

OPEN ACCESS

Volume: 5

Issue: 4

Month: October

Year: 2026

ISSN: 2583-7117

Published: 07.10.2026

Citation:

Yashvant Kumar, Dr. Satkar Prasad, Priyanka More, Khushboo Chouhan "Assessment of the Antidiabetic Effects of Neomarica gracilis Leaf Extract in Streptozotocin-Induced Diabetic Rats: A Biochemical and Histopathological Study" International Journal of Innovations in Science Engineering and Management, vol.5, no.4, 2026, pp. 72-87

DOI:

10.69968/ijsem.2026v5i472-87



This work is licensed under a Creative Commons Attribution-Share Alike 4.0 International License

Assessment of the Antidiabetic Effects of Neomarica gracilis Leaf Extract in Streptozotocin-Induced Diabetic Rats: A Biochemical and Histopathological Study

Yashvant Kumar¹, Dr. Satkar Prasad², Priyanka More³, Khushboo Chouhan⁴

¹Reserach Scholar, Pharmacology, RKDF School of Pharmaceutical Science

²Principal, RKDF School of Pharmaceutical Science

³Asst Professor, Pharmacology, RKDF School of Pharmaceutical Science

⁴Assistant Professor, RKDF SPS (Pharmacology)

Abstract

The present study evaluated the antioxidant and antidiabetic activities of the methanolic leaf extract of *Neomarica gracilis*. Methanolic extraction of 400 g of dried leaves yielded 30.2 g of extract (7.55%). Phytochemical screening confirmed the presence of alkaloids, glycosides, flavonoids, phenolics, carbohydrates, and proteins. The extract exhibited appreciable total phenolic (37.22 mg GAE/g) and flavonoid (23.15 mg RE/g) contents. In the DPPH assay, the extract showed significant antioxidant activity with an IC_{50} value of 28.81 μ g/mL, compared with 17.10 μ g/mL for ascorbic acid. Acute oral toxicity studies indicated that the extract was safe up to 2000 mg/kg body weight, with no mortality observed. In streptozotocin-induced diabetic rats, the extract significantly reduced blood glucose levels and improved the lipid profile in a dose-dependent manner. The 400 mg/kg dose showed greater efficacy, reducing blood glucose to 96.79 ± 0.36 mg/dL by day 21, comparable to Metformin (92.80 ± 0.095 mg/dL). Histopathological findings also demonstrated protection and restoration of pancreatic tissue architecture. These findings suggest that the methanolic leaf extract of *Neomarica Gracilis* possesses significant antioxidant and antidiabetic activities and may be a promising natural agent for the management of diabetes mellitus.

Keywords; *Neomarica gracilis*, Antioxidant activity, Antidiabetic activity, DPPH, Streptozotocin, Methanolic extract.

INTRODUCTION

Herbal plants

Herbal plants, commonly referred to as herbs, are valued for their therapeutic, nutritional, aromatic, culinary, and cosmetic applications. They have been used in traditional medicine for thousands of years and continue to contribute to modern healthcare and wellness practices. Herbal medicines are used to prevent, manage, alleviate, or treat various diseases, particularly conditions traditionally associated with locally available medicinal plants. Although herbal products are often considered natural and safe, they may produce adverse effects or interact with conventional medicines; therefore, their use should be approached with appropriate medical guidance. Despite numerous traditional claims regarding the therapeutic potential of medicinal plants, only a limited proportion have been scientifically investigated and validated for their efficacy and safety (Mosihuzzaman et al., 2012).

Medicinal plants contain a wide range of phytochemicals and biologically active compounds that contribute to plant defense and may possess pharmacological properties. However, the complex chemical composition of whole plants makes their therapeutic effects, mechanisms of action, and safety difficult to establish without systematic investigation. The historical significance of medicinal plants is well documented, beginning with ancient Sumerian records and the Ebers Papyrus, which described numerous plant-based remedies. Later, Dioscorides documented more than 600 medicinal plants and numerous remedies in *De materia medica*,

establishing an important foundation for subsequent pharmacopoeias. Ethnobotanical research has also contributed to the discovery of several important drugs derived from natural sources, including aspirin, digoxin, opium, and quinine (Ahn et al., 2017).

Medicinal plants remain an important global source of therapeutic compounds, particularly in developing countries where a large proportion of the population depends on traditional herbal remedies. Increasing demand for natural health products has further expanded their utilization. However, overharvesting, habitat destruction, population growth, and increasing consumption have placed many medicinal plant species at risk of depletion and extinction. Consequently, conservation and sustainable utilization have become essential for maintaining these valuable biological resources. Integrated conservation approaches combining in situ and ex situ strategies, species inventories, population monitoring, and sustainable harvesting practices are particularly important for protecting medicinal plants and ensuring their continued availability (Chen et al., 2016).

Diabetes

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistently elevated blood glucose levels (hyperglycemia), resulting from impaired insulin secretion, reduced insulin action, or both. Persistent hyperglycemia causes disturbances in carbohydrate, lipid, and protein metabolism and can progressively impair the normal functioning of multiple organs. If inadequately controlled, diabetes may lead to serious complications, including retinopathy, neuropathy, nephropathy, cardiovascular disorders, and ulceration (Whiting et al., 2010).

Insulin and glucagon, secreted by the pancreatic islets of Langerhans, play major roles in maintaining blood glucose homeostasis. Insulin released by β -cells in response to increased blood glucose promotes glucose uptake and utilization by tissues, whereas glucagon secreted by α -cells during low blood glucose stimulates the release of stored glucose, particularly from the liver (Gupta and Amartya, 2012).

Diabetes includes several forms, such as type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes, neonatal diabetes, maturity-onset diabetes of the young (MODY), and diabetes secondary to other conditions or medications. T1DM is primarily associated with inadequate insulin production and commonly develops earlier in life, whereas T2DM involves impaired insulin action and/or secretion and is frequently

associated with lifestyle and dietary factors. Each form differs in its underlying pathophysiology, clinical presentation, and management requirements (Rajaei et al., 2019).

DIABETES MELLITUS TYPES

Type 1 diabetes mellitus (T1DM) is characterized by little or no insulin production, making lifelong insulin replacement essential for survival. It is broadly classified into type 1a and type 1b. Type 1a, comprising most cases, results from autoimmune destruction of pancreatic β -cells and may be associated with autoimmune disorders such as Hashimoto's thyroiditis, Addison's disease, and Graves' disease. Type 1b is idiopathic, with no identified cause, but involves severe insulin deficiency and a risk of ketoacidosis (Singab et al., 2014).

Type 1 diabetes

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder in which the immune system destroys the insulin-producing β -cells of the pancreas, resulting in little or no insulin production. It primarily affects children and young adults and may develop rapidly, making lifelong insulin replacement necessary for survival. Autoimmune markers such as glutamic acid decarboxylase (GAD), islet-cell, and insulin autoantibodies can help identify the underlying β -cell destruction. The rate of β -cell loss varies among individuals, with some experiencing rapid and others gradual progression. Untreated insulin deficiency causes persistent hyperglycemia and may lead to serious complications, including retinopathy, nephropathy, neuropathy, and vascular disease. T1DM may also occur with other autoimmune conditions, including Graves' disease, Hashimoto's thyroiditis, and Addison's disease. Although autoimmune mechanisms are strongly implicated, the precise cause remains incompletely understood (Gordon et al., 2020).

Type 2 diabetes

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes and is primarily characterized by insulin resistance combined with inadequate insulin secretion. Genetic predisposition and environmental factors contribute to its development, while prolonged metabolic stress may progressively impair pancreatic β -cell function (Türkvtan et al., 2013). It occurs mainly in adults and older individuals and is often associated with unhealthy dietary habits, physical inactivity, obesity, and other metabolic conditions. Unlike type 1 diabetes, T2DM is generally not dependent on ketosis and can often be managed through lifestyle

modification, oral hypoglycemic medicines, and, when necessary, insulin therapy. Other forms of diabetes may result from genetic defects affecting pancreatic β -cells or insulin receptors, pancreatic diseases such as pancreatitis and cystic fibrosis, endocrine disorders such as Cushing's syndrome and acromegaly, or certain medications, including glucocorticoids, interferon, and niacin (Craig et al., 2009).

