Phytochemical and Antidiabetic Activity of *Mimosa tenuiflora* leaves

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Abstract- Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both, leading to severe microvascular and macrovascular complications. The increasing global prevalence of diabetes and the limitations associated with synthetic antidiabetic drugs have prompted the exploration of plant-based therapeutics. Mimosa tenuiflora, a medicinal plant belonging to the Fabaceae family, is rich in bioactive phytoconstituents such as flavonoids, tannins, alkaloids, and phenolic compounds, which are known to exhibit antioxidant and antidiabetic properties. *Mimosa tenuiflora* is a medicinal plant known for its pharmacological properties. This study focuses on extraction, isolation, and identification of bioactive compounds and evaluation of antidiabetic activity using in vitro and in vivo models. The results indicated significant glucose-lowering potential due to presence of flavonoids and phenolics.

The present study was undertaken to extract, isolate, and identify bioactive compounds from *Mimosa tenuiflora* and evaluate their antidiabetic potential. The plant material was subjected to hydro-alcoholic extraction followed by phytochemical screening, chromatographic separation (TLC, column chromatography), and advanced analytical techniques such as HR-LC-MS for identification of active constituents. The extract and isolated fractions were evaluated for in vitro antioxidant activity using DPPH and other free radical scavenging assays, as well as in vitro antidiabetic activity through α-amylase and α-glucosidase inhibition studies. Furthermore, in vivo antidiabetic activity was assessed using streptozotocin-induced diabetic Wistar rats by evaluating biochemical parameters including blood glucose, lipid profile, and oxidative stress markers such as SOD, GSH, and MDA.

The results demonstrated that *Mimosa tenuiflora* extract possesses significant antioxidant and antidiabetic activity, which may be attributed to the presence of polyphenolic compounds and flavornoids. The study supports the traditional use of this plant and highlights its potential as a source of novel, safe, and effective antidiabetic agents.

Keywords: Mimosa tenuiflora, Antidiabetic Activity, Phytochemicals, Extraction, Isolation, HR-LC-MS, Flavonoids, Antioxidant, Streptozotocin, Wistar Rats

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide and represents a major public health challenge due to its increasing incidence, long-term complications, and associated healthcare burden. Chronic hyperglycemia leads to disturbances in carbohydrate, lipid, and protein metabolism, ultimately resulting in serious complications such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy. The global burden of diabetes has risen dramatically over the past few decades, with developing countries like India experiencing a particularly rapid increase in prevalence due to urbanization, sedentary lifestyle, and dietary changes.

Diabetes mellitus is broadly classified into Type-1 diabetes mellitus (T1DM), Type-2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and other specific types. Among these, Type-2 diabetes is the most common, accounting for approximately 90–95% of all cases. It is primarily associated with insulin resistance and impaired insulin secretion. Genetic predisposition, obesity, lack of physical activity, unhealthy dietary habits, and aging are major risk factors contributing to the development of the disease. In addition, chronic stress and hormonal imbalance further exacerbate metabolic dysfunction, leading to progressive deterioration of glucose homeostasis.

The pathophysiology of diabetes involves a complex interplay of metabolic, hormonal, and molecular mechanisms. Insulin resistance in peripheral tissues such as skeletal muscle, adipose tissue, and liver leads to decreased glucose uptake and increased hepatic glucose production. Simultaneously, pancreatic β-cell dysfunction results in inadequate insulin secretion. Persistent hyperglycemia triggers oxidative stress through the excessive generation of reactive oxygen species (ROS), which causes cellular damage, lipid peroxidation, protein oxidation, and DNA damage. Oxidative stress plays a crucial role in the progression of diabetes and its complications by activating inflammatory pathways and impairing cellular function.

In addition to oxidative stress, chronic low-grade inflammation is a key contributor to the development and progression of diabetes. Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) interfere with insulin signaling pathways, leading to increased insulin resistance. The accumulation of advanced glycation end products (ages) further aggravates oxidative stress and inflammation, resulting in vascular damage and tissue dysfunction. These interconnected mechanisms highlight the multifactorial nature of diabetes and emphasize the need for therapeutic strategies that can target multiple pathways simultaneously.

Conventional antidiabetic therapies, including insulin and oral hypoglycemic agents such as metformin and sulfonylureas, are widely used for the management of diabetes. Although these drugs are effective in controlling blood glucose levels, they are often associated with various side effects such as hypoglycemia, gastrointestinal disturbances, weight gain, and long term complications. Furthermore, these treatments primarily focus on glycemic control and may not adequately address the underlying oxidative stress and inflammation involved in disease progression. These limitations have prompted researchers to explore alternative therapeutic approaches, particularly those derived from natural sources.

Medicinal plants have gained considerable attention as potential sources of novel antidiabetic agents due to their therapeutic efficacy, safety, and multi-target mechanisms of action. Plant-based therapies are rich in bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which exhibit a wide range of pharmacological activities. These phytoconstituents have been reported to exert antidiabetic effects through various mechanisms, including enhancement of insulin secretion, improvement of insulin sensitivity, inhibition of carbohydrate digesting enzymes, and reduction of oxidative stress. Additionally, the antioxidant properties of plant-derived compounds help protect pancreatic β-cells from oxidative damage and improve overall metabolic function.

Among various medicinal plants, *Mimosa tenuiflora* has emerged as a promising candidate due to its rich phytochemical profile and traditional medicinal uses. It belongs to the family Fabaceae and is commonly known as Jurema preta or Tepezcohuite. The plant is widely distributed in tropical and semi-arid regions and has been traditionally used for the treatment of wounds, burns, inflammation, and infections. Phytochemical investigations of *Mimosa tenuiflora* have revealed the presence of tannins, flavonoids, alkaloids, and saponins, which are known to possess antioxidant, antimicrobial, and anti-inflammatory properties. These bioactive compounds are likely to contribute to its potential antidiabetic activity.

Recent studies have demonstrated that extracts of *Mimosa tenuiflora* exhibit significant antioxidant activity, which plays a crucial role in mitigating oxidative stress associated with diabetes. Antioxidants neutralize reactive oxygen species and prevent cellular damage, thereby protecting pancreatic β-cells and improving insulin function. In addition, certain phytochemicals present in the plant may inhibit key carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, leading to reduced glucose absorption and improved glycemic control. These findings suggest that *Mimosa tenuiflora* may act through multiple mechanisms to exert its antidiabetic effects.

Despite the promising therapeutic potential of Mimosa tenuiflora, there is a need for systematic scientific investigation to identify and characterize the specific bioactive compounds responsible for its pharmacological activity. Extraction, isolation, and identification of these compounds are essential steps in

understanding their mechanism of action and developing standardized herbal formulations. Advanced analytical techniques such as chromatography and mass spectrometry play a crucial role in the separation and characterization of phytoconstituents. Furthermore, evaluation of antidiabetic activity using in vitro and in vivo models provides scientific validation for the traditional use of the plant.

Therefore, the present study focuses on the extraction, isolation, and identification of bioactive compounds from *Mimosa tenuiflora* and the evaluation of their antidiabetic activity. By integrating phytochemical analysis with pharmacological evaluation, this study aims to contribute to the development of safe, effective, and plant-based therapeutic agents for the management of diabetes mellitus. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. Medicinal plants provide alternative therapies. *Mimosa tenuiflora* contains bioactive compounds like flavonoids, tannins, and alkaloids responsible for therapeutic effects.

Material and Methods

Collection of plant- Fresh leaves of *Mimosa tenuiflora* were collected from an appropriate geographical region during the optimal growing season to ensure maximum phytochemical content and biological activity. Seasonal variation plays a crucial role in the concentration of active constituents, and hence, collection at the correct time enhances the reliability and reproducibility of pharmacological studies. After collection, the plant material was carefully examined and authenticated by a qualified taxonomist to confirm its botanical identity. Proper authentication is essential to avoid misidentification, which could lead to erroneous experimental outcomes and affect the validity of the research findings.

Furthermore, a voucher specimen of the plant was prepared and deposited in an institutional herbarium. This specimen serves as a permanent reference for the plant material used in the study and allows future researchers to verify the identity of the species. The deposition of voucher specimens is considered a standard practice in pharmacognostic and phytochemical research, as it ensures transparency, traceability, and reproducibility of scientific investigations. It also aids in the standardization of herbal drugs by providing a documented source of the plant material used in experimental procedures. Therefore, proper collection, authentication, and documentation of *Mimosa tenuiflora* leaves are critical steps that contribute significantly to the credibility and scientific validity of the study.

Preparation of plant extract- The collected leaves of *Mimosa tenuiflora* were initially washed thoroughly with distilled water to eliminate dust, soil particles, and other extraneous contaminants that could interfere with subsequent extraction and analysis. The cleaned leaves were then shade dried at room temperature to preserve heat sensitive phytoconstituents such as flavonoids, phenolics, and glycosides, which might degrade under direct sunlight or high temperatures. Once completely dried, the plant material was coarsely powdered using a mechanical grinder to increase the surface area, thereby enhancing the efficiency of

solvent penetration during extraction.

The powdered material was subjected to Soxhlet extraction, a widely used continuous extraction technique that ensures exhaustive extraction of bio-active compounds. A hydroalcoholic solvent system consisting of ethanol and water was employed, as it effectively dissolves a broad range of polar and semi-polar phytochemicals. The extraction process was continued until the solvent in the siphon tube became colorless, indicating complete extraction. The obtained extract was then concentrated under reduced pressure using a rotary evaporator, which helps in removing the solvent at a lower temperature, thus preventing thermal degradation of active constituents.

Finally, the concentrated extract was transferred into an airtight container and stored at 4°C to maintain its stability and prevent microbial growth or chemical degradation. This standardized extraction procedure ensures the preservation of bioactive compounds and reproduceibility of experimental results[1].

Preliminary phytochemical screening- The prepared extract of Mimosa tenuiflora leaves was subjected to preliminary qualitative phytochemical screening to identify the presence of various bioactive constituents responsible for its therapeutic potential. This screening is an essential step in pharmacognostic studies, as it provides a preliminary understanding of the chemical composition of the plant extract and helps correlate its constituents with biological activities. Standard qualitative tests were performed to detect major classes of phytochemicals such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, and glycosides.

