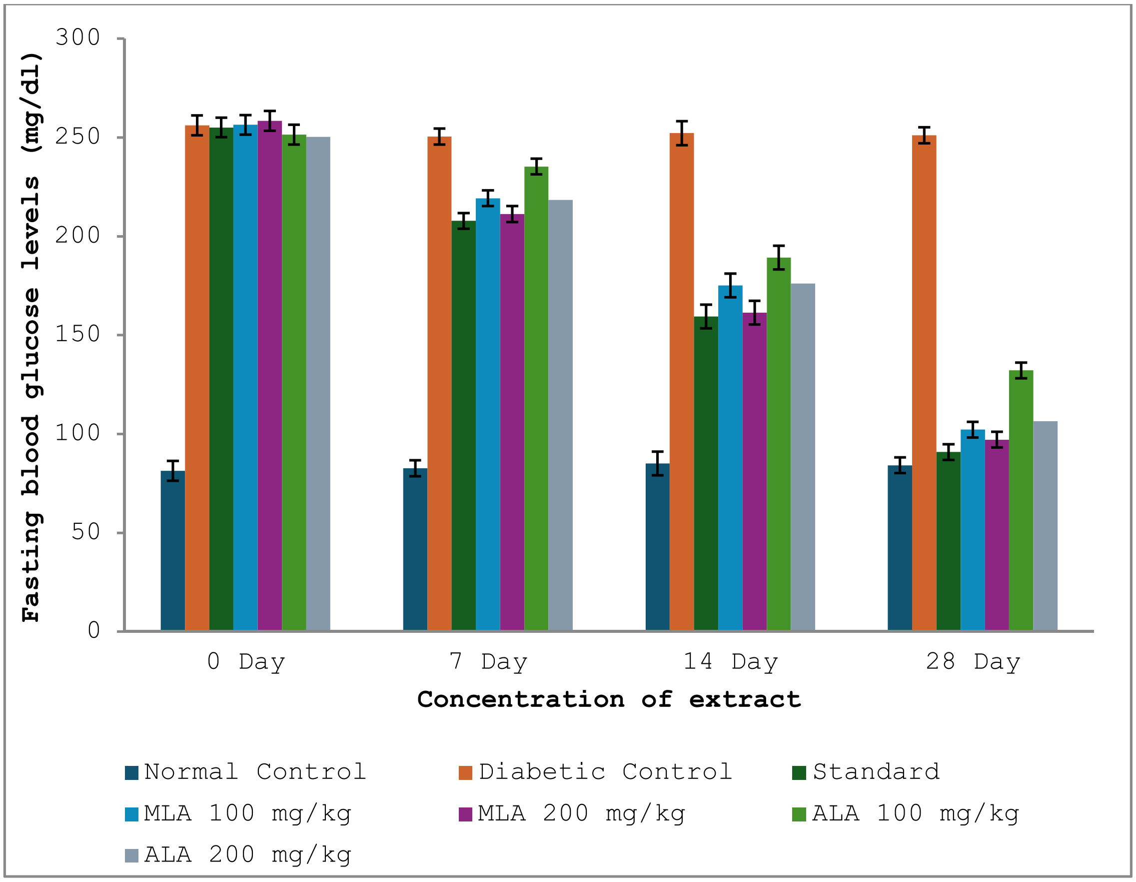

Figure 5.27: Effect of Leea asiatica on the blood glucose levels in diabetic rats

Body weight assessment

Body weight change is a key physiological indicator in diabetes studies because uncontrolled hyperglycemia leads to protein breakdown, muscle wasting, dehydration, and impaired glucose utilization, resulting in progressive weight loss in diabetic animals. The body weight of the normal rats before and after completion of experiment remained unchanged, but a significant reduction in body weight of STZ-induced diabetic rats was noted and maximum reduction was observed on 28th day.