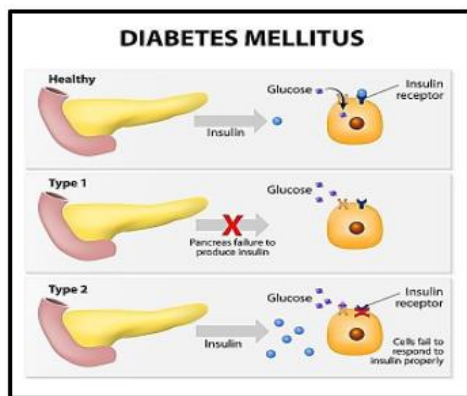


Figure 1: Diabetes mellitus

Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) refers to glucose intolerance or diabetes first recognized during pregnancy, most commonly in the second or third trimester. It is distinct from pre-existing diabetes, although previously undiagnosed type 2 diabetes may sometimes be identified during pregnancy. Current diagnostic approaches distinguish overt diabetes detected during pregnancy from GDM, particularly among women at high risk because of factors such as obesity. GDM generally develops during pregnancy and often resolves after delivery or termination of pregnancy (Noctor et al., 2016). However, maternal hyperglycemia during pregnancy can affect fetal development and increase the child's later risk of obesity and metabolic disorders. Therefore, appropriate detection and management of GDM are important for protecting both maternal and offspring health.

PLANT PROFILE



Figure 2: Neomarica Gracilis

Table 1: Plant Profile

Plant Name	Neomarica Gracilis
Scientific classification of Neomarica Gracilis	Domain: Eukaryota Kingdom: Plantae Clade: Angiosperms Clade: Monocots Order: Asparagales Family: Iridaceae Genus: Neomarica Species: Neomarica Gracilis (Herb.) Sprague
Synonyms	<i>Marica gracilis</i> , <i>Cipura gracilis</i> , and <i>Cypella gracilis</i> .
Common name	<i>Walking Iris</i> , <i>Apostle Plant</i> , or <i>Fan Iris</i> .

Description:

Neomarica Gracilis is a rhizomatous, evergreen herb characterized by long, narrow, sword-shaped leaves arranged in a fan-like manner. The leaves are glossy green, arching, and can grow up to 60–90 cm in length, giving the plant a graceful, grass-like appearance. The flowering stalks arise from the leaf axils and bear attractive, short-lived but showy flowers.

Distribution Neomarica gracilis:

Neomarica Gracilis (commonly known as walking iris or apostle plant) is native to tropical regions of Central and South America, particularly Brazil, where it grows naturally in warm, humid environments. The plant is widely distributed in rainforests, forest margins, and shaded woodland areas, thriving in well-drained soils. Due to its attractive foliage and ornamental flowers, Neomarica Gracilis has been extensively introduced and cultivated in many parts of the world, including Asia, Africa, Australia, and subtropical regions, where it adapts well to gardens, landscapes, and indoor conditions in frost-free climates (Maldonado, 2025).

Habitat of Neomarica gracilis

Neomarica Gracilis is native to the tropical regions of Central and South America, particularly Brazil. It naturally grows in warm, humid environments such as forest margins, understories, and shaded open woodlands. The plant thrives in well-drained, fertile soils rich in organic matter and prefers partial shade to filtered sunlight. It is commonly found in areas with moderate rainfall and consistently moist soil conditions, although it can tolerate short dry periods (Odenwald and, Turner 2006)

Uses of Neomarica gracilis

Neomarica gracilis, commonly known as the "Walking Iris," is primarily an ornamental plant valued for its attractive flowers and foliage. Its uses in brief: Neomarica

Gracilis is mainly used for decorative and landscaping purposes due to its striking blue or purple flowers and lush green foliage. It is planted in gardens, patios, and indoor pots to enhance aesthetic appeal. Additionally, it can be used for border planting and ground cover because of its hardy growth and spreading habit (MacCubbin and Tasker 2013).

Table 2: Pharmacological activities of *Neomarica Gracilis*

Antimicrobial activity	Shows potential against certain bacterial and fungal strains.
Antioxidant activity	Exhibits free radical scavenging properties.
Anti-inflammatory activity	May reduce inflammation through inhibition of inflammatory mediators.
Cytotoxic activity	Some studies suggest potential activity against certain cancer cell lines.
Hepatoprotective activity	Provides protective effects against liver damage in experimental models (Mound and Marullo 1996).

OBJECTIVES

1. To collect and authenticate *Neomarica Gracilis* leaves.
2. To prepare the leaf extract using suitable extraction methods.
3. To perform phytochemical screening of the leaf extract.
4. To induce diabetes in Wistar rats using streptozotocin.
5. To administer the leaf extract and assess its effect on fasting blood glucose levels.
6. To monitor changes in body weight of diabetic rats after treatment.
7. To evaluate the effect of the extract on selected biochemical parameters (lipid profile, liver and kidney function tests).
8. To examine pancreatic tissue histopathology for protective or restorative effects.
9. To compare the efficacy of the leaf extract with standard antidiabetic treatment.

MATERIAL AND METHODS

List of reagent and chemicals used

Table 3: List of reagent and chemicals

Reagent / Chemical	Purpose	Typical Supplier / Source
Methanol	Extraction solvent for phytochemicals	Merck
Distilled Water	Solvent & reagent preparation	Laboratory distilled

Phytochemical Tests Reagents		
Molisch's reagent	Carbohydrate detection	Hi-Media / Merck
Fehling's solutions A & B	Reducing sugar test	Hi-Media
Benedict's reagent	Reducing sugar test	Hi-Media
Barfoed's reagent	Monosaccharide test	Prepared in-lab
Borntrager's reagent	Glycoside test	Hi-Media / Sigma-Aldrich
Legal's reagent	Cardiac glycoside test	Hi-Media
Keller-Killiani reagent	Glycoside test	Prepared in-lab
Dragendorff's reagent	Alkaloid detection	Hi-Media
Mayer's reagent	Alkaloid detection	Hi-Media
Wagner's reagent	Alkaloid detection	Hi-Media
Hager's reagent	Alkaloid detection	Hi-Media
Ferric chloride (FeCl ₃)	Phenolic test	Merck / Research Fine Chem
Lead acetate solution	Triterpenoid/steroid test	Merck
Foam test reagents	Saponin detection	In-lab preparations
Quantitative Assay Reagents		
Folin-Ciocalteu reagent	Total Phenolic Content (TPC)	Sigma-Aldrich (Germany) (ScienceDirect)
Gallic acid standard	TPC standard	Sigma-Aldrich
Aluminum chloride	Flavonoid complexation (TFC)	Sigma-Aldrich
Rutin standard	TFC standard	Sigma-Aldrich
Sodium carbonate (Na ₂ CO ₃)	TPC assay	Merck
Dimethyl sulfoxide (DMSO)	Solvent for in-vitro assays	Merck / Hi-Media
Antidiabetic & Biochemical Reagents		
Streptozotocin (STZ) / Alloxan monohydrate	Diabetes induction in rats	Sisco Research
Normal saline (0.9% NaCl)	Vehicle for STZ/Alloxan	In-lab/Local pharmacy
Glucose solution	Oral glucose tolerance test (OGTT)	Merck
Glibenclamide	Positive control	Local pharmacy
Biochemical assay kits (e.g., blood glucose, lipid profile)	Serum analysis	Roche Diagnostics