Specific chemical reagents were used for each class of compounds; for instance, alkaloids were identified using Dragendorff's or Mayer's reagent, which produces a characteristic precipitate. Flavonoids were detected by the Shinoda test, showing color changes upon reaction, while tannins and phenolics were confirmed using ferric chloride, resulting in a dark blue or green coloration. Saponins were identified through the frothing test, indicating their surfactant properties, and glycosides were detected using tests such as Keller–Killiani for cardiac glycosides. These qualitative assays are simple, rapid, and effective in establishing the presence or absence of key phyto-constituents.

The identification of these compounds is significant, as many of them are known to possess pharmacological activities, including antioxidant and antidiabetic effects. Thus, preliminary phyto-chemical screening serves as a found-ation for further quantitative analysis and bio-activity guided studies, ensuring scientific validation of the plant's medicinal properties[2-4].

Preparation of Hydroalcoholic Extract by Soxhlet Extraction- The hydroalcoholic extracts of *Eclipta alba* and *Ziziphus jujuba* leaves were prepared using the Soxhlet extraction method. The collected plant materials were first washed thoroughly with distilled water to remove adhering impurities and then shade-dried at room temperature. For several days. The dried leaves were coarsely powdered using a mechanical grinder and stored in airtight

containers until further use.

Approximately 100 g of the powdered plant material was placed in a Soxhlet apparatus and extracted with a hydro-alcoholic solvent consisting of ethanol and water in a suitable ratio (commonly 70:30 v/v). The extraction process was carried out for 6–8 hours until the solvent in the siphon tube became colorless, indicating complete extraction of phyto-constituents.

After completion of extraction, the extract was filtered and concentrated under reduced pressure using a rotary evaporator or by evaporating the solvent on a water bath at controlled temperature. The concentrated extract was then dried to obtain a semi-solid mass. The obtained extracts were stored in airtight containers at low temperature for further pharmacological evaluation. The same procedure was followed for both *Eclipta alba* and *Ziziphus jujuba* to ensure uniformity in extraction conditions[7].

Physico-chemical Evaluation- Physicochemical parameters were determined for crude drug according to the procedures given in WHO guidelines. Ash values including acid insoluble ash, water soluble ash, total ash and extractive values such as water & alcohol soluble extractive value were determined by hot extraction and cold maceration method[5,6].

The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Evaluation of Ash Values- The quality of crude medication can be effectively evaluated by ash values. Ash content signifies the inorganic substance adheres with the crude drug. Sometimes the inorganic substances can be intentionally mixed with the drug to get profit. Accordingly, assurance of ash value of unrefined medication gives the character and cleanliness of a medication and gives data in respect to its degradation with inorganic substances.

Acid Insoluble Ash Value- To total obtained ash 25ml of hydrochloric acid was added followed by boiling for 5 min. around five milliliters of hot water was poured on the watch glass for its complete washing and transferred to the crucible. The crucible matter was filtered on an ash less filter paper and residue so obtained was rinsed with hot water until the filtrate became neutral. This ashless filter paper comprising acid insoluble substance was placed in the crucible, dried on a hot plate to obtain the constant weight. The crucible containing acid insoluble ash was placed in desiccators and weighed. The % of acid insoluble ash [% w/w] was calculated by ratio of weight of ash to the weight of sample multiplied by 100.

Water Soluble Ash Value- To the total ash obtained as mentioned in above procedure, 25 ml of water was added and boiled for 5min. The crucible content was filtered on an ash less filter paper and the residue was washed with hot water. The filter paper comprising insoluble content was placed in the original crucible and ignited in muffle furnace at 450°C for 15 min. The weight of this residue in mg was subtracted from the weight of total ash. The percentage of water soluble ash with reference to air dried plant material was calculated.

Total Ash Value- Two-four gram plant material powder was placed in a tared silica crucible and spreaded uniformly. Then this silica crucible was placed in a muffle furnace and ignited at 500-600ºc until all the material was completely free from carbon content and converted to white color ash. It was transferred to desiccator to avoid moisture absorbance and weighed. Total ash [% w/w] was calculated by the ratio of weight of ash to the weight of sample multiplied by 100.

The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Loss on Drying- This parameter is used to determine the amount of moisture present in particular sample. The drug was powdered and the sample (10 gm) was placed on a pre-weighed evaporating dish. The evaporating dish was already dried at 105˚C for 6 hrs in an oven. The drying was sustained till two consecutive reading matches or the variation between two consecutive weightings was less than 0.25% of constant weight. The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Extractive Values- Extractive values of

crude drug determine the presence of chemical constituents in the crude drugs.

Water Soluble Extractive Value- About four gram of coarsely powdered plant material was kept in a conical flask and macerated with 100 ml water for 24 h, with occasional shaking. After completion of 24 h, the content in the flask was filtered and 25ml of filtrate was placed in a previously tared china dish for the evaporation of solvent. The residual extract obtained was heat dried at 105ºc for six hours, placed in a crucible for thirty minutes and its weight was taken. For the determination of extractive value, the results were determined in terms of mg/g of air-dried material.

Alcohol Soluble Extractive Value- 100 ml of methanol was added to glass stoppered conical flask containing 4 g coarsely, dried powdered plant material and macerated for 24 h with occasional shaking. After completion of 24 h, the content in the flask was filtered and 25ml of filtrate was placed in a previously tared flat-bottomed china dish and the solvent was allowed to evaporate. The residual extract so obtained was dried at 105ºC for 6 h and placed in a desiccator for 30 min and weighed.

The extractive value was determined in terms of mg/g of air-dried material. The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Fluorescence Analysis- The powdered drug from Carissa spinarum L. Flowers was perceived in daylight and UV light to determine its fluorescence properties. The drug was mixed with various chemical reagents to study the changes in fluorescent colour of solution.

The above methods were uniformly applied to both plant extracts to ensure consistency in evaluation.

Table-1 Qualitative Phytochemical Screening of Plant Extract

Sr. No	Phytochemical Constituents	Test Name	Observation	Inference
1	Alkaloids	Wagner's Test	Reddish-brown precipitate	Presence of alkaloids
		Dragendorff's Test	Orange-red precipitate	Presence of alkaloids
		Hager's Test	Yellow precipitate	Presence of alkaloids
		Mayer's Test	Cream-colored precipitate	Presence of alkaloids
		Legal's Test	Pink to red coloration	Presence of alkaloids
2	Glycosides	Keller–Killiani Test	Pale blue layer formation	Cardiac glycosides present
		Borntrager's Test	Red coloration	Anthraquinone glycosides present
		Ninhydrin Test	Blue coloration	Amino glycosides present
3	Proteins	Molisch's Test	Purple/violet ring	Presence of proteins
4	Carbohydrates & Reducing Sugars	Fehling's Test	Brick-red precipitate	Reducing sugars present
		Benedict's Test	Red precipitate	Reducing sugars present
		Lead Acetate Test	White precipitate	Carbohydrates present
5	Phenolics & Tannins	Ferric Chloride Test	Dark blue/greenish-black color	Phenolics/tannins present
		Potassium Ferricyanide Test	Red coloration	Phenolics present
		Ammonia Test	Fluorescence	Phenolics present
6	Flavonoids	Amyl alcohol Test	Yellow color (disappears with acid)	Flavonoids present
		Shinoda's Test	Cherry-red color	Flavonoids present
7	Steroids & Terpenoids	Libermann–Burchard Test	Bluish-green color	Steroids present
		Salkowski Test	Blue-green color	Steroids present
8	Saponins	Froth Test	Persistent froth	Saponins present
		Foam Test	Stable foam layer (1 cm)	Saponins present

Thin Layer Chromatography (TLC) Analysis

I. Preparation of TLC Plates- Pre-coated silica gel 60 F_{254} aluminum plates were used as the stationary phase. The plates were activated by drying in a hot air oven at 110°C for 30 minutes prior to use and allowed to cool to room temperature.

II. Preparation of Sample Solution- The hydroalcoholic extract of *Mimosa tenuiflora* (MT-HAE) was dissolved in hydroalcoholic solvent (ethanol:water) to obtain a concentration of 10 mg/ml. The solution was filtered before application.

III. Mobile Phase Optimization- Different solvent systems were tried, and the optimized mobile phase was selected based on clear separation of spots.

MT-HAE =Chloroform : Methanol : Water (7:3:0.5 v/v/v)

IV. Chromatographic Development

• A line was drawn 1 cm above the lower edge of the TLC plate.

•Using a capillary tube, 5–10 µl of the extract was spotted on the plate. The plate was placed in a TLC chamber pre-

saturated with mobile phase vapor for 20 minutes.

• Development was carried out up to 8 cm from the point of application.

• The plate was removed and air-dried.

V. Detection of Spots: The developed plates were observed under:

• UV light at 254 nm

• UV light at 366 nm

For better visualization, plates were sprayed with detecting reagents such as vanillin–sulphuric acid or anisaldehyde reagent and heated at 105°C for color development.

VI. Calculation of Rf Value- Rf values were calculated for each visible spot and recorded.

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

In vitro Antioxidant Assay In vitro antioxidant study for different extracts were carried out by using some standard protocols including hydrogen-donating assay, hydrogen peroxide antioxidant assay, superoxide radicals antioxidant assay and reducing power assay.

I. Hydrogen Donating Activity- 2, 2-diphenyl-1-picryl-hydrazil stable radical (DPPH) assay was used to perform the hydrogen donating activity of extracts. Around 50 µm of DPPH was added to solution of different proportions of plant extracts and standard ascorbic acid individually. The solutions were shaken thoroughly and kept at dark for thirty minutes. Then 2ml of methanol and 2ml of DPPH was added to prepare the control soln. The absorbance of all the reaction mixtures and control solution was determined at 517 nm. The % inhibition was calculated by following equation[8].

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of extract or standard}}{\text{Absorbance of control}}$$

The graph was plotted between % inhibition vs different concentrations of both plant extracts and ascorbic acid. IC 50 was evaluated from the graph.

II. H_2O_2 Antioxidant Assay- H_2O_2 antioxidant assay was performed by the following method: The capability of extract to reduce hydrogen peroxide (H_2O_2) was evaluated spectro-photo-metrically at 230 nm. Varying concentrations of extract or standard were dissolved in 3.4 ml of (0.1 M) PBS at pH 7.4 and added with 0.6 ml of 40 mm H_2O_2. Absorbance at 230 nm was recorded 10 minutes later. A separated blank sample was used for each concen-tration, for background subtraction[9-10].