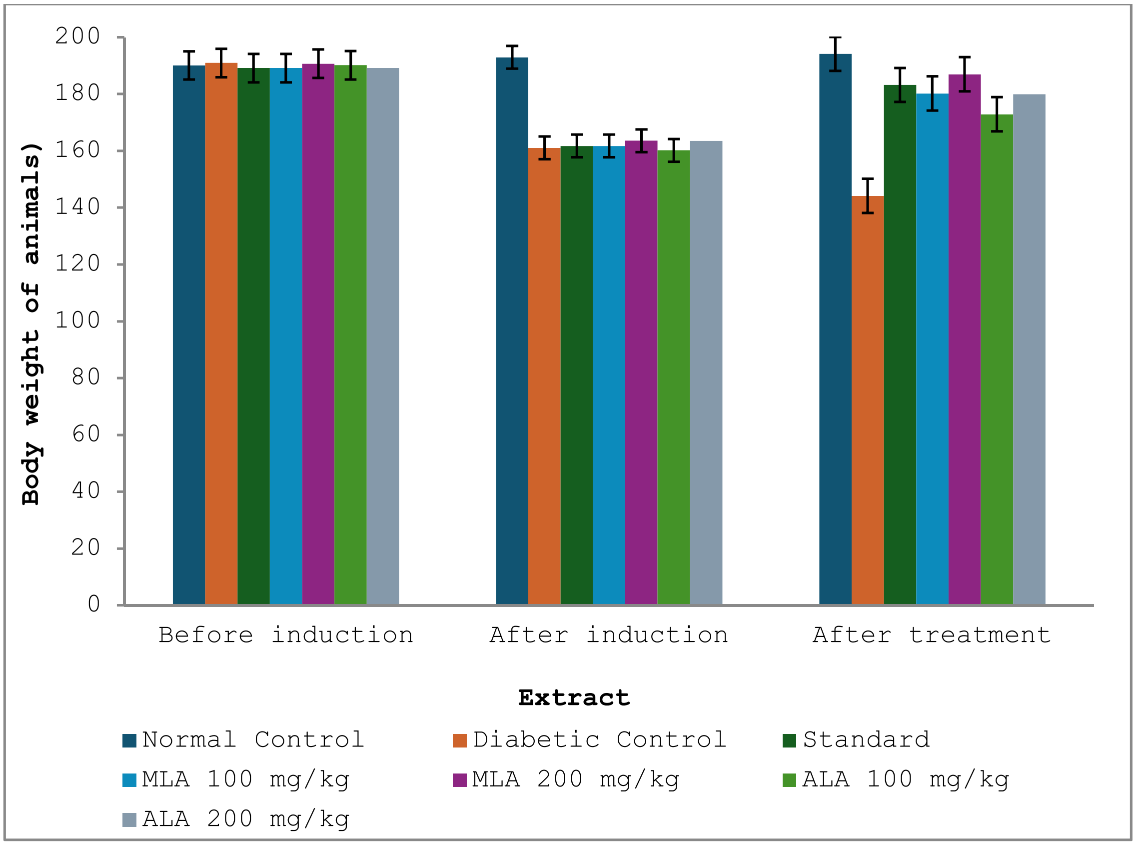

Figure 5.29: Effect of *Leea asiatica* on body weight of animals

Leea asiatica extracts show significant potential in managing both blood glucose levels and associated body weight changes in diabetic, in Streptozotocin-induced, models. Diabetes often causes significant body weight loss due to muscle wasting and increased adipose lipolysis, and methanol extract of *Leea asiatica* have been found to reverse this, promoting a regain or stabilization of body weight alongside reducing hyperglycemia

Estimation of insulin level

After 28th day of treatment blood samples were withdrawn by in order to examine the insulin levels. Serum insulin was measured. The decrease in serum insulin level indicates hyperglycemia in STZ-induced diabetic rats. The STZ-induced diabetic rats exhibited maximum decrease in serum insulin levels on 28th day.