Table 4: List of glassware's

S. No.	Glassware / Equipment	Company / Brand
1	Graduated pipette	Borosilicate
2	Glass rod	Borosilicate
3	Volumetric flask	Borosilicate
4	Beakers	Borosilicate
5	Test tubes	Borosilicate
6	Petri dishes	Borosilicate
7	Conical flasks	Borosilicate
8	Measuring cylinders	Borosilicate
9	Funnels (glass)	Borosilicate
10	Micropipettes / Micropipette tips	Eppendorf / Borosilicate
11	Test tube racks	Plastic / Borosilicate
12	Mortar & pestle	Porcelain / Borosilicate
13	Dropper bottles	Borosilicate / Plastic
14	Reagent bottles	Borosilicate
15	Glass stoppers	Borosilicate
16	Test tube clamps & holders	Borosilicate / Metal

Table 5: List of Instrument's

S. No.	Instrument	Indian Company
1	Soxhlet apparatus	Borosil Scientific
2	Analytical balance	Essae / Shimadzu India
3	Hot air oven	Labtech Instruments
4	Rotary evaporator	Borosil / Perfit Scientific
5	UV-Vis spectrophotometer	Systronics / Elico
6	pH meter	Systronics
7	Viscometer	Labindia Instruments
8	Centrifuge	Remi
9	Glucometer	Roche Diabetes Care India
10	Refrigerator	Godrej
11	Microscope	Olympus
12	Microtome	Leica
13	Water bath	Borosil
14	Glassware	Borosil
15	Gavage needle	Hindustan Syringes

Collection of Plant Material

A total of 400 g of *Neomarica Gracilis* leaves were collected for the present study. The collected plant material was carefully inspected and thoroughly cleaned to remove dust, soil particles, and other extraneous matter. After proper washing and cleaning, the leaves were shade-dried at room temperature for three days to facilitate gradual removal of moisture while preserving heat-sensitive and bioactive phytoconstituents. Following drying, the plant material was stored in airtight glass containers and kept in a cool and dry place to prevent contamination, microbial growth, moisture absorption, and degradation of active constituents. The

containers were appropriately labeled for easy identification and future reference. The authenticity and botanical identity of *Neomarica Gracilis* leaves were confirmed by a qualified plant taxonomist before their use in the study. This authentication ensured that the correct plant species was selected, thereby maintaining the accuracy, reproducibility, and scientific reliability of the experimental work.

Extraction

Powdered leaves of *Neomarica gracilis* were extracted by continuous hot percolation using a Soxhlet apparatus. The plant material was placed in a Soxhlet thimble and extracted with methanol at approximately 60°C to obtain bioactive phytoconstituents, including flavonoids, phenolics, glycosides, and other secondary metabolites. Extraction was continued until the siphoned solvent became colorless, indicating exhaustion of the plant material. Completion was further confirmed by evaporating a small amount of solvent and observing the absence of residue. The methanolic extract was concentrated using a rotary vacuum evaporator at 40°C under reduced pressure and subsequently dried and weighed to determine the percentage yield. The extract was assessed for color, odor, and consistency and stored in airtight containers under cool, dry conditions for further phytochemical and pharmacological evaluation (Kumar et al., 2023).



Figure 3: Continuous Hot Extraction Process

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Phytochemical Investigation of Neomarica Gracilis Leaf Extract

The methanolic leaf extract of *Neomarica Gracilis* was subjected to preliminary qualitative phytochemical screening to identify the presence of major classes of bioactive constituents such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenolics, proteins, amino acids, saponins, steroids, and triterpenoids. All tests were carried out using standard phytochemical procedures under controlled laboratory conditions, and results were recorded based on characteristic color changes or precipitate formation (Shrikant et al., 2024).

1. Tests for Alkaloids

- **Dragendorff's Test:** 1 mL of extract was mixed with acetic acid and Dragendorff's reagent. Formation of orange-red precipitate indicated alkaloids.
- **Mayer's Test:** Extract was treated with Mayer's reagent; formation of cream or dull white precipitate confirmed alkaloids.
- **Wagner's Test:** Extract was mixed with Wagner's reagent; brown or reddish-brown precipitate indicated alkaloids.
- **Hager's Test:** Addition of Hager's reagent to extract produced yellow precipitate indicating alkaloids.

2. Tests for Carbohydrates

- **Molisch's Test:** Extract was treated with Molisch reagent and concentrated H_2SO_4 was added carefully. A violet ring at the interface confirmed carbohydrates.
- **Fehling's Test:** Extract was heated with Fehling's A and B solution; formation of brick-red precipitate indicated reducing sugars.
- **Benedict's Test:** Extract was heated with Benedict's reagent; green/yellow/red precipitate confirmed carbohydrates.
- **Barfoed's Test:** Extract was heated with Barfoed's reagent; red precipitate indicated monosaccharides.

3. Tests for Flavonoids

- **Alkaline Reagent Test:** Addition of NaOH to extract produced yellow coloration, which disappeared on addition of acid, confirming flavonoids.

- **Lead Acetate Test:** Extract treated with lead acetate solution; yellow precipitate indicated flavonoids.

4. Tests for Tannins and Phenolic Compounds

- **Ferric Chloride Test:** Extract treated with FeCl_3 solution; blue/green coloration indicated phenolics and tannins.

5. Tests for Proteins and Amino Acids

- **Biuret Test:** Extract treated with NaOH and CuSO_4 solution; violet color indicated proteins.
- **Ninhydrin Test:** Heating extract with ninhydrin solution produced blue/violet color indicating amino acids.

6. Tests for Saponins

- **Froth Test:** Extract was shaken vigorously with distilled water; stable persistent foam confirmed saponins.

7. Tests for Steroids and Triterpenoids

- **Salkowski's Test:** Extract dissolved in chloroform and treated with concentrated H_2SO_4 ; reddish-brown coloration indicated steroids/triterpenoids.
- **Liebermann-Burchard's Test:** Extract treated with acetic anhydride and H_2SO_4 ; green or bluish-green color confirmed steroids.

Quantitative Phytochemical Estimation of Neomarica Gracilis leaf Extract

The methanolic leaf extract of *Neomarica Gracilis* was subjected to quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) using standard UV-Visible spectrophotometric methods. All estimations were carried out in triplicate and results were expressed as mean values (Sachan et al., 2025).

1. Total Phenolic Content (TPC) Estimation (Folin-Ciocalteu Method)

- The methanolic extract was prepared at a known concentration (commonly 1 mg/mL stock solution) in methanol.
- From this stock, 0.2 mL of extract was taken for analysis.
- To this, 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) was added.
- After 5 minutes, 2 mL of 7.5% sodium carbonate solution was added to provide alkaline conditions.
- The reaction mixture was made up to final volume (approximately 7 mL) with distilled water.

- The solution was incubated at room temperature for 2 hours for full color development.
- Absorbance was measured at 760 nm using a UV–Visible spectrophotometer.
- A calibration curve was prepared using gallic acid standard solutions (10, 30, 50, 70, and 90 µg/mL). (Lawag et al., 2023).
- The results were expressed as mg gallic acid equivalent (GAE) per gram of dry extract.

2. Total Flavonoid Content (TFC) Estimation (Aluminium Chloride Method)

- The methanolic extract was prepared at a concentration of 1 mg/mL stock solution.
- From this, 0.5 mL of extract solution was taken for analysis.
- To this, 2 mL of distilled water was added.
- Then 0.15 mL of 5% sodium nitrite (NaNO₂) was added and allowed to stand for 6 minutes.
- After that, 0.15 mL of 10% aluminium chloride (AlCl₃) was added and kept for another 6 minutes.
- Subsequently, 2 mL of 4% sodium hydroxide (NaOH) was added, and volume was adjusted with distilled water (final volume ~5–6 mL).
- The solution was mixed thoroughly and absorbance was measured at 510 nm.
- A standard curve was prepared using rutin solutions (10, 30, 50, 70, and 90 µg/mL).
- The results were expressed as mg rutin equivalent (RE) per gram of dry extract (Aparna and Hema 2022).