III. Superoxide Radical Antioxidant Assay- The superoxide radical anti-oxidant assay of extracts was evaluated by the following method: Around 0.1 ml quantity of extract were dissolved with 468 µm of 1 ml NADH in 100 mm PBS at ph 7.8 and 156 µm of 1 ml nitroblue tetrazolium solution. Further, the reac-tion was started with addition of 100 µl of PMS into different reaction mixtures. Then for 5 min, the mixture was kept at the temperature of 25°C and subseq-uently the absorbance was taken at wavelength of 560 nm by spectro-photometer. In the presence of extract, the absorbance was gradually decreased that indicated the inhibition of super-oxide radicals against the control. The percentage inhibition was deter-mined and IC_{50} values for various ex-tracts were estimated. The % inhibition of superoxide free radicals was evaluated by the formula illustrated in previous

section[11,12].

IV. Reducing Power Assay- Around 10–100 µg/ml of extract was added with 2.5 ml of PBS at ph 6.6 and 2.5 ml of 1% $kfecn_3$. The solution was properly mixed and placed in an incubator for 20 min at 50ºc. After incubation, the resulting solution was cooled and 2.5 ml of 10% trichloroacetic acid was mixed with the reaction mixture, accompanied by centrifugation at a speed of 3000 rpm for ten minutes. After centrifugation, an equal volume of distilled water was mixed with 2.5 ml of supernatant and finally 0.5 ml of 0.1% $fecl_3$ was added. Then the mixture was stirred thoroughly and kept at room temperature for ten minutes. The absorbance was taken at a wavelength of 700 nm.

V. Quantitative Analysis of Carissa spinarum L. Root bark-Quantitative estimation of ethyl acetate and methanol was done for the presence of total flavonoids, alkaloids, saponins and phenols.

VI.Total Phenolic Content Estimation- Around 1.5 ml of Folin's reagent was mixed with 1 ml of each sample and standard & then incubated for five minutes at room temperature. Further, 4 ml of Na_2CO_3 and 3.5 ml of distilled water were added to the solution. This reaction mixture was kept for thirty minutes at room temperature after shaking. Then absorbance was measured at a wavelength of 765 nm. For estimation of total phenolic content from the extracts, gallic acid graph was taken as a standard and the value was expressed as mg GAE/g (gallic acid eqv/g of dry wt. of extract)[13].

VII. Total Flavonoid Content Estimation- Around 1 ml of each standard and sample solutions, 3 ml methanol, 0.2 ml $alcl_3$, 5.6 ml distilled water and 0.2 ml of CH_3CO_2K were added. The reaction mixture was kept for thirty minutes at room temperature after shaking. Then absorbance of reaction mixtures was determined at wavelength of 415 nm. For the estimation of total flavonoid content from the extracts, quercetin graph was taken as standard and the value was expressed as mg QE/g [quercetin eqv/gm of dry weight of extract].

VIII. Total Alkaloid Content Estimation- Test sample was dissolved in DMSO and then 1ml of 2 N hydrochloric acid was added and filtered. Around 5 ml of bromocresol green solution and 5 ml of PBS was mixed to the above solution. The solution was vigorously stirred with the constant addition of 1–4 ml of chloroform, then this mixture was kept in 10 ml of volumetric flask. Absorbance was determined at wavelength of 470 nm by utilizing UV spectroscopy. Standard graph of atropine was used for the determination of total alkaloid content from the extracts.

IX. Total Saponin Content Estimation-Test sample was dissolved in DMSO, then 1 ml of 2 N hydrochloric acid was added and filtered. Around 5 ml of bromocresol green solution and 5 ml of PBS was mixed with the above solution. About 1–4 ml of chloroform was constantly mixed to the solution with thorough stirring. The solution was kept in a 10 ml volumetric flask and the volume was made up with chloroform. Absorbance was determined at wavelength of 470 nm by using UV spectroscopy. Different concentrations

of diosgenin solution (50, 62.5, 75, 87.5, 100, 112.5 and 125.5 µg/ml) were made by adding diosgenin distilled water & methanol at 4:6 ratio respectively. 0.25 ml of 8% vanillin reagent and 2.5 ml of 72% sulphuric acid were blended with each sample. The samples were kept at 60 °C in water bath and incubated for ten minutes. Then absorbance of solution was determined at wavelength of 544 nm. Further, 0.1 g of extract was heated in aqueous methanol and the solution was prepared up to 0.25 ml. The total saponins present in the prepared solution were estimated at 544 nm.

Experimental animals- Healthy adult Wistar rats weighing between 180–220 g of either sex were selected for the experimental study. These animals are widely used in pharmacological and toxicological research due to their well-characterized physiology, ease of handling, and consistent response to experimental conditions. The rats were procured from a certified animal facility and maintained under standard laboratory conditions to ensure uniformity and minimize environmental stress. The animals were housed in clean polypropylene cages under controlled temperature (22 ± 2°C) and relative humidity (55 ± 5%), with a 12-hour light and dark cycle to mimic natural circadian rhythms. Proper environmental conditions are essential for maintaining normal metabolic and physiological functions, thereby ensuring the reliability of experimental outcomes. The animals were provided with a standard pellet diet and had free access to clean drinking water ad libitum.

Prior to the commencement of the experiment, all animals were acclimatized to the laboratory environment for one week. This acclimatization period is crucial as it allows the animals to adjust to new surroundings, reducing stress-induced variability in experimental results. Proper care and handling of laboratory animals in accordance with ethical guidelines are fundamental to obtaining reproducible and scientifically valid data. These standardized conditions help ensure consistency, reduce experimental bias, and improve the reproducibility of pharmacological studies.

Acute oral toxicity- The acute oral toxicity study of the *Mimosa tenuiflora* leaf extract was conducted in accordance with OECD Guideline 423, also known as the Acute Toxic Class Method. This standardized protocol is widely accepted for evaluating the safety profile of test substances and determining their approximate lethal dose. The extract was administered orally to experimental animals at graded dose levels of 100, 200, 500, 1000, and 2000 mg/kg body weight to assess dose-dependent toxic effects. Following administration, the animals were closely monitored for a period of 14 days for any signs of toxicity. Observations included changes in behavioral parameters such as locomotor activity, alertness, and grooming, as well as neurological responses like tremors, convulsions, and coordination. Addition-ally, autonomic responses such as salivation, lacrimation, respiration, and changes in food and water intake were recorded. Mortality, if any, was also noted to estimate the safety margin of the extract.

This systematic observation period is crucial for identifying both immediate and delayed toxic effects. Based on the

absence or presence of toxic symptoms and mortality, the extract's. Safety profile was established, and sui-table doses were selected for further pharmacological evaluation. Thus, the acute toxicity study provides essential baseline data to ensure the safe administration of the plant extract in subsequent experimental models[14].

Induction of experimental diabetes- Experimental diabetes was induced in Wistar rats using Streptozotocin (STZ), a well-known diabetogenic agent that selectively destroys insulin-producing β-cells of the pancreas. A single intraperitoneal injection of STZ was administered at a dose of 50 mg/kg body weight. The compound was freshly prepared in cold citrate buffer (0.1M, pH 4.5) to maintain its stability, as STZ is highly unstable in aqueous solutions at neutral ph. Prior to administration, the animals were fasted overnight to enhance the effectiveness of STZ and ensure consistent induction of diabetes. Following STZ injection, the animals were provided with a 5% glucose solution for 24 hours to prevent sudden hypoglycemia, which may occur due to the acute release of insulin from damaged pancreatic cells. This step is critical for reducing mortality during the initial phase of diabetes induction. After 72 hrs, fasting blood glucose levels were measured using a standard glucometer. Rats exhibiting fasting blood glucose levels greater than 250 mg/dl were considered diabetic and selected for further experimental studies. This method of diabetes induction is widely used due to its reproducibility and its ability to mimic human diabetes mellitus, particularly insulin-dependent diabetes. It provides a reliable experi-mental model for evaluating the anti-diabetic potential of plant extracts and pharmacological agents.

Experimental design- In this study, Wistar rats were randomly divided into six groups, with six animals in each group (n = 6), to evaluate the antidiabetic potential of the plant extract in a controlled and systematic manner. Such grouping ensures proper comparison between normal, diabetic, standard, and treatment groups, thereby improving the reliability of experimental outcomes.

Group I served as the normal control and received only the vehicle, maintaining physiological conditions without any treatment.

Group II acted as the diabetic control, in which diabetes was induced using Streptozotocin (STZ), but no treatment was administered.

Group III was treated with a standard antidiabetic drug such as Metformin or Glibenclamide to serve as a positive control for comparison.

Groups IV to VI were designated as test groups and received low, medium, and high doses of the plant extract, respectively. All treatments were administered orally once daily for a duration of 21 days. This duration is sufficient to observe the therapeutic effects of the extract on blood glucose levels and other biochemical parameters. The structured experimental design allows for dose-dependent evaluation and comparison with standard treatment, thereby helping to establish the efficacy of the plant extract in managing diabetes.

Table-2 Experimental Design

Group	Description	Treatment Given
Group I	Normal control	Vehicle only
Group II	Diabetic control	STZ only
Group III	Standard drug-treated	Metformin/ Glibenclamide
Group IV	Treatment(Low dose) 100 Mg/kg p.o.	Low dose of extract
Group V	Treatment(Low dose)200 Mg/kg p.o.	Medium dose of extract
Group VI	Treatment(Low dose) 400 Mg/kg p.o.	High dose of extract

This design ensures clear differentiation between control and treatment effects, enabling accurate assessment of anti-diabetic activity.

Results

Evaluation of antidiabetic activity- The antidiabetic activity of the plant extract was evaluated by monitoring key physiological and biochemical parameters in Wistar rats throughout the study period. Fasting blood glucose levels were measured at regular intervals using a standard glucometer, which provides a rapid and reliable assessment of glucose concentration in blood. Measurements were typically taken at baseline (day 0) and at predetermined intervals (such as day 7, 14, and 21) to observe the progression or reduction of hyperglycemia in response to treatment. Regular monitoring of blood glucose is essential for determining the efficacy of the extract in controlling diabetes.