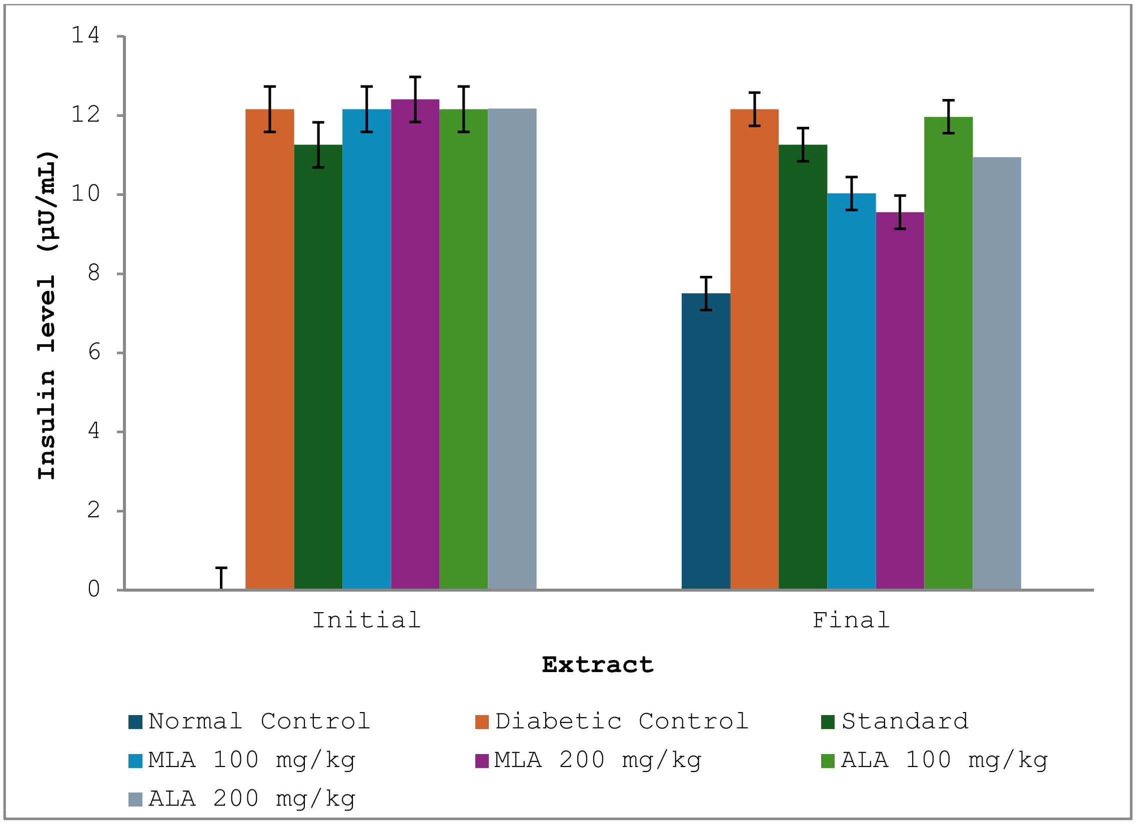

Figure 5.31: Effect of Leea asiatica on insulin level of animals

Treatment with *Leea asiatica* extracts at different doses (100 mg/kg and 200 mg/kg) showed decrease of insulin level dose dependently. The administration of *Leea asiatica* extract restored the insulin level compared to diabetic control, which was as significant as standard treated group

Pancreatic weights assessment

After sacrifice pancreas, was dissected and rapidly stored in cold saline until further use. Pancreas was isolated and store in ice cold saline, blotted and weighted. Pancreatic weight is an important indicator of structural integrity and functional status of pancreatic tissue in diabetes studies. Streptozotocin/alloxan-induced diabetes is typically associated with pancreatic atrophy due to β-cell destruction, oxidative stress, inflammation, and reduced insulin production. Therefore, restoration of pancreatic weight reflects protection or regeneration of pancreatic tissue.