In Vitro Antioxidant Activity of Neomarica Gracilis Leaf Extract

The in vitro antioxidant activity of the methanolic leaf extract of *Neomarica Gracilis* was evaluated using standard spectrophotometric methods, including the DPPH radical scavenging assay and reducing power assay. In the DPPH assay, different concentrations of the extract (10, 20, 30, 40, 50, µg/mL) were prepared in methanol. To each concentration, an equal volume of 0.1 mM DPPH solution was added, mixed thoroughly, and incubated in the dark for 30 minutes at room temperature. The absorbance was then measured at 517 nm using a UV–Visible spectrophotometer, with ascorbic acid used as the standard reference. The percentage inhibition was calculated using the standard formula based on control and sample absorbance values. In the reducing power assay, similar concentrations (10–100 µg/mL) of the extract were mixed with phosphate buffer and potassium ferricyanide solution and incubated at 50°C for 20

minutes. After treatment with trichloroacetic acid and centrifugation, the upper layer was reacted with ferric chloride, and absorbance was measured at 700 nm. An increase in absorbance indicated enhanced reducing ability. The antioxidant activity observed in both assays confirmed the presence of bioactive phytoconstituents in *Neomarica Gracilis* leaf extract capable of scavenging free radicals and exhibiting electron-donating potential (Aslam et al., 2023).

Acute Oral Toxicity Study (OECD 423)

The acute oral toxicity study of the methanolic leaf extract of *Neomarica Gracilis* was carried out according to OECD guideline 423 (Acute Toxic Class Method) to evaluate its safety profile. Healthy adult Wistar rats (180–220 g) of either sex were acclimatized under standard laboratory conditions with free access to food and water prior to the experiment. The animals were fasted overnight before dosing, while water was provided ad libitum. The extract was administered orally in a stepwise manner at fixed dose levels of 5, 50, 300, and 2000 mg/kg body weight. Each dose level was administered to a small group of animals, and they were observed continuously for the first 4 hours after dosing and periodically during the first 24 hours for any signs of toxicity. The animals were further monitored daily for 14 days for delayed toxic effects. Observations included changes in behavior, locomotor activity, skin and fur condition, eye and mucous membrane, respiratory pattern, salivation, body posture, food and water intake, and mortality. Body weight and general health status were also recorded throughout the study period. The absence or presence of toxic signs and mortality was used to assess the safety of the extract and to classify its toxicity level (Ranjini et al., 2025).

Experimental work

• Animals Protocol

IAEC Approval: All animal experiments were approved by Institutional Animal Ethics Committee (IAEC). (CCSEA Approval number PBRI/IAEC/ 21042026/010)

• Animal used

Weight: 180–220g gm; Strain: Wistar rat; Sex: Male

• Housing Condition

The study was conducted using male Wistar albino rats aged 2–3 months, with body weights ranging from 180–220g. The experiment, the animals were maintained under standard laboratory conditions, including a 12-hour light–

dark cycle, with free access to standard rodent chow and water. The animals were acclimatized to the laboratory environment for several days before the start of the study to minimize stress and ensure stable physiological conditions.

• Streptozotocin Induced Diabetes

Streptozotocin (STZ) was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and freshly prepared by dissolving in 0.1 M cold citrate buffer (pH 4.5) immediately before use to maintain its stability. Diabetes was induced in overnight-fasted Wistar rats by a single administration of STZ at a dose of 50 mg/kg body weight. The solution was administered intraperitoneally using a sterile 1 mL tuberculin syringe fitted with a 24-gauge needle in a total volume of 0.4 mL. After STZ injection, animals were provided with 5% glucose solution for 24 hours to prevent initial hypoglycemic shock. The normal control group received an equal volume of distilled water. Fasting blood glucose levels were measured on the 3rd day after STZ administration using a glucometer. Animals exhibiting elevated blood glucose levels were confirmed as diabetic and included for further experimental studies. This protocol ensures reliable induction of diabetes while minimizing degradation of STZ, which is unstable in aqueous conditions (Luty et al., 2025).

Table 6: Experimental design of Antidiabetic Activity

Group	Description/ Treatment	Dosage / Route	Purpose
I	Normal control	1 mL distilled water orally via feeding tube	Establish baseline physiological parameters
II	Diabetic control	Streptozotocin (STZ) 50 mg/kg b.w.	Serve as untreated diabetic control
III	standard drug	Metformin 100 mg/kg mg/kg b.w./day orally for 21 days	Positive control for comparison with test extract
IV	Test group – Low dose	<i>Neomaria Gracilis</i> leaf extract 200 mg/kg b.w./day orally for 21 days	Evaluate antidiabetic effect at low dose
V	Test group – High dose	<i>Neomaria Gracilis</i> leaf extract 400 mg/kg b.w./day orally for 21 days	Evaluate antidiabetic effect at high dose

The extracts were administered to the animals once every morning via mandatory oral intubation prior to meals. The

treatment was continued for 21 consecutive days (Gavanji et al., 2024).

In-Vivo Antidiabetic Activity

Collection of Blood Samples for Glucose Analysis

Blood glucose levels in STZ-induced diabetic rats were measured after overnight fasting (12 hours).

- **Sampling schedule:** Blood was collected from the tail vein on day 0 (three days after STZ administration) and on days 1, 3, 5, 7, 14 and 21.
- **Sampling technique:** The first drop of blood was wiped away to avoid contamination. The second drop was applied to a glucose strip in an Accu-Chek glucometer for immediate measurement of blood glucose levels.
- **Post-sampling care:** The tail was rubbed with ethanol to prevent infection and promote proper healing.

This method allows accurate, minimally invasive, and repeated monitoring of blood glucose in experimental rats (Van et al., 2022).

Biochemical Parameters of Antidiabetic Activity (Jaber et al., 2023).

Serum lipid levels were analyzed in overnight-fasted rats on days 0 and 21.

- **Blood collection:** Blood samples were obtained under diethyl ether anesthesia using the retro-orbital plexus puncture method.
- **Clotting:** Collected blood was allowed to stand for 30 minutes at room temperature to clot.
- **Serum separation:** The samples were centrifuged at 2500 rpm for 10 minutes at 25°C to separate the serum.
- **Lipid analysis:** The serum was tested for triglycerides (TG), high-density lipoprotein (HDL), and total cholesterol (TC) using the Mission Cholesterol Meter.

This method provides accurate measurement of serum lipid parameters in experimental rats with minimal sample handling.

Very low-density lipoprotein (VLDL) cholesterol was calculated using the formula:

$$VLDL = \frac{TG}{5}$$

Low-density lipoprotein (LDL) cholesterol was estimated using the Friedewald equation:

$$LDL = TC - (HDL + VLDL)$$

Histopathological Examination

At the end of the experimental study, animals were sacrificed by humane euthanasia, and the pancreas was carefully excised as it is the primary organ involved in insulin secretion and glucose regulation. The collected pancreatic tissue was washed with normal saline to remove blood and fixed in 10% neutral buffered formalin for 24–48 hours to preserve tissue architecture. After fixation, the tissues were processed through a graded series of alcohol for dehydration, followed by clearing in xylene and embedding in paraffin wax to form tissue blocks.

Thin sections of approximately 4–5 µm thickness were cut using a rotary microtome and mounted on clean glass slides. The sections were then deparaffinized and stained with hematoxylin and eosin (H&E) for routine histological evaluation. The stained slides were examined under a light microscope to observe structural changes in the islets of Langerhans, β-cell integrity, cellular degeneration, and inflammatory alterations.

Histopathological findings of treated groups (Neomaria Gracilis leaf extract, standard drug, and polyherbal/individual formulations) were compared with normal and diabetic control groups to assess the protective and regenerative effect on pancreatic tissue architecture (Aljazzaf et al., 2023).

RESULTS AND DISCUSSION

Percentage Yield

Percentage yield is an important parameter used to assess the efficiency of extraction. The methanolic extract of Neomaria Gracilis leaves showed a good yield, indicating effective extraction of bioactive constituents. This suggests the presence of appreciable amounts of phytochemicals such as phenolics, flavonoids, and other polar compounds. Variations in yield may be attributed to solvent polarity and the chemical nature of the plant material. Overall, the results confirm that methanol is a suitable solvent for efficient extraction and further pharmacological evaluation of the plant.