In addition to glucose levels, body weight of the animals was recorded periodically, as weight loss is a common symptom associated with diabetes due to altered metabolism and protein catabolism. Improvement or stabilization of body weight indicates a positive therapeutic effect of the treatment. Furthermore, general health conditions such as activity level, grooming behavior, food and water intake, and any signs of distress or illness were carefully observed and recorded. These parameters provide supportive evidence regarding the safety and overall beneficial effects of the extract. Thus, the combined evaluation of blood glu-cose levels, body weight, and general health status offers a comprehensive assessment of the antidiabetic potential of the test extract over the experimental period.

These tables facilitate systematic recording and comparison of experimental data, ensuring clarity and accuracy in evaluating the antidiabetic activity of the extract[15,16].

Biochemical estimation- At the end of the experimental period, blood samples were collected from Wistar rats under appropriate conditions, typically via retro-orbital puncture or cardiac puncture under mild anesthesia. The collected blood was allowed to clot and then centrifuged to separate the serum, which was used for various biochemical estimations. These analyses are crucial for evaluating the overall metabolic status and organ function in diabetic conditions and after treatment.

Serum glucose levels were measured to confirm the antidiabetic effect of the extract. In addition, the lipid profile, including total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), was assessed to evaluate the impact on lipid metabolism, as diabetes is often associated with dyslipidemia. Renal function markers such as Serum creatinine and blood urea nitrogen (BUN) were also estimated, since prolonged hyperglycemia can lead to kidney damage or diabetic nephropathy. All bio-chemical parameters were analyzed using standard commercially available diagnostic kits, following the manu-facturer's protocols to ensure accuracy and reproducibility. These kits are designed to provide precise quantitative measurements and are widely used in experimental and clinical studies. The combined evaluation of glucose, lipid profile, and renal markers provides a comprehensive understanding of the therapeutic efficacy and safety of the plant extract in managing diabetes and its associated complications[17,18].

Table-3 Biochemical Parameters Evaluated

Parameter	Significance
Serum Glucose	Assessment of antidiabetic activity
Total Cholesterol	Indicator of lipid metabolism
Triglycerides	Marker of dyslipidemia
HDL (High-density lipoprotein)	Protective lipid fraction
LDL (Low-density lipoprotein)	Atherogenic lipid fraction
Serum Creatinine	Indicator of kidney function
Blood Urea Nitrogen (BUN)	Marker of renal function

These biochemical estimations provide essential insights into both metabolic control and organ protection offered by the treatment.

Estimation of oxidative stress parameters- At the end of the experimental study, pancreatic tissue from Wistar rats was carefully excised, washed with ice-cold saline, and homogenized to prepare tissue samples for the assessment of oxidative stress para-meters. Tissue homogenization was carried out in an appropriate buffer under cold conditions to preserve enzyme activity and prevent degradation of antioxidant molecules. The resulting homogenate was then centrifuged, and the supernatant was used for biochemical analysis.

The estimation of antioxidant enzymes is crucial, as oxidative stress plays a significant role in the pathogenesis of diabetes and its complications. Key antioxidant parameters evaluated included superoxide dismutase (SOD), which catalyzes the dismutation of superoxide radicals into hydrogen peroxide; catalase (CAT), which further converts hydrogen peroxide into water and oxygen; and reduced glutathione (GSH), a major intracellular antioxidant that protects cells against oxidative damage. Additionally, lipid peroxidation (LPO) levels were measured, commonly expressed as malondialdehyde (MDA), to assess the extent of oxidative damage to cellular membranes.

All these parameters were determined using standard biochemical methods, ensuring accuracy and reproducibility of results. An increase in antioxidant enzyme levels along with a decrease in lipid peroxidation indicates a protective effect of the plant extract against oxidative stress. Therefore, the evaluation of these markers provides important insights into the antioxidant potential and therapeutic efficacy of the extract in diabetic conditions[17,18].

Table-4 Oxidative Stress Parameters

Parameter	Full Form	Significance
SOD	Superoxide Dismutase	Converts superoxide radicals to H_2O_2
CAT	Catalase	Breaks down hydrogen peroxide into Water and O_2
GSH	Reduced Glutathione	Major intracellular antioxidant

Histopathological examination- Histopathological evaluation of pancreatic tissue was performed by fixing excised samples in 10% neutral buffered formalin, followed by dehydration, clearing, and paraffin embedding. Thin sections (4–5 µm) were prepared using a microtome and stained with hematoxylin and eosin (H&E) for microscopic examination. The stained sections were analyzed to assess structural changes in β-cells of the islets of Langerhans, including degeneration, necrosis, inflammation, and restoration of normal architecture. This evaluation provides visual confirmation of pancreatic damage and the protective effects of the treatment, supporting the biochemical findings.

Statistical analysis- All experimental data obtained from studies on Wistar rats were expressed as mean ± standard error of the mean (SEM), which reflects the variability and reliability of the results. This format allows easy comparison between different experimental groups and improves the interpretation of data precision. Statistical analysis was performed using one way ANOVA to evaluate differences among multiple groups. Post hoc tests such as Tukey's or Dunnett's were applied to identify specific group differences.

Standardization of Plant Material (Preliminary Pharmacognostical Studies)- The safety, quality, and therapeutic efficacy of herbal formulations depend on proper identification, authentication, and standardization of the plant material used. Therefore, preliminary pharmacognostical and physicochemical evaluation of the powdered leaves of *Mimosa tenuiflora* was carried out to establish its identity, purity, and quality prior to pharmacological investigation.

The physicochemical parameters of the powdered leaf material were determined using standard pharmacognostical procedures. The total ash value was found to be 6.8 ± 0.12%, indicating the presence of inorganic constituents such as carbonates, phosphates, and silicates. The acid-insoluble ash value was 1.1 ± 0.10%, suggesting minimal contamination with siliceous matter and confirming the purity of the crude drug.

The water-soluble ash value was observed to be 2.3 ± 0.20%, indicating the presence of water-soluble inorganic salts. The loss on drying was found to be 9.6 ± 0.75%, reflecting acceptable moisture content and suitability for storage without significant risk of microbial growth.

The extractive values were determined to estimate the amount of active phyto-constituents present in the plant material. The alcohol-soluble extractive value was found to be 15.2 ± 0.58%, while the water-soluble extractive value was 20.6 ± 0.70%, indicating the predominance of polar constituents.

Fluorescence analysis of the powdered leaf material was carried out as an additional parameter for authentication and quality evaluation. The powder exhibited characteristic color changes under daylight and ultraviolet light upon treatment with different chemical reagents. These fluorescence character-ristics serve as an important tool for identification and detection of adult-eration.

Table-5 Physicochemical Parameters of Powdered Leaves of Mimosa tenuiflora

Parameters (% w/w)	***Mimosa tenuiflora***
Total ash	7.48 ± 0.12
Acid insoluble ash	1.36 ± 0.05
Water soluble ash	3.14 ± 0.09
Loss on drying	6.72 ± 0.11
Alcohol extractive value	10.47 ± 0.13
Water extractive value	12.83 ± 0.15

Values are expressed as mean ± SEM (n = 3)

Table-6 Fluorescence Characteristics of Powdered Leaves of *Mimosa tenuiflora*

Treatment	**Day Light**	**UV Light (254 nm)**	**UV Light (366 nm)**
Powder as such	Brown	Dark brown	Greenish brown
Powder + Distilled water	Light brown	Dark green	Green
Powder + 1N hcl	Reddish brown	Dark brown	Orange brown
Powder + 1N naoh	Dark brown	Green	Yellowish green
Powder + Methanol	Brown	Greenish brown	Light green
Powder + Ethanol	Brown	Dark green	Yellowish green
Powder + Chloroform	Dark brown	Green	Light green
Powder + Acetic acid	Brown	Dark brown	Greenish brown

Extraction of Plant Material- Extraction of plant material was carried out to isolate the bioactive phytoconstituents present in the leaves of *Mimosa tenuiflora* for further pharmacological evaluation. The dried and powdered leaves of Mimosa tenuiflorawere subjected to Soxhlet

extraction using a hydroalcoholic solvent system. The extract obtained was concentrated and dried to obtain a semi-solid residue. The percentage yield of the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) was calculated based on the initial weight of the powdered drug. The percentage yield was found to be 11.2% w/w.

Qualitative Phytochemical Screening- Preliminary phytochemical screening of the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) was carried out to identify the presence of major bioactive constituents. The phytochemical investigation revealed the presence of secondary metabolites such as proteins, carbohydrates, flavonoids, glycosides, saponins, polyphenols, tannins, and alkaloids in the extract. Steroids were found to be present, while fats and oils were absent. The results of qualitative phytochemical screening of HAEMT are presented in Table 7.

Table-7 Phytochemical Constituents of Hydroalcoholic Extract of *Mimosa tenuiflora*

Phytoconstituents	HAEMT
Proteins	+
Carbohydrates	+
Flavonoids	+
Glycosides	+
Saponins	+
Polyphenols	+
Tannins and phenolic compounds	+
Alkaloids	+
Steroids	+
Fats and oils	-

(+ = Present, − = Absent)

Quantitative Analysis of Hydro-alcoholic Extract- Quantitative estimation of bioactive constituents was carried out for the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) following preliminary phytochemical screening. The total phenolic content (TPC) and total flavonoid content (TFC) were determined using standard procedures.

Estimation of Total Phenolic Content (TPC)- The total phenolic content of HAEMT was determined using the Folin–Ciocalteu method, with gallic acid as the standard. The calibration curve obtained from gallic acid was used to calculate the phenolic content of the extract. The total phenolic content of HAEMT was found to be 212.85 ± 0.88 mg/g extract (gallic acid equivalent).

Estimation of Total Flavonoid Content (TFC)- The total flavonoid content of HAEMT was determined using rutin as the standard. The calibration curve of rutin was used to estimate the flavonoid content in the extract. The total flavonoid content of HAEMT was found to be 76.94 ± 3.84 mg/g extract (rutin equivalent)

Estimation of Total Alkaloid Content- The total alkaloid content of the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) was determined using atropine as the standard. The calibration curve of atropine was used to quantify the alkaloid content in the extract. The total alkaloid content of HAEMT was found to be 27.63 ± 2.48 mg/g extract (atropine equivalent)

Estimation of Total Saponin Content- The total saponin content of the hydroalcoholic extract of Mimosa tenuiflora(HAEMT) was determined using the vanillin–sulfuric acid method. Diosgenin was used as the standard reference compound, and the results were expressed as mg/g diosgenin equivalent using the standard calibration curve. The total saponin content of HAEMT was found to be 54.72 ± 1.28 mg/g extract (diosgenin equivalent).