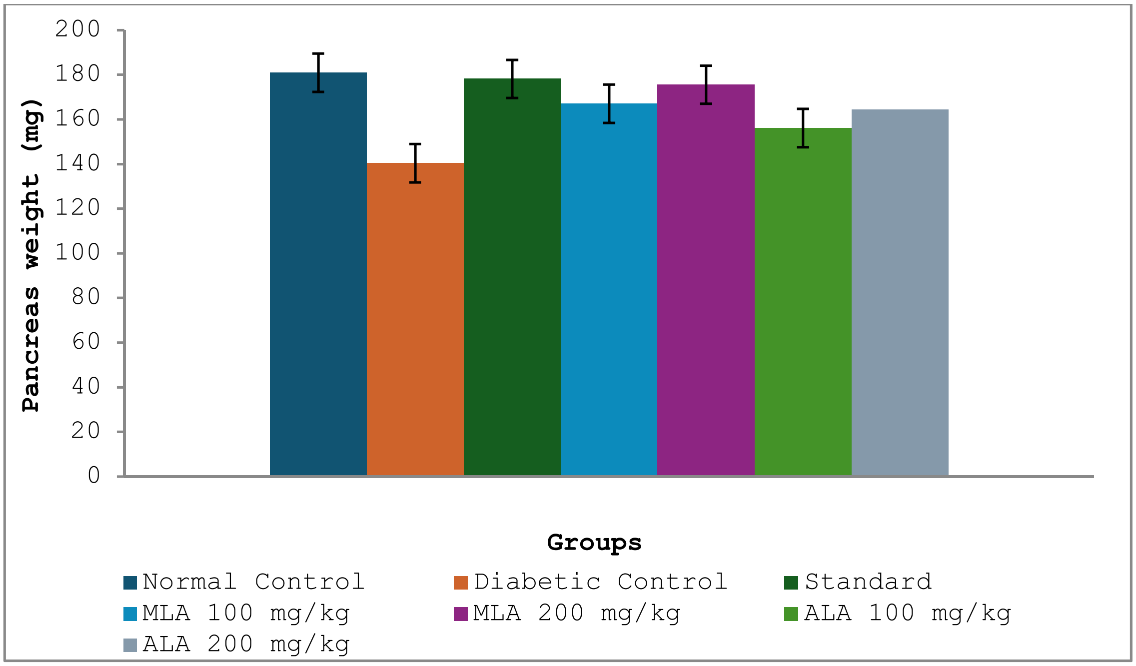

Figure 5.33: Effect of Leea asiatica on Pancreas weight

There was a remarkable reduction in the pancreatic weights in diabetic control animals compared to Normal Control animals. Their were maximum decreased in the pancreatic weight in diabetic control animals. Treatment with both plant extracts at different doses (100 mg/kg and 200 mg/kg) showed increase in pancreatic weight dose dependently.

Treatment with Methanol extract of *Leea asiatica* were found more effective in restored pancrease weight compared to diabetic control than aqueous extract.

Estimation of biochemical parameters

Estimation of glycosylated hemoglobin (HbA1c)

Diabetes is one of the most common diseases worldwide. This serious complication may result from increased glycation of healthy proteins as a consequence of associated chronic hyperglycemia. Most proteins (including hemoglobin) react with glucose and form covalent combinations without the requirement of enzymes. HbA1C is an inductor combination, which is reversible, but after the inner reformation of this combination, stable HbA1C is formed.

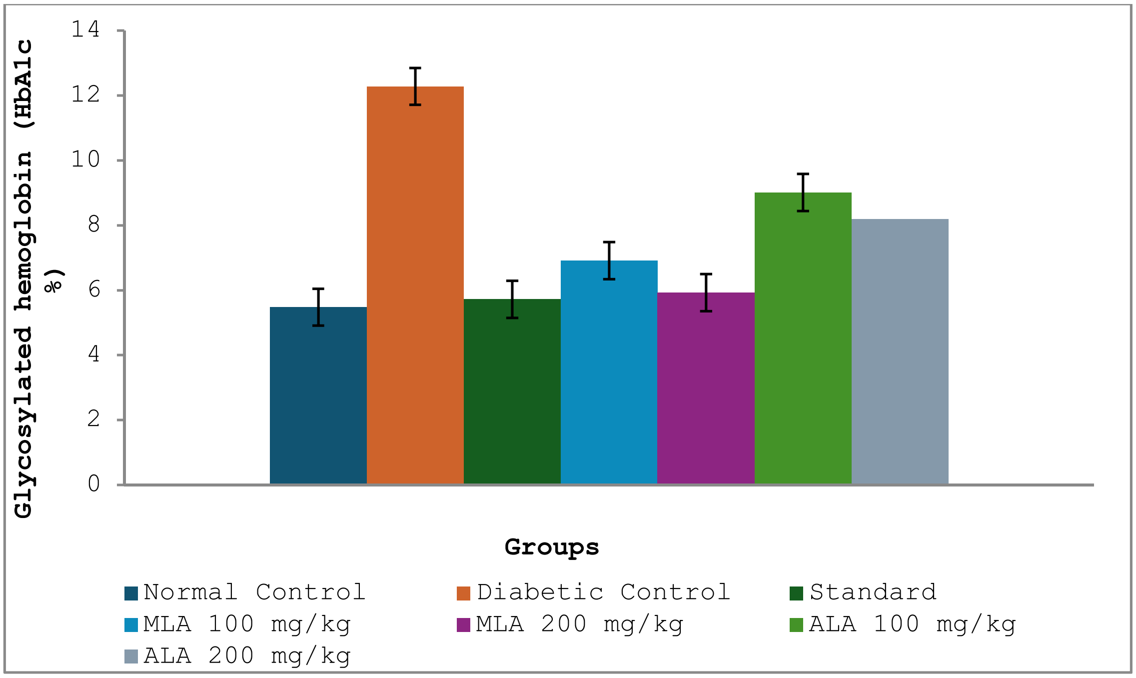

Figure 5.35: **Effect of Leea asiatica on glycosylated hemoglobin levels in animals**

DISCUSSION

The present investigation demonstrated significant antioxidant and antidiabetic activities of Leea asiatica extracts through both in-vitro and in-vivo experimental studies. The pharmacognostic and physicochemical evaluations confirmed the authenticity, purity, and quality of the plant material. Microscopic characteristics such as anisocytic stomata, lignified fibers, cluster crystals, starch grains, and tannin-containing cells serve as important diagnostic parameters for identification and standardization of the plant drug.