Table 7: Percentage Yield of crude extracts of Neomaria Gracilis leaves extracts

S. No.	Plant name	Solvent	Theoretical weight	Yield (gm)	% yield
1.	<i>Neomaria gracilis</i>	Methanol	400	30.2	7.55%



Figure 4: Percentage Yield of Crude Methanolic Extract of Neomaria Gracilis Leaves Discussion

The percentage yield of the crude methanolic extract of Neomaria Gracilis leaves was evaluated using 400 g of plant material, which produced 30.2 g of extract corresponding to a yield of 7.55%. This indicates that the methanol solvent was effective in extracting a considerable amount of bioactive constituents from the leaves. The obtained yield reflects the presence of appreciable phytochemicals, particularly polar compounds such as phenolics, flavonoids, glycosides, carbohydrates, and other secondary metabolites. The relatively good yield also suggests efficient extraction conditions and proper processing of the plant material. Overall, the results confirm that Neomaria Gracilis leaves are a good source of extractable compounds suitable for further phytochemical screening and pharmacological investigations.

Preliminary Phytochemical study

Table 8: Phytochemical Screening Results of Methanolic Leaf Extract of Neomaria gracilis

S. No.	Phytochemical Group	Test Name	Result
1	Alkaloids	Dragendorff's test	Present (+)
		Mayer's test	Present (+)
		Wagner's test	Present (+)
		Hager's test	Present (+)
2	Glycosides	Borntrager's test	Present (+)

		Legal's test	Present (+)
		Keller–Killiani test	Present (+)
3	Carbohydrates	Molisch's test	Present (+)
		Fehling's test	Present (+)
		Benedict's test	Present (+)
		Barfoed's test	Present (+)
4	Proteins & Amino acids	Biuret test	Present (+)
		Ninhydrin test	Present (+)
5	Flavonoids	Alkaline reagent test	Present (+)
		Lead acetate test	Present (+)
6	Phenolics & Tannins	Ferric chloride test	Present (+)
7	Saponins	Foam (froth) test	Absent (–)
8	Triterpenoids & Steroids	Salkowski's test	Absent (–)
		Liebermann–Burchard test	Absent (–)

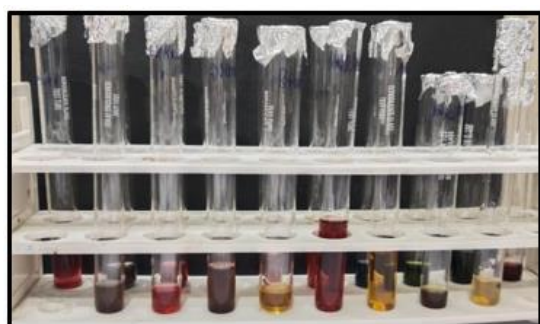


Figure 5: Phytochemical Screening of Methanolic Extract of Neomarica gracilis

Discussion

The preliminary phytochemical screening of the methanolic leaf extract of Neomarica Gracilis revealed the presence of several important bioactive constituents. The extract showed positive results for alkaloids, glycosides, carbohydrates, proteins and amino acids, flavonoids, and phenolic compounds, indicating a rich profile of secondary metabolites.

Alkaloids and glycosides detected in the extract are known for various pharmacological activities, including antidiabetic, anti-inflammatory, and antioxidant effects. The presence of carbohydrates and proteins reflects the nutritional and biochemical nature of the plant material.

Flavonoids and phenolic compounds, which were also present, are well recognized for their strong antioxidant potential and may contribute significantly to the therapeutic efficacy of the plant.

However, saponins, triterpenoids, and steroids were found to be absent in the methanolic extract, suggesting that these compounds are either not present or not efficiently extracted by methanol under the given conditions.

Overall, the results indicate that Neomarica Gracilis leaf extract is a rich source of pharmacologically active phytoconstituents, which may be responsible for its potential antidiabetic and antioxidant activities observed in further studies.

Quantitative Analysis

Preliminary phytochemical tests of the crude extracts revealed the presence of phenolic compounds and flavonoids. Further quantitative analysis measured the total phenolic content (TPC) and total flavonoid content (TFC), providing an estimate of the main bioactive constituents. These findings offered insights into the antioxidant capacity and potential therapeutic benefits of the plant extracts.

Total Phenolic content (TPC) estimation

Table 9: Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	10	0.162
2.	30	0.198
3.	50	0.246
4.	70	0.289
5.	90	0.334

Total Phenolic Content in Neomarica Gracilis leaf extract

Table 10: Total Phenolic Content

S. No.	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.209	37.22 mg/gm
2	0.219	
3	0.231	

Discussion

The total phenolic content (TPC) of the methanolic leaf extract of Neomarica Gracilis was estimated using the Folin–Ciocalteu method, with gallic acid as the standard reference compound. The standard calibration curve of gallic acid showed good linearity, confirming the reliability of the method for quantitative analysis.

The results revealed that *Neomarica Gracilis* leaf extract contains a considerable amount of phenolic compounds, with an average TPC value of 37.22 mg/g equivalent of gallic acid (GAE). Phenolic compounds are well known for their strong antioxidant properties due to their ability to donate hydrogen atoms or electrons, thereby neutralizing free radicals and reducing oxidative stress.

The presence of a significant level of phenolics suggests that the plant may possess notable antioxidant, anti-inflammatory, and antidiabetic potential. Therefore, the observed TPC supports the traditional and pharmacological relevance of *Neomarica Gracilis* and justifies further *in vitro* and *in vivo* studies to explore its therapeutic applications.

Total Flavonoids content (TFC) estimation

Table 11: Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	10	0.118
2.	30	0.157
3.	50	0.201
4.	70	0.238
5.	90	0.276

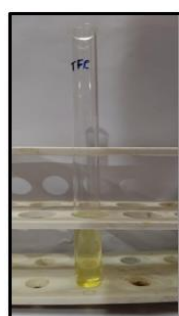
Total Flavonoid Content in *Neomarica Gracilis* leaf extract

Table 12: Total Flavonoid Content

S. No.	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.133	23.15 mg/gm
2	0.145	
3	0.158	



(a)



(b)

Figure 6: (a) Total Phenolic Content; (b) Total Flavonoid Content *Neomarica Gracilis* leaf extract

Discussion

The total flavonoid content (TFC) of the methanolic leaf extract of *Neomarica Gracilis* was estimated using the aluminium chloride colorimetric method, with rutin as the standard reference compound. The standard calibration

curve of rutin showed good linearity, indicating the accuracy and reliability of the method for flavonoid estimation.

The results showed that the leaf extract of *Neomarica Gracilis* contains a moderate amount of flavonoids, with an average value of 23.15 mg/g equivalent of rutin (RE). Flavonoids are important secondary metabolites widely recognized for their antioxidant activity due to their ability to scavenge free radicals and chelate metal ions.

The presence of flavonoids in appreciable quantity suggests that *Neomarica Gracilis* may possess significant antioxidant and therapeutic potential, particularly in conditions associated with oxidative stress such as diabetes mellitus. Therefore, the obtained results support further pharmacological evaluation of the plant for its bioactive and disease-modulating properties.