Thin Layer Chromatography (TLC) Results of Hydroalcoholic Extract- Thin Layer Chromatography of the hydroalcoholic extract of *Mimosa tenuiflora* (MT-HAE) was performed using chloroform: methanol: water (7:3:0.5 v/v/v) as the mobile phase. The developed TLC plates showed distinct and well-resolved spots indicating the presence of multiple phytoconstituents in the extract. The chromatogram was observed under UV light at 254 nm and 366 nm, followed by spraying with vanillin–sulphuric acid reagent, which resulted in the appearance of colored spots corresponding to different classes of compounds.

Table-8 TLC Profile of MT-HAE

Spot No.	Rf Value	Color (After Spraying)	Observation under UV	Probable Phytoconstituent
1	0.18	Light yellow	Faint fluorescence	Phenolic compounds
2	0.32	Yellow-orange	Bright fluorescence	Flavonoids
3	0.45	Brown	Moderate	Tannins
4	0.58	Greenish-yellow	Strong fluorescence	Polyphenols
5	0.71	Violet	Visible	Terpenoids
6	0.86	Dark brown	Weak	Glycosides

Interpretation- The TLC profile of the hydroalcoholic extract revealed the presence of multiple phytoconstituents with different polarities, as indicated by varying Rf values. The appearance of yellow, orange, and green fluorescent spots suggests the presence of flavonoids and phenolic compounds, which are known for their antioxidant activity. The brown colored spots indicate tannins, while violet coloration may correspond to terpenoid compounds. The distribution of spots across the TLC plate indicates that the hydroalcoholic extract contains a complex mixture of bioactive compounds, supporting its potential pharmacological activity.

Figure-1 TLC Analysis

In vitro Antioxidant Activity

DPPH Radical Scavenging Activity- The hydroalcoholic extract of *Mimosa tenuiflora* (MT-HAE) exhibited significant hydrogen donating ability in

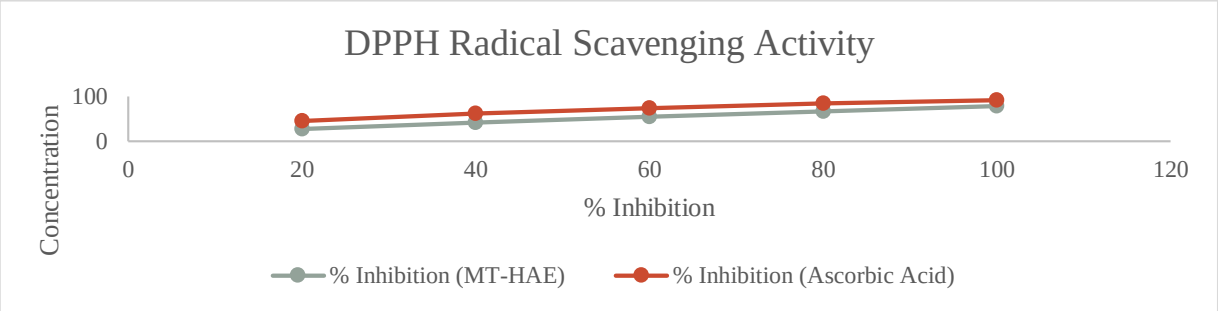
the DPPH assay. The percentage inhibition increased in a concentration-dependent manner when compared with standard ascorbic acid.

Table-9 DPPH Radical Scavenging Activity

Concentration (µg/ml)	% Inhibition (MT-HAE)	% Inhibition (Ascorbic Acid)
20	28.4 ± 1.2	45.6 ± 1.1
40	41.7 ± 1.4	62.3 ± 1.3
60	55.2 ± 1.5	74.8 ± 1.2
80	66.9 ± 1.3	85.2 ± 1.1
100	78.5 ± 1.2	92.6 ± 1.0

IC_{50} value:

- MT-HAE → **~58 µg/ml**
- Ascorbic acid → **~32 µg/ml**

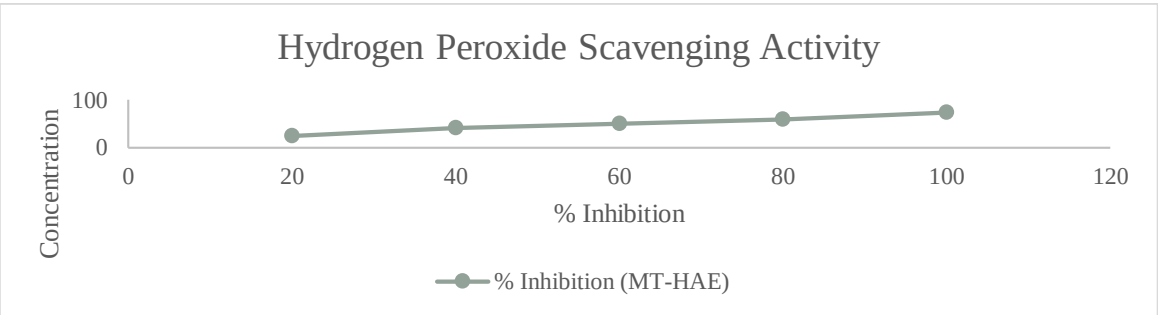


Graph-1 DPPH Radical Scavenging Activity

II. Hydrogen Peroxide Scavenging Activity-The extract demonstrated moderate to strong H_2O_2 scavenging activity, indicating its ability to neutralize reactive oxygen species.

Table-10 Hydrogen Peroxide Scavenging Activity

Concentration (µg/ml)	% Inhibition (MT-HAE)
20	25.6 ± 1.0
40	38.2 ± 1.2
60	49.7 ± 1.3
80	61.3 ± 1.4
100	72.8 ± 1.2

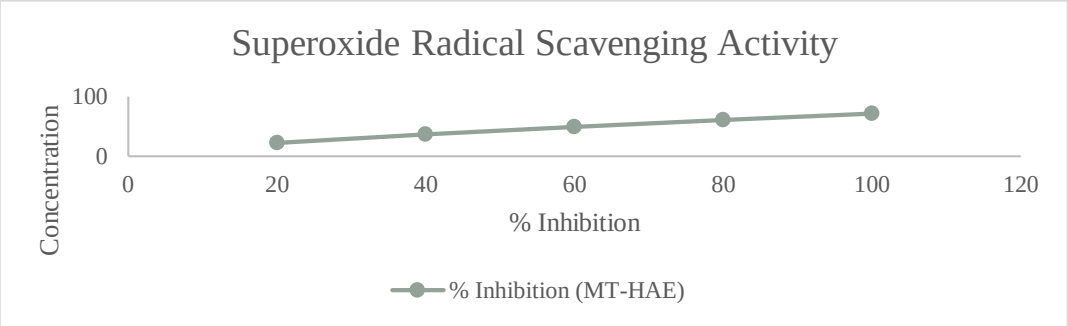


Graph- 2 Hydrogen Peroxide Scavenging Activity

III. Superoxide Radical Scavenging Activity- The extract showed effective inhibition of superoxide radicals, confirming its antioxidant potential.

Table-11 Superoxide Radical Scavenging Activity

Concentration (µg/ml)	% Inhibition (MT-HAE)
20	22.3 ± 1.1
40	36.8 ± 1.2
60	48.9 ± 1.4
80	60.5 ± 1.3
100	71.2 ± 1.1

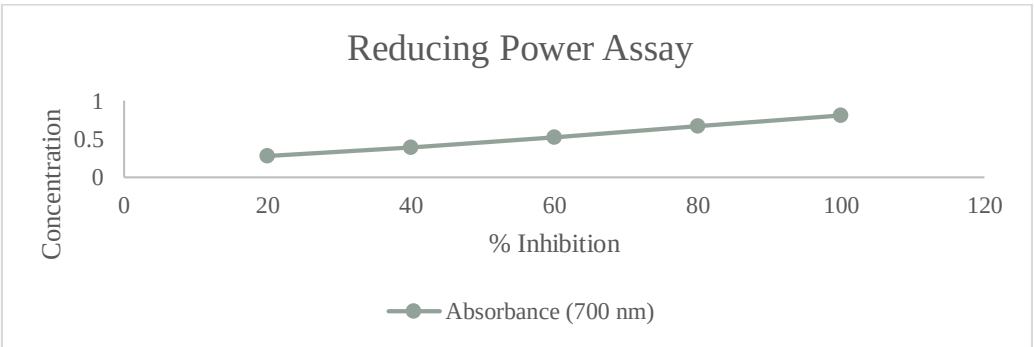


Graph- 3 Superoxide Radical Scavenging Activity

IV. Reducing Power Assay- The reducing power of MT-HAE increased with increasing concentration, indicating strong electron-donating capacity.

Table-12 Reducing Power Assay

Concentration (µg/ml)	Absorbance (700 nm)
20	0.28 ± 0.01
40	0.39 ± 0.02
60	0.52 ± 0.02
80	0.67 ± 0.01
100	0.81 ± 0.02



Graph-4 Reducing Power Assay

Quantitative Phytochemical Estimation- The quantitative phytochemical evaluation of the hydroalcoholic extract of *Mimosa tenuiflora* (MT-HAE) was carried out to determine the concentration of major bioactive constituents.

These phyto-constituents are responsible for important pharmacological activities, especially antioxidant and antidiabetic effects. The results were expressed as mean ± SEM to ensure accuracy, reliability, and reproducibility.

Total Phenolic Content- The total phenolic content was determined using the Folin–Ciocalteu method with gallic acid as the standard. The extract showed a phenolic content of 82.5 ±2.1mg GAE/g, indicating a high abundance of phenolic compounds. This suggests strong antioxidant potential, which may help in reducing oxidative stress, particularly under diabetic conditions.

Total Flavonoid Content- The total flavonoid content was estimated using the aluminium chloride colorimetric method with quercetin as the standard. The extract exhibited 64.3 ± 1.8 mg QE/g, indicating a substantial presence of flavonoids. These compounds contribute to antioxidant, anti-inflammatory, and antidiabetic activities by improving glucose uptake and scavenging free radicals.