Phytochemical screening revealed the presence of various bioactive constituents including flavonoids, phenolics, tannins, alkaloids, glycosides, and triterpenoids, particularly in the methanol extract. Phenolic and flavonoid compounds are well known for their free radical scavenging and antidiabetic properties. The higher total phenolic and flavonoid contents observed in the methanol extract may be responsible for its potent biological activity. Phenolic compounds can donate hydrogen atoms or electrons to neutralize reactive oxygen species and thereby reduce oxidative stress associated with diabetes.

The methanol extract exhibited strong nitric oxide and superoxide radical scavenging activities in a concentration-dependent manner. Excessive production of reactive oxygen species contributes to pancreatic β-cell damage and insulin resistance in diabetes mellitus. Therefore, the antioxidant potential of Leea asiatica may play a crucial role in protecting pancreatic tissues against oxidative injury. The lower IC50 values observed for the methanol extract indicate its higher antioxidant efficiency compared to other extracts.

The oral glucose tolerance test demonstrated that treatment with Leea asiatica extracts significantly improved glucose utilization and reduced postprandial hyperglycemia. Improvement in glucose tolerance suggests enhancement of peripheral glucose uptake and possible stimulation of insulin secretion. The methanol extract at higher dose produced effects comparable to glibenclamide, indicating strong antihyperglycemic activity.

In streptozotocin-induced diabetic rats, administration of methanol and aqueous extracts significantly reduced fasting blood glucose levels in a dose-dependent manner. Streptozotocin induces diabetes by selective destruction of pancreatic β-cells through oxidative stress and DNA alkylation, resulting in insulin deficiency and hyperglycemia. Restoration of blood glucose levels following treatment indicates protective or regenerative effects of the extracts on pancreatic β-cells.

Diabetic animals showed marked reduction in body weight due to increased protein catabolism and impaired glucose utilization. Treatment with Leea asiatica extracts improved body weight significantly, suggesting enhanced glycemic control and improved metabolic status. Similarly, restoration of pancreatic weight in treated groups indicates possible protection against pancreatic atrophy and β-cell degeneration caused by diabetes.

The reduction in serum insulin levels observed in diabetic control rats confirms β-cell dysfunction induced by streptozotocin. Treatment with Leea asiatica extracts significantly restored insulin levels, suggesting stimulation of residual β-cells or regeneration of pancreatic tissue. Improvement in glycosylated hemoglobin (HbA1c) further confirmed long-term glycemic control following treatment.

Overall, the methanol extract showed superior activity compared to the aqueous extract, which may be attributed to higher concentrations of phenolic and flavonoid compounds extracted in methanol. The observed antidiabetic activity may involve multiple mechanisms including antioxidant action, enhancement of insulin secretion, inhibition of carbohydrate metabolizing enzymes, improvement of peripheral glucose uptake, and protection of pancreatic β-cells from oxidative stress.

CONCLUSION

The present study scientifically validated the traditional use of Leea asiatica as a medicinal plant with significant antioxidant and antidiabetic potential. Pharmacognostic and physicochemical studies confirmed the quality and authenticity of the plant material, while phytochemical investigations revealed the presence of important bioactive constituents such as flavonoids and phenolic compounds. Among all extracts, the methanol extract exhibited the highest antioxidant activity and demonstrated potent antihyperglycemic effects in streptozotocin-induced diabetic rats. Treatment with Leea asiatica extracts significantly reduced blood glucose and HbA1c levels while improving serum insulin levels, body weight, pancreatic weight, and glucose tolerance. The antioxidant activity of the extracts may contribute to protection of pancreatic β-cells against oxidative damage and improvement of overall metabolic function. Acute toxicity studies further established the safety profile of the extracts. The findings of the present study suggest that Leea asiatica could serve as a promising natural source for the development of safer and effective antidiabetic formulations. However, further studies are required to isolate and characterize the active phytoconstituents responsible for the observed pharmacological effects and to elucidate their exact molecular mechanisms of action through advanced preclinical and clinical investigations.

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