Evaluation of Antioxidant Activity

By DPPH method

Table 13: Antioxidant activity of Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
10	0.564	43.145
20	0.451	54.536
30	0.395	60.181
40	0.322	67.540
50	0.216	78.225
Control	0.992	
IC ₅₀	17.10	

Table 14: DPPH radical scavenging activity of methanolic leaf extract of *Neomarica gracilis*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.512	48.490
40	0.466	53.118
60	0.434	56.338
80	0.387	61.066
100	0.311	68.712
Control	0.994	
IC ₅₀	28.81	

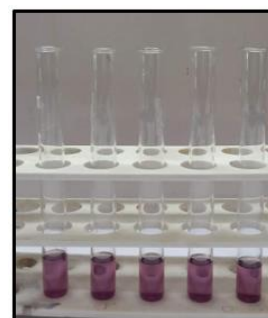


Figure 7: DPPH radical scavenging activity of methanolic leaf extract of *Neomarica gracilis*

Discussion

The antioxidant potential of the standard ascorbic acid and the methanolic leaf extract of *Neomarca Gracilis* was evaluated using the DPPH radical scavenging assay. The results clearly showed a concentration-dependent increase in percentage inhibition for both the standard and the plant extract, indicating strong free radical scavenging activity.

Ascorbic acid exhibited higher antioxidant activity with % inhibition ranging from 43.145% at 10 µg/ml to 78.225% at 50 µg/ml, and an IC₅₀ value of 17.10 µg/ml. This lower IC₅₀ value confirms its strong and rapid hydrogen donating ability, making it a potent standard antioxidant. In comparison, the methanolic leaf extract of *Neomarca Gracilis* also showed significant DPPH radical scavenging activity, with % inhibition increasing from 48.490% at 20µg/ml to 68.712% at 100 µg/ml. The IC₅₀ value was found to be 26.52µg/ml, indicating moderate antioxidant potential compared to ascorbic acid. The higher IC₅₀ value suggests that the plant extract requires a higher concentration to achieve 50% radical scavenging activity.

The observed antioxidant activity may be attributed to the presence of phytochemical constituents such as flavonoids, phenolics, and other bioactive compounds in the methanolic extract. These compounds are known to donate hydrogen atoms or electrons to neutralize free radicals, thereby preventing oxidative damage.

Overall, the results suggest that *Neomarca Gracilis* possesses notable antioxidant properties, although its activity is lower than that of ascorbic acid. This supports its potential use as a natural source of antioxidant agents in pharmaceutical and nutraceutical applications.

In vivo acute oral toxicity (OECD 423)

Table 15: Acute Oral Toxicity Evaluation of *Neomarca Gracilis* Leaf Extract at Different Dose Levels

S. No.	Parameters Observed	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
1.	Body Weight (14th Day)	Normal	Normal	Normal	Normal
2.	Skin and Fur	Normal	Normal	Normal	Mild alteration
3.	Eyes	Normal	Normal	Normal	Mild alteration
4.	Mucous Membrane	Normal	Normal	Normal	Normal
5.	Salivation	Normal	Normal	Normal	Normal
6.	Stool	Normal	Normal	Mild alteration	Mild alteration
7.	Urination	Normal	Normal	Mild alteration	Mild alteration

8.	Sleep Pattern	Normal	Normal	Normal	Normal
9.	Behaviour	Normal	Normal	Mild alteration	Mild alteration
10.	Sensory/Motor Activity	Normal	Normal	Normal	Normal
11.	Food Intake	Normal	Normal	Slight reduction	Slight reduction
12.	Water Intake	Normal	Normal	Normal	Normal
13.	Mortality	No mortality	No mortality	No mortality	No mortality

Discussion

The acute oral toxicity study of the methanolic leaf extract of *Neomarca Gracilis* was carried out according to OECD guideline 423 to assess its safety profile at dose levels of 5, 50, 300, and 2000 mg/kg body weight. The animals were observed for changes in general behavior, physiological parameters, food and water intake, and mortality throughout the observation period. At lower doses (5 and 50 mg/kg), no signs of toxicity or abnormal behavioral changes were observed. The animals exhibited normal body weight gain, skin and fur condition, eye appearance, mucous membranes, salivation, stool consistency, urination, sleep pattern, sensory/motor activity, food intake, and water consumption, indicating good tolerability of the extract. At higher doses (300 and 2000 mg/kg), mild alterations such as changes in stool consistency, urination pattern, behavior, slight reduction in food intake, and minor changes in skin and eye appearance were observed. However, these effects were not severe and appeared to be reversible. Importantly, no mortality was observed even at the highest tested dose of 2000 mg/kg body weight, suggesting that the methanolic leaf extract possesses a wide margin of safety. Overall, the findings indicate that the methanolic leaf extract of *Neomarca Gracilis* is relatively safe and can be considered practically non-toxic under the experimental conditions employed, supporting its further evaluation for pharmacological activities.

In vivo anti-diabetic study

Table 16: Effect of methanolic leaf extract of *Neomarca Gracilis* on Body weight of the rats

Body weight (gms)						
Groups	Treatments	1 day	3 days	7 days	14 days	21 days
I	Normal Control	185.13	186.37	184.34	189.66	196.99
		±0.2	±0.2	±0.2	±0.2	±0.2
		2	11	5	9	52
II	Diabetic Control	181.35	178.89	165.22	161.67	159.33

	(Streptozotocin 50 mg/kg)	±0.1 9	±0.1 62	±0.2 3	±0.2 61	±0.2 64
III	Standard (Metformin 100 mg/kg)	182.23 ±0.2 2	179.78 ±0.1 06	175.67 ±0.2 4	185.90 ±0.2 7	191.90 ±0.2 73
IV	Neomarica Gracilis leaf extract (200 mg/kg)	172.25 ±0.2 0	170.91 ±0.6 03	169.90 ±0.2 2	176.34 ±0.2 5	183.06 ±0.2 42
V	Neomarica Gracilis leaf extract (400 mg/kg)	177.88 ±0.2 1	176.79 ±0.2 75	171.56 ±0.2 3	181.23 ±0.2 6	187.98 ±0.2 29

Discussion

The effect of the methanolic leaf extract of *Neomarica Gracilis* on body weight was evaluated in streptozotocin-induced diabetic rats for 21 days. The diabetic control group showed a progressive decline in body weight from 181.35 ± 0.19 g to 159.33 ± 0.264 g, indicating diabetes-induced metabolic disturbances and increased protein catabolism. In contrast, the normal control group exhibited a gradual increase in body weight throughout the study period. Treatment with Metformin (100 mg/kg) significantly improved body weight, reaching 191.90 ± 0.273 g on day 21. Similarly, rats treated with *Neomarica Gracilis* leaf extract at 200 mg/kg and 400 mg/kg showed dose-dependent improvement in body weight, increasing to 183.06 ± 0.242 g and 187.98 ± 0.229 g, respectively. The higher dose produced a better effect and was comparable to the standard drug. Overall, the methanolic leaf extract of *Neomarica Gracilis* effectively prevented diabetes-associated body weight loss, suggesting its potential antidiabetic activity.

Blood Glucose Level

Table 17: Effect of methanolic leaf extract of *Neomarica Gracilis* on Blood Glucose Level in experimental rats

Blood Glucose Level (gms)						
Groups	Treatment	1 day	3 days	7 days	14 days	21 days
Group I	Normal Control	86.09 ±0.19 4	88.47 ±0.21 0	91.99 ±0.36	98.99 ±0.19 6	102.78 ±0.271
Group II	Diabetic Control (Streptozotocin in 50 mg/kg)	135.79 ±0.18 1	179.87 ±0.26 2	189.79 ±0.24 0	210.67 ±0.30 8	238.81 ±0.23 0
Group III	Standard (Metformin 100 mg/kg)	91.06 ±0.22 7	94.78 ±0.10 6	96.79 ±0.49 8	99.91 ±0.28 9	108.80 ±0.09 5
Group IV	Neomarica Gracilis leaf	99.79 ±0.09 7	103.91	107.99	111.91	115.47

	extract (200 mg/kg)		±0.60 3	±0.20 3	±0.23 7	±0.21 0
Group V	Neomarica Gracilis leaf extract (400 mg/kg)	96.89 ±0.37 1	98.79 ±0.27 5	99.45 ±0.22 2	105.23 ±0.25 8	112.79 ±0.36

Discussion

The present study demonstrates that Streptozotocin (STZ) administration successfully induced diabetes in rats, as evidenced by a significant and progressive increase in blood glucose levels in the diabetic control group compared to the normal control. The normal group maintained stable glycemic levels throughout the study, indicating normal glucose homeostasis, while the Metformin-treated group showed a marked reduction in blood glucose, confirming its standard antihyperglycemic effect.