Total Alkaloid Content- The total alkaloid content was determined using a spectrophotometric method with atropine as the reference standard. The extract contained 21.6 ± 1.2 mg/g of alkaloids, showing a moderate level of these compounds. Although present in smaller quantities, alkaloids may contribute synergistically to the overall pharmacological effects of the extract.

Total Saponin Content- The total saponin content was estimated using standard colorimetric methods. The extract showed 18.9 ±1.0 mg/g, indicating a moderate presence of saponins. These compounds are known to enhance glucose metabolism and improve insulin sensitivity, supporting the antidiabetic potential of the extract.

In vivo Pharmacological Evaluation- The in vivo study was carried out to evaluate the pharmacological activity of the hydroalcoholic extract of *Mimosa tenuiflora* (HAEMT) in experimental animals.

Acute Oral Toxicity Study- The acute oral toxicity study of the hydroalcoholic extract of *Mimosa tenuiflora*(HAEMT) was carried out in experimental animals in accordance with OECD guideline 423.

The extract was administered orally at a limit dose of 2000 mg/kg body weight. The animals were observed continuously for the first 4 hours for any immediate signs of toxicity and periodically up to 24 hours, followed by daily observations for a period of 14 days.

No mortality was observed in any of the treated animals during the entire study period. The animals did not exhibit any signs of toxicity such as tremors, convulsions, salivation, diarrhea, lethargy, or coma. No abnormal behavioral changes were observed, and the animals remained active and healthy throughout the study.

There were no noticeable changes in skin, fur, eyes, or mucous membranes. Food and water intake were found to be normal, and no significant variation in body weight was observed during the observation period.

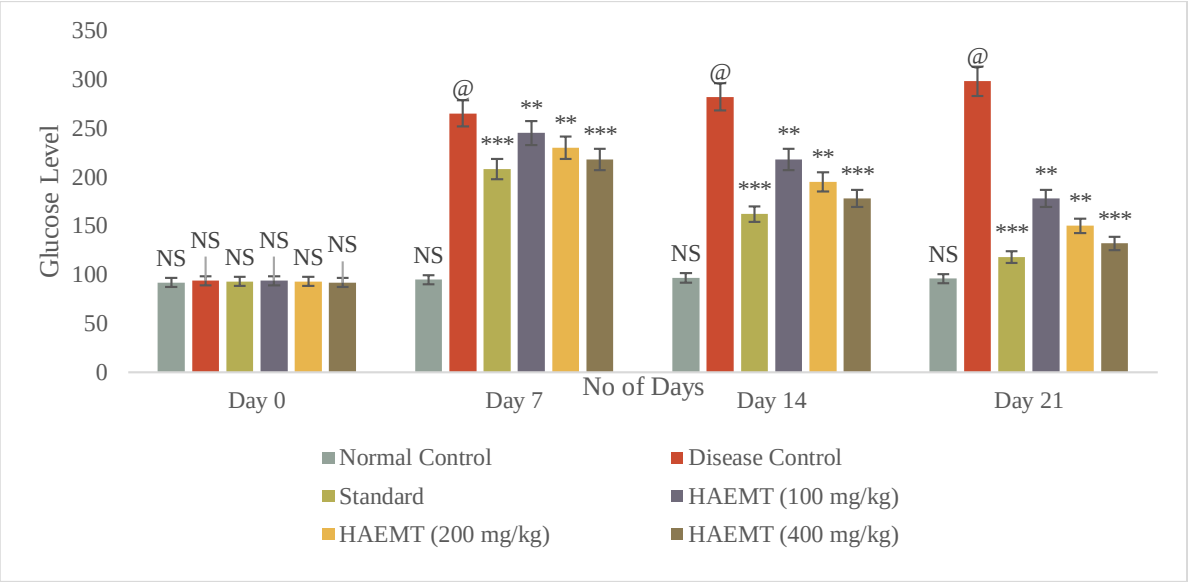
Based on these observations, the hydroalcoholic extract of *Mimosa tenuiflora* was found to be safe at the dose of 2000 mg/kg body weight, and the median lethal dose (LD_{50}) was considered to be greater than 2000 mg/kg.

Change in Blood Glucose Levels- The effect of HAEMT on blood glucose levels was evaluated over a period of 21 days. The observations are presented in Table-13.

Table-13 Effect of Extract on Blood Glucose Levels (mg/dl)

Group	Day 0	Day 7	Day 14	Day 21
Normal Control	92 ± 3	95 ± 4	97 ± 3	96 ± 2
Disease Control	94 ± 8	265 ± 11	282 ± 12	298 ± 13
Standard	93 ± 8	208 ± 7	162 ± 6	118 ± 5
HAEMT (100 mg/kg)	94 ± 7	245 ± 9	218 ± 8	178 ± 7
HAEMT (200 mg/kg)	93 ± 7	230 ± 8	195 ± 7	150 ± 6
HAEMT (400 mg/kg)	92 ± 6	218 ± 7	178 ± 6	132 ± 5

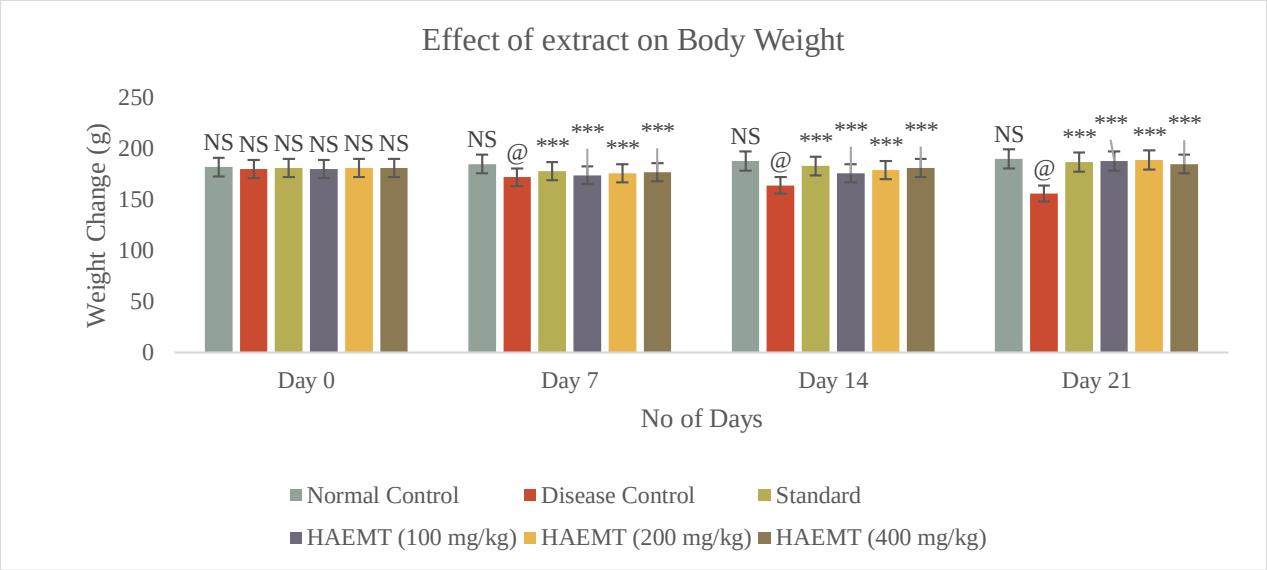
Values are expressed as mean ± SEM (n = 6)



Graph-5 Effect of Extract on Blood Glucose Levels (mg/dl)

Table-14 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Body Weight (g)

Group	Day 0	Day 7	Day 14	Day 21
Normal Control	182 ± 5	185 ± 6	188 ± 5	190 ± 6
Disease Control	180 ± 6	172 ± 5	164 ± 6	156 ± 7
Standard	181 ± 5	178 ± 5	183 ± 5	187 ± 6
HAEMT (Low Dose)	180 ± 6	174 ± 5	176 ± 6	179 ± 5
HAEMT (Medium Dose)	181 ± 5	176 ± 5	179 ± 5	183 ± 5
HAEMT (High Dose)	181 ± 5	177 ± 5	181 ± 5	185 ± 6



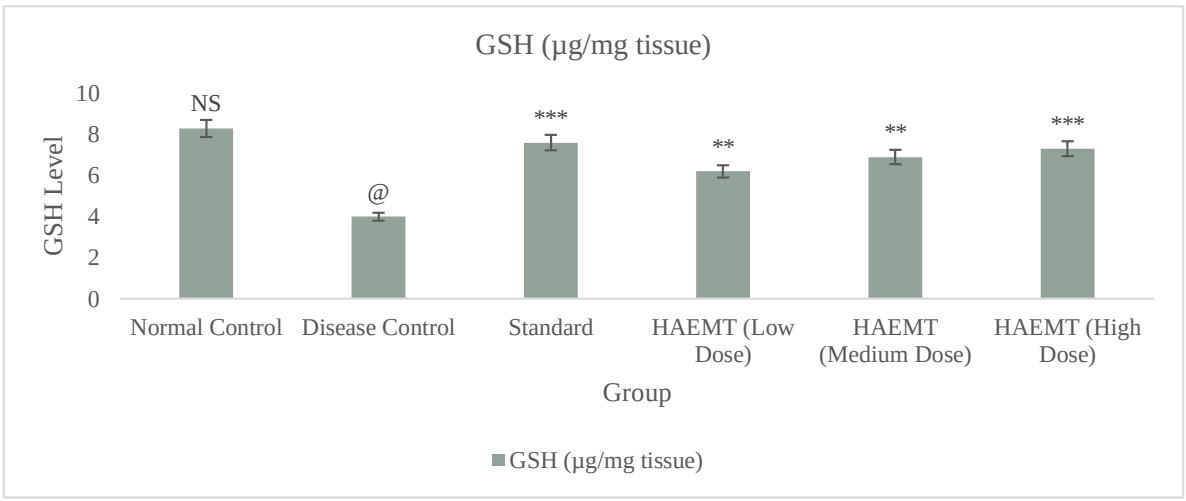
Graph-6 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Body Weight (g)

