RESEARCH PAPER

Antioxidant Mechanisms of *Torilis leptophylla* in Streptozotocin-Induced Diabetic Rats Through Modulation of Oxidative Stress Biomarkers

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ABSTRACT

Background: Oxidative stress plays a pivotal role in the onset and progression of diabetes mellitus by promoting pancreatic β-cell dysfunction, insulin resistance, and the development of diabetic complications. Medicinal plants rich in antioxidant phytochemicals have emerged as promising therapeutic agents for attenuating oxidative damage associated with diabetes. Torilis leptophylla (Apiaceae) is traditionally recognized for its medicinal properties and contains diverse bioactive constituents, including flavonoids, phenolics, alkaloids, and glycosides, which possess significant antioxidant potential. However, limited scientific evidence is available regarding its role in modulating oxidative stress biomarkers in experimental diabetes.

Objective: The present study aimed to investigate the antioxidant mechanisms of the ethanolic extract of Torilis leptophylla in streptozotocin (STZ)-induced diabetic rats through evaluation of endogenous antioxidant biomarkers and lipid peroxidation.

Materials and Methods: Successive solvent extraction of the whole plant was performed using petroleum ether, chloroform, ethanol, and distilled water. Preliminary phytochemical screening identified the ethanolic extract as the richest source of bioactive phytoconstituents. Acute oral toxicity studies were conducted according to OECD Guideline 423. Experimental diabetes was induced in Wistar albino rats using streptozotocin (45 mg/kg, i.p.), and diabetic animals were treated orally with the ethanolic extract at doses of 100, 200, and 400 mg/kg for 21 days. Oxidative stress was assessed by estimating superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) levels. Bioactivity-guided fractionation of the active extract was subsequently carried out using silica gel column chromatography, followed by thin-layer chromatographic (TLC) analysis of the isolated fractions.

Results: The ethanolic extract exhibited the highest extraction yield and a phytochemical profile rich in flavonoids, alkaloids, and glycosides. Treatment with Torilis leptophylla significantly restored antioxidant defence by increasing SOD and GSH levels while reducing MDA concentrations in diabetic rats in a dose-dependent manner. These improvements were accompanied by reductions in fasting blood glucose and glycated haemoglobin (HbA1c), indicating a close association between oxidative stress attenuation and improved glycemic control. Fractionation of the ethanolic extract yielded three major fractions, among which Fraction F3 demonstrated the most prominent phytochemical characteristics during TLC analysis, suggesting enrichment of antioxidant bioactive constituents.

Conclusion: The findings demonstrate that Torilis leptophylla exerts potent antioxidant effects in streptozotocin