Treatment with methanolic leaf extract of *Neomarica Gracilis* produced a significant, dose-dependent reduction in blood glucose levels at both 200 mg/kg and 400 mg/kg doses. The higher dose exhibited greater efficacy, although it did not fully restore glucose levels to those observed in the standard drug group. This antihyperglycemic effect may be attributed to phytoconstituents such as flavonoids, alkaloids, and phenolic compounds, which could enhance insulin secretion, improve peripheral glucose utilization, and exert antioxidant protection on pancreatic β -cells.

Overall, the results indicate that the methanolic leaf extract of *Neomarica Gracilis* possesses notable antidiabetic potential in STZ-induced diabetic rats, with stronger activity observed at higher dosage. Further studies are required to isolate active compounds and clarify the underlying mechanism of action.

Biochemical Parameters

Table 18: Effect of test samples of methanolic leaf extract of *Neomarica Gracilis* on Biochemical Parameters in experimental rats

Groups	Treatment groups	TG (mg/d L)	TC (mg/d L)	HDL (mg/d L)	LDL (mg/d L)
Group 1	Normal Control	58.35 ± 1.26	82.07 ± 0.85	41.76 ± 0.28	29.446 ± 0.80
Group 2	Diabetic Control (Streptozotocin 50 mg/kg)	180.66 ± 2.06	191.83 ± 2.73	18.24 ± 0.21	137.65 8 ± 1.67
Group 3	Standard (Metformin 100 mg/kg)	67.61 ± 1.53	88.66 ± 1.49	37.54 ± 0.37	40.178 ± 0.95

Group 4	Neomarina Gracilis leaf extract (200 mg/kg)	87.53 ± 1.86	104.67 ± 2.04	22.56 ± 0.46	52.604 ± 1.18
Group 5	Neomarina Gracilis leaf extract (400 mg/kg)	75.98 ± 1.96	93.23 ± 2.10	33.05 ± 0.51	45.184 ± 1.23

Discussion

The effect of the methanolic leaf extract of Neomarina Gracilis on lipid biochemical parameters was evaluated in streptozotocin-induced diabetic rats. The diabetic control group showed a marked increase in triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL) levels, along with a significant decrease in high-density lipoprotein (HDL) levels, indicating diabetes-associated dyslipidemia.

Treatment with the standard drug, Metformin (100 mg/kg), significantly improved the lipid profile by reducing TG (67.61 ± 1.53 mg/dL), TC (88.66 ± 1.49 mg/dL), and LDL (40.178 ± 0.95 mg/dL) levels, while increasing HDL (37.54 ± 0.37 mg/dL) levels. Similarly, Neomarina Gracilis leaf extract at both 200 mg/kg and 400 mg/kg doses improved the altered biochemical parameters compared to the diabetic control group. The 400 mg/kg dose exhibited better efficacy, with TG (75.98 ± 1.96 mg/dL), TC (93.23 ± 2.10 mg/dL), HDL (33.05 ± 0.51 mg/dL), and LDL (45.184 ± 1.23 mg/dL) values approaching those of the standard treatment.

Overall, the methanolic leaf extract of Neomarina Gracilis effectively ameliorated diabetes-induced dyslipidemia in a dose-dependent manner, suggesting its potential role in improving lipid metabolism and reducing the risk of diabetic complications.

Histopathological examination

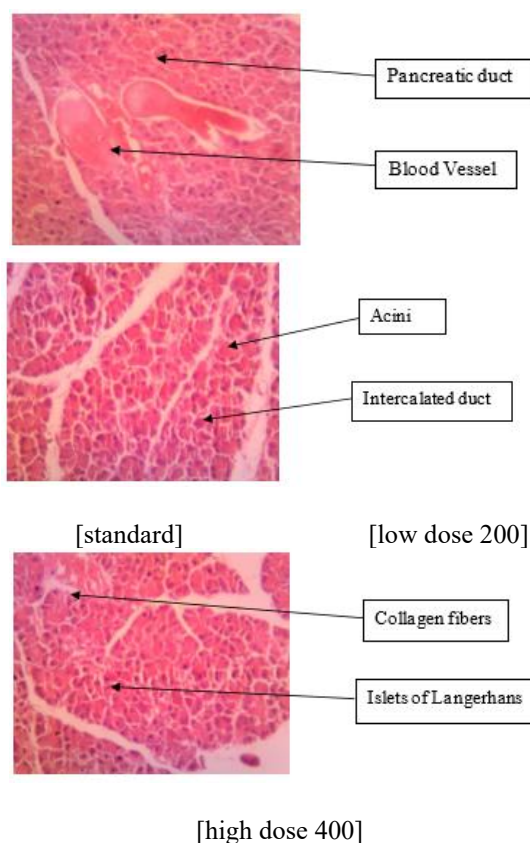
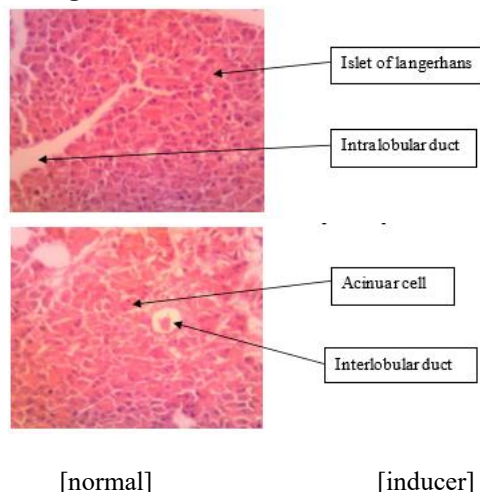


Figure 8: Histopathological examination

Discussion

Histopathological examination of pancreatic tissues revealed normal architecture in the normal control group (Group I), with well-organized pancreatic islets and intact acinar cells. In contrast, the diabetic control group (Group II) showed marked pathological alterations, including degeneration of β -cells, reduced islet size, and disruption of normal pancreatic architecture, confirming streptozotocin-induced pancreatic damage.

The standard treatment group (Metformin, Group III) exhibited considerable restoration of pancreatic tissue architecture, with improved islet cell integrity and reduced cellular damage. Similarly, treatment with the methanolic leaf extract of Neomarina Gracilis produced protective effects on pancreatic tissues. The 200 mg/kg dose (Group IV) showed moderate improvement in pancreatic structure with partial regeneration of islet cells, whereas the 400 mg/kg dose (Group V) demonstrated more pronounced restoration of normal histological features and reduced tissue damage.

Overall, the histopathological findings support the biochemical and blood glucose results, indicating that the

methanolic leaf extract of *Neomarica Gracilis* exerts a protective effect against streptozotocin-induced pancreatic injury. The higher dose (400 mg/kg) showed greater efficacy and was comparable to the standard treatment, suggesting significant antidiabetic potential.

SUMMARY

The present study evaluated the antidiabetic potential of the methanolic leaf extract of *Neomarica Gracilis* using in vitro antioxidant assays, phytochemical screening, acute toxicity studies, and in vivo experiments in streptozotocin (STZ)-induced diabetic rats. Phytochemical analysis revealed the presence of flavonoids, phenolics, alkaloids, glycosides, carbohydrates, proteins, and amino acids. The extract exhibited antioxidant activity in the DPPH assay with an IC_{50} value of 26.52 μ g/mL, while the standard ascorbic acid showed an IC_{50} of 17.10 μ g/mL. Quantitative analysis showed total phenolic content (TPC) of 37.22 mg/g GAE and total flavonoid content (TFC) of 23.15 mg/g RE, indicating a rich presence of antioxidant phytoconstituents. Acute oral toxicity studies (OECD 423) confirmed safety up to 2000 mg/kg body weight, with no mortality observed and only mild reversible behavioral changes at higher doses.