Effect on Antioxidant Parameters

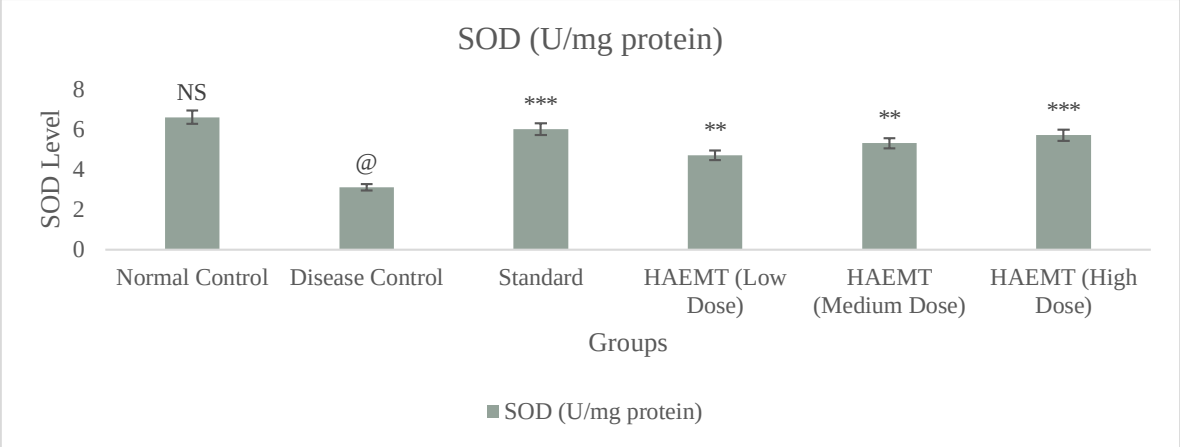
Table-15 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on GSH, SOD, and MDA Levels.

Group	GSH (µg/mg tissue)	SOD (U/mg protein)	MDA (nmol/mg tissue)
Normal Control	8.3 ± 0.4	6.6 ± 0.3	2.0 ± 0.1
Disease Control	4.0 ± 0.3	3.1 ± 0.2	5.9 ± 0.3
Standard	7.6 ± 0.3	6.0 ± 0.3	2.5 ± 0.2
HAEMT (Low Dose)	6.2 ± 0.3	4.7 ± 0.2	3.8 ± 0.2
HAEMT (Medium Dose)	6.9 ± 0.3	5.3 ± 0.2	3.3 ± 0.2
HAEMT (High Dose)	7.3 ± 0.3	5.7 ± 0.3	2.9 ± 0.2

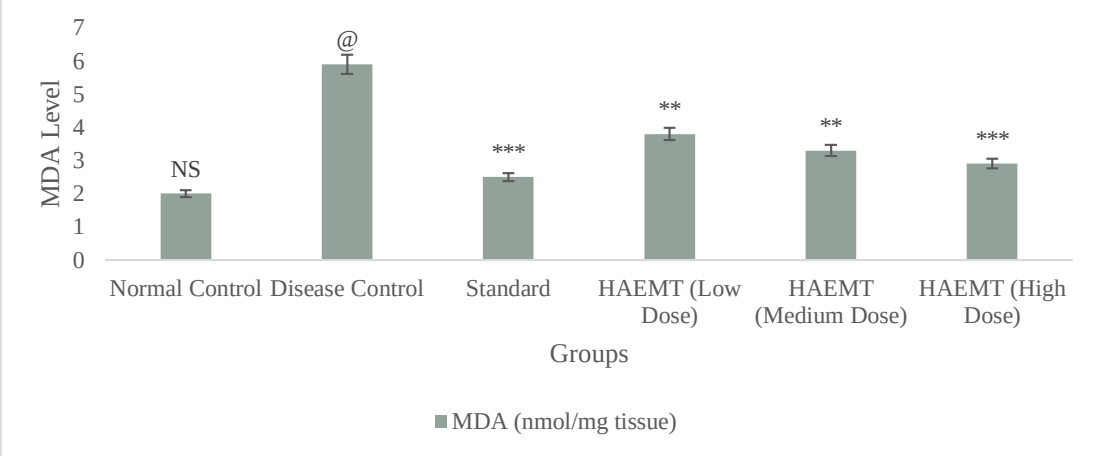
Values are expressed as mean ± SEM (n = 6)



Graph- 7 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on GSH Levels



Graph-8 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on SOD Levels

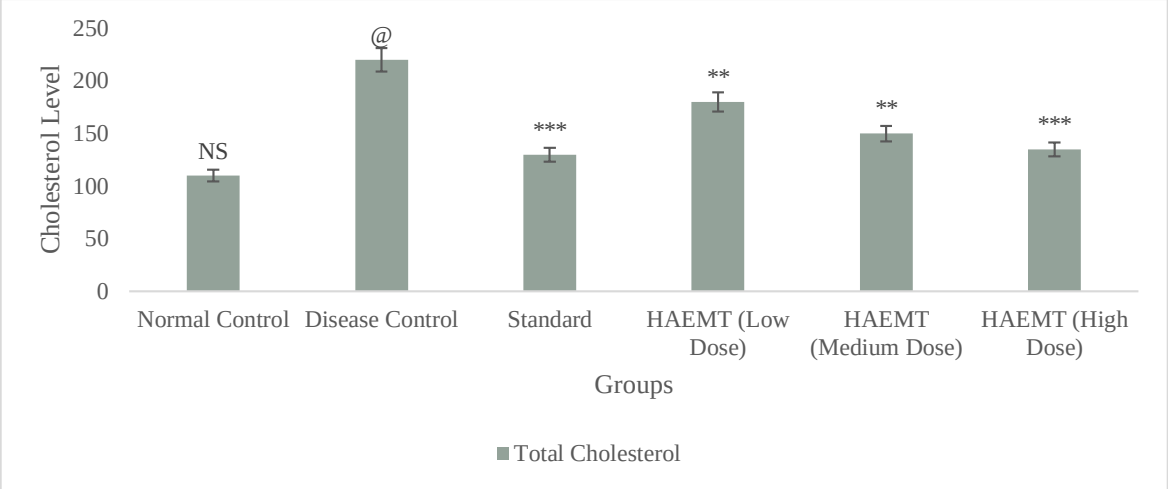


Graph-9 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on MDA Levels

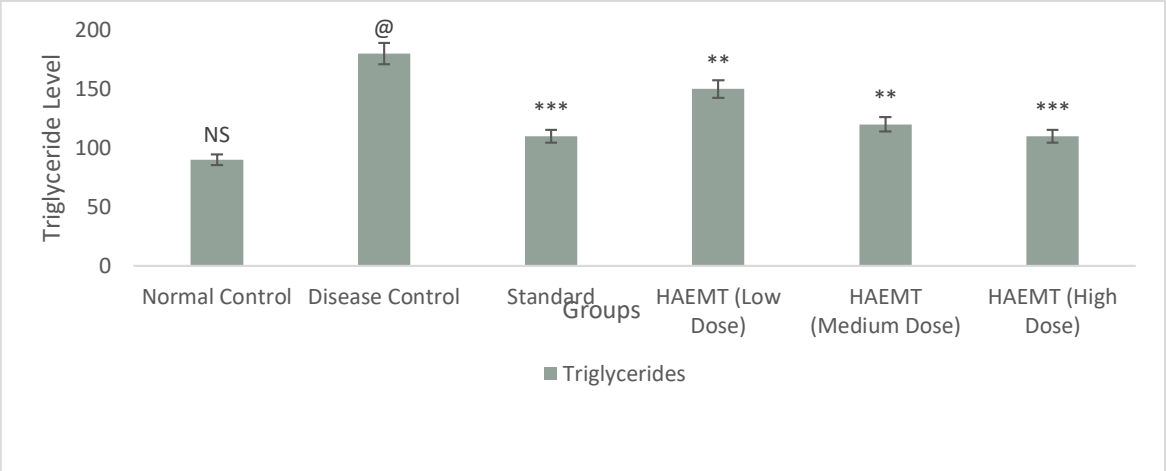
Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Lipid Profile

Table-16 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Lipid Profile

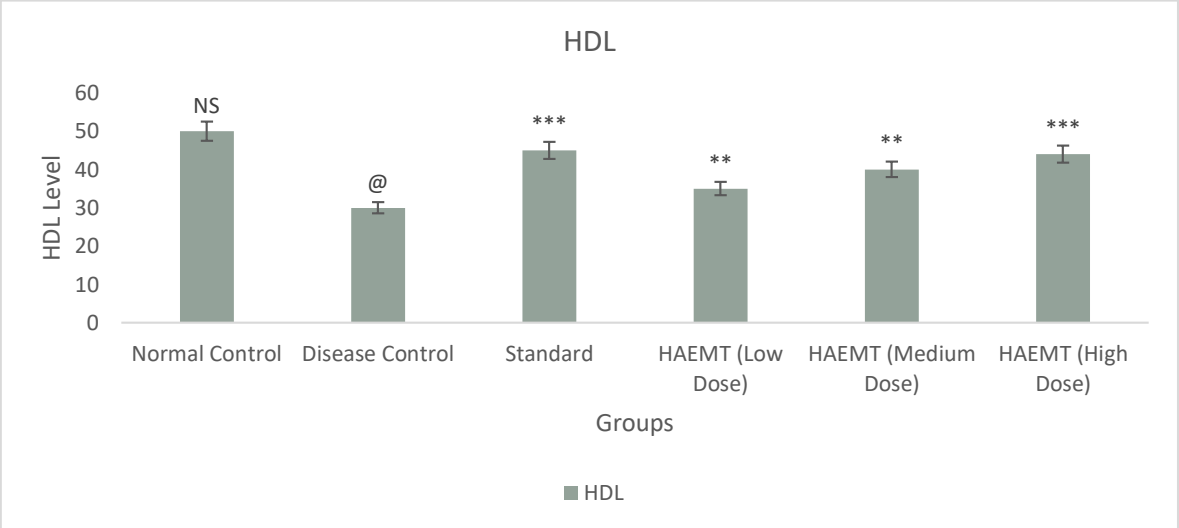
Group	Total Cholesterol	Triglycerides	HDL	LDL
Normal Control	110 ± 3	90 ± 2	50 ± 2	40 ± 2
Disease Control	220 ± 5	180 ± 4	30 ± 1	140 ± 3
Standard	130 ± 3	110 ± 3	45 ± 2	60 ± 2
HAEMT (Low Dose)	180 ± 4	150 ± 3	35 ± 2	100 ± 3
HAEMT (Medium Dose)	150 ± 3	120 ± 3	40 ± 2	80 ± 2
HAEMT (High Dose)	135 ± 3	110 ± 2	44 ± 2	65 ± 2