In vivo studies showed that STZ significantly increased blood glucose levels from 135.79 ± 0.18 mg/dL to 238.81 ± 0.23 mg/dL, along with body weight reduction from 181.35 ± 0.19 g to 159.33 ± 0.26 g in the diabetic control group. Treatment with *Neomarica Gracilis* extract significantly improved these parameters. At 400 mg/kg, blood glucose was reduced to 112.79 ± 0.36 mg/dL, and body weight improved to 187.98 ± 0.23 g by day 21. Lipid profile analysis also showed improvement at 400 mg/kg, with triglycerides (75.98 ± 1.96 mg/dL), total cholesterol (93.23 ± 2.10 mg/dL), HDL (33.05 ± 0.51 mg/dL), and LDL (45.18 ± 1.23 mg/dL) compared to the diabetic control group (TG 180.66 ± 2.06 mg/dL, TC 191.83 ± 2.73 mg/dL, HDL 18.24 ± 0.21 mg/dL, LDL 137.65 ± 1.67 mg/dL).

Histopathological studies further confirmed partial restoration of pancreatic architecture in the extract-treated groups, especially at 400 mg/kg, compared to severe β -cell damage in diabetic controls.

CONCLUSION

It can be concluded that the methanolic leaf extract of *Neomarica Gracilis* exhibits significant antidiabetic potential in STZ-induced diabetic rats. The extract effectively reduced hyperglycemia, improved body weight (up to 187.98 ± 0.23 g), ameliorated dyslipidemia, and

protected pancreatic tissue structure. These effects are likely due to its rich phytochemical content, including phenolics (37.22 mg/g GAE) and flavonoids (23.15 mg/g RE), which contribute to antioxidant activity ($IC_{50} = 26.52$ μ g/mL). The findings validate the traditional medicinal relevance of *Neomarica Gracilis* and suggest its potential as a natural source for antidiabetic drug development. However, further studies are required to isolate active compounds, elucidate molecular mechanisms, and establish long-term safety and clinical efficacy.

REFERENCES

- [1] Mosihuzzaman M. Herbal medicine in healthcare-an overview. Natural product communications. 2012 Jun; 7(6):628.
- [2] Ahn K. The worldwide trend of using botanical drugs and strategies for developing global drugs. BMB reports. 2017 Mar 31;50(3):111.
- [3] Chen SL, Yu H, Luo HM, Wu Q, Li CF, Steinmetz A. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chinese medicine. 2016 Jul 30; 11(1):37.
- [4] Whiting D, Unwin N, Roglic G. Diabetes: equity and social determinants. Equity, social determinants and public health programmes. 2010 Jul 7; 77:94.
- [5] Gupta PD, Amartya De AD. Diabetes mellitus and its herbal treatment. (2012).
- [6] Rajaei E, Jalali MT, Shahrabi S, Asnafi AA, Pezeshki S. HLAs in autoimmune diseases: dependable diagnostic biomarkers?. Current rheumatology reviews. 2019 Nov 1; 15(4):269-76.
- [7] Singab AN, Youssef FS, Ashour ML. Medicinal plants with potential antidiabetic activity and their assessment. Med Aromat Plants. 2014; 3(151):2167-0412.
- [8] Gordon HS, Sharp LK, Schoenthaler A. "They are talking from the Encyclopedia Britannica brain": diabetes patients' perceptions of barriers to communicating with physicians. BMC health services research. 2020 Mar 13; 20(1):211.
- [9] Türkvan A, Erden A, Türkoğlu MA, Yener Ö. Congenital variants and anomalies of the pancreas and pancreatic duct: imaging by magnetic resonance cholangiopancreatography and multidetector computed tomography. Korean journal of radiology. 2013 Nov 5;14(6):905.
- [10] Craig ME, Hattersley A, Donaghue KC. Definition, epidemiology and classification of diabetes in children and adolescents. Pediatr diabetes. 2009 Sep 1; 10(Suppl 12):3-12.

- [11] Noctor E, Crowe C, Carmody LA, Saunders JA, Kirwan B, O'Dea A, Gillespie P, Glynn LG, McGuire BE, O'Neill C, O'Shea PM. Abnormal glucose tolerance post-gestational diabetes mellitus as defined by the International Association of Diabetes and Pregnancy Study Groups criteria. *European journal of endocrinology*. 2016 Oct;175(4):287-97.
- [12] Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T, K S, Oz F. Major phytochemicals: recent advances in health benefits and extraction method. *Molecules*. 2023 Jan 16;28(2):887.
- [13] Shrikant NV, Sangram SV. LC-HRMS and Phytochemicals Analysis of *Gnidia glauca* L. leaf crude extract with different solvents. *CURRENT TRENDS IN BIOTECHNOLOGY AND PHARMACY*. 2024 Oct 1;18(4):2048-61.
- [14] Sachan A, Anand S, Varma R, Kumar RR, Mohanty AK. Comparative extraction efficiency of solvent systems for phytochemicals from *Moringa oleifera*, *Madhuca indica*, and *Ficus nemoralis*.
- [15] Lawag IL, Nolden ES, Schaper AA, Lim LY, Locher C. A modified folin-ciocalteu assay for the determination of total phenolics content in honey. *Applied Sciences*. 2023 Feb 7;13(4):2135.
- [16] Aparna B, Hema BP. Preliminary screening and quantification of flavonoids in selected seeds of *Apiaceae* by UV-visible spectrophotometry with evaluation study on different aluminium chloride complexation reaction. *Indian Journal of Science and Technology*. 2022 May 25;15(18):857-68.
- [17] Aslam J, Shahzad MI, Ali HM, Ramzan M, Mustafa MZ, Iqbal RK, Hirad AH, Alarfaj AA, Danish S. Assessing the in vitro and in vivo antioxidant and anti-inflammatory effects of aerial and floral extracts of *Neurada procumbens*. *J. King Saud Univ. Sci*. 2023;102822.
- [18] Ranjini HS, Azar KA, Haq SF, Goudappala P. Acute Oral Toxicity Evaluation of Hydroalcoholic Extract of *Salvia Officinalis* Roots in Wistar Rats as per OECD 423 TG. *Pharmacognosy Journal*. 2025 Sep;17(5).
- [19] Luty RS, Al-Zubaidy AA, Malik AS, Ridha-Salman H, Abbas AH. Protective effect of orientin on diabetic nephropathy in rat models of high-fat diet and streptozotocin-induced diabetes. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2025 Aug;398(8):10769-84.
- [20] Gavanji S, Bakhtari A, Abdel-latif R, Bencurova E, Othman EM. Experimental approaches for induction of diabetes mellitus and assessment of antidiabetic activity: An in vitro and in vivo methodological review. *Fundamental & Clinical Pharmacology*. 2024 Oct;38(5):842-61.
- [21] Van LV, Pham EC, Nguyen CV, Duong NT, Le Thi TV, Truong TN. In vitro and in vivo antidiabetic activity, isolation of flavonoids, and in silico molecular docking of stem extract of *Merremia tridentata* (L.). *Biomedicine & Pharmacotherapy*. 2022 Feb 1;146:112611.
- [22] Jaber SA. In vitro alpha-amylase and alpha-glucosidase inhibitory activity and in vivo antidiabetic activity of *Quercus coccifera* (Oak tree) leaves extracts. *Saudi Journal of Biological Sciences*. 2023 Jul 1;30(7):103688.
- [23] Aljazzaf B, Regeai S, Elghmasi S, Alghazir N, Balgasim A, Hdud Ismail IM, Eskandrani AA, Shamlan G, Alansari WS, Al-Farga A, Alghazeer R. Evaluation of antidiabetic effect of combined leaf and seed extracts of *Moringa oleifera* (Moringaceae) on Alloxan-induced diabetes in mice: A biochemical and histological study. *Oxidative Medicine and Cellular Longevity*. 2023;2023(1):9136217.