Graph -10 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on total cholesterol Level



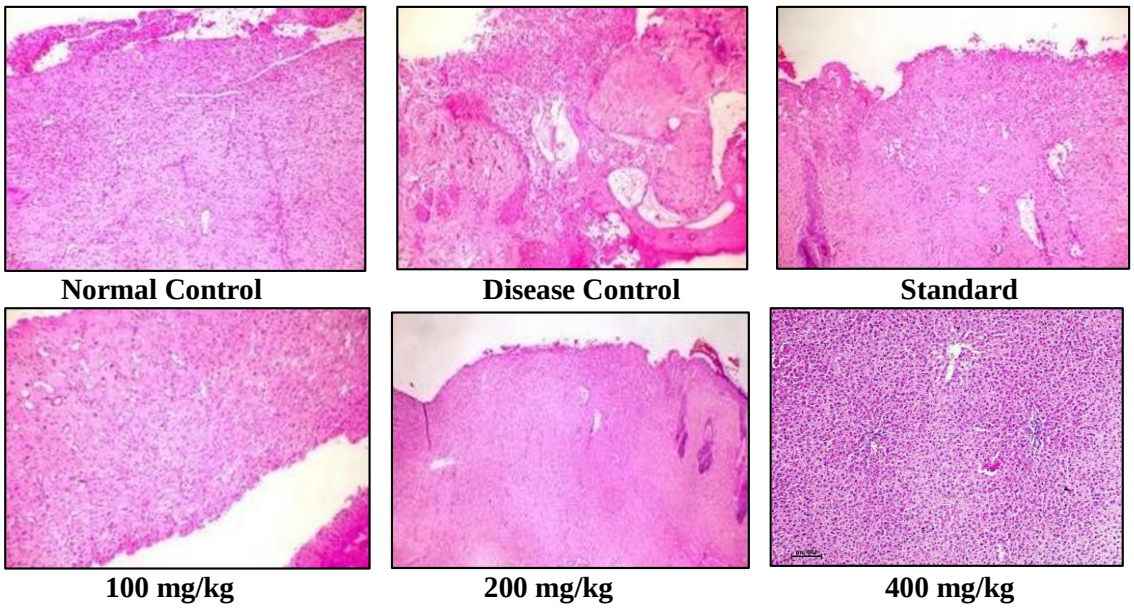
Graph-11 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on Triglycerides Level



Graph-12 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on HDL Level

Graph-13 Effect of Hydroalcoholic Extract of *Mimosa tenuiflora* on LDL Level

Histopathological Analysis



Phytochemicals such as flavonoids and tannins were identified. Extract showed significant α-amylase and α-glucosidase inhibition and reduced blood glucose in animal models.

Discussion

The present study was conducted to evaluate the antidiabetic potential of *Mimosa tenuiflora* through extraction, isolation, and identification of its bio-active compounds, along with pharma-cological assessment using in vitro and in vivo models. The findings of this study clearly demonstrate that the plant extract possesses significant antidiabetic and antioxidant activity, which can be attributed to its rich phytochemical composition. Diabetes mellitus is a multifactorial metabolic disorder in which chronic hyperglycemia leads to oxidative stress and inflammation, playing a crucial role in disease progression and complications. Persistent elevation of blood glucose levels results in excessive production of reactive oxygen species (RO-S), which disrupt cellular homeo-stasis and damage lipids, proteins, and DNA. This oxidative damage contributes to the development of complications such as neuropathy, nephropathy, and cardio-

vascular disorders.

In the present study, phytochemical screening revealed the presence of flavo-noids, phenolic compounds, tannins, and alkaloids, which are well known for their antioxidant and anti-diabetic properties. These compounds play a significant role in neutralizing ROS and protecting pan-creatic β-cells from oxidative damage. The antioxidant activity observed in assays such as DPPH supports the ability of the extract to scavenge free radicals and reduce oxidative stress. The anti-diabetic activity of *Mimosa tenuiflora* can be explained through multiple mec-hanisms. Firstly, flavonoids and phen-olic compounds may enhance insulin secretion and improve insulin sens-itivity in peripheral tissues. Secondly, inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase delays glucose absorption, thereby controlling postprandial hyper-glycemia. Additionally, certain phyto-constituents may reduce hepatic glucose production, contributing to overall glyc-emic control. The in vivo results obtained from strep-tozotocin-induced diabetic Wistar rats further validate the antidiabetic potential of the plant extract. A significant reduction in blood glucose levels, along with improvement in biochemical para-meters such as lipid profile and anti-oxidant enzymes (SOD, GSH, MDA), indicates the effectiveness of the extract in managing diabetes and associated oxi-dative stress. These findings are consis-tent with previous reports that highlight the role of plant-derived antioxidants in improving metabolic function and pre-venting diabetic complications. The selection of plant-based therapy in this study is justified by the limitations associated with conventional anti-diabetic drugs, which often produce adverse effects and target single pathways. In contrast, phytochemicals exhibit a multi-target mode of action, addressing various aspects of diabetes, including insulin resistance, oxidative stress, and inflammation. The synergistic interaction of multiple bioactive compounds present in the extract enhances its therapeutic efficacy and provides a holistic approach to disease management.

Overall, the results of the present study strongly support the therapeutic poten-tial of *Mimosa tenuiflora* as a natural source of antidiabetic agents. The integration of phytochemical analysis with pharma-cological evaluation provides a scientific basis for its traditional use and highlights its potential for further development into safe and effective herbal formulations.

Conclusion

The present study was carried out to investigate the extraction, isolation, and identification of bioactive compounds from *Mimosa tenuiflora* and to evaluate its antidiabetic potential using both in vitro and in vivo approaches. The findings of the study clearly indicate that *Mimosa tenuiflora* is a rich source of pharmacologically active phyto-constituents, including flavonoids, phen-olic compounds, tannins, and alkaloids, which are known to possess significant antioxidant and therapeutic properties.

Phytochemical analysis confirmed the presence of these bioactive compounds, while chromatographic and analytical techniques facilitated their separation and identification. The antioxidant studies demonstrated that the extract possesses strong free radical scavenging activity, suggesting its ability to reduce oxidative stress, which is a key factor in the

pathogenesis of diabetes mellitus. The in vitro antidiabetic assays further revealed that the extract effectively inhibits carbohydrate digesting enzymes, thereby contributing to improved glyce-mic control.

The in-vivo evaluation using streptozotocin-induced diabetic Wistar rats provided substantial evidence of the antidiabetic efficacy of the plant extract. A significant reduction in blood glucose levels, along with improvement in lipid profile and antioxidant parameters such as SOD, GSH, and MDA, highlights its potential in managing both hyper-glycemia and associated metabolic dis-turbances. These effects may be attri-buted to multiple mechanisms, inclu-ding enhancement of insulin secretion, improvement of insulin sensitivity, reduction of oxidative stress, and protection of pancreatic β-cells.

Overall, the study supports the potential of *Mimosa tenuiflora* as a promising natural source for the development of novel antidiabetic agents. Its multi-target mechanism of action and favorable safety profile make it a valuable candidate for further research. Future studies focusing on isolation of pure compounds, clinical validation, and formulation development are warranted to establish its therapeutic application in diabetes management. Mimosa tenui-flora shows promising antidiabetic activity and can be a potential source for herbal drug development.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. American Diabetes Association. Diag-nosis and classification of diabetes mellitus. *Diabetes Care*; 2014; 37(Suppl 1):S81–S90.
2. Definition and diagnosis of diabetes mellitus. *World Health Organization;* Geneva: WHO; 2006.
3. Atkinson, M.A; Eisenbarth, G.S.; Michels, A.W. Type-1 diabetes. *Lancet.;* 2014; 383(9911):69–82.
4. DeFronzo, R.A.; Ferrannini, E. and Groop, L et al. Type 2 diabetes mellitus. *Nat. Rev. Dis. Primers;* 2015; 1:15019.
5. Buchanan, T. A. and Xiang, A.H. Gestational diabetes mellitus. Journal Clin. Inves.; 2005; 115(3):485–491.
6. American Diabetes Association. Classification and diagnosis of dia-betes. *Diabetes Care;* 2017; 40(Suppl 1) :S11–S24.
7. International Diabetes Federation. *IDF Diabetes Atlas;* 10th ed.; Brussels: IDF; 2021.
8. Saeedi. P.; Petersohn, I.; Salpea, P. et al. Diabetes prevalence. *Diabetes Res. Clin. Pract.;* 2019; 157:107843.
9. Anjana, R. M.; Deepa, M.; Pradeepa, R. et al. Diabetes in India. *Lancet Diabetes Endocrinol.;* 2017; 5(8):585–596.
10. Mohan, V. and Pradeepa, R. Epide-miology in India. *Health Adm.;* 2009; 22 (1):1–18.
11. Bommer, C.; Heesemann, E.; Sagalova, V. et al. Economic burden of diabetes. *Lancet Diabetes Endocrinol.*; 2017; 5(6) :423–430.
12. Prasad, R.B. and Groop, L. Genetics of type 2 diabetes. *Genes.*; 2015; 6(1):87–1 23.
13. Mohan, V. and Deepa, R. Genetic predisposition. *Indian J. Med. Res.;* 2006; 125(3):259–274.

14. Kahn, S.E.; Hull, R.L. and Utzschneider, K.M. Obesity and insulin resistance. *Nature*; 2006; 444:840–846.

15. Colberg, S.R.; Sigal, R.J.; Yardley, J.E. et al. Exercise and diabetes. *Diabetes Care*; 2016; 39(11):2065–2079.

16. Ley, S.H.; Hamdy, O.; Mohan, V. and Hu, F.B. Prevention of diabetes. *Lancet*; 2014; 383:1999–2017.

17. Chang, A.M. and Halter, J.B. Aging and Insulin secretion. *Am. J. Physiol*.; 2003; 284:E7–E12.

18. Hackett, R. A. and Steptoe, A. Stress and diabetes. *Nat. Rev. Endocrinol.* 2017; 13 :547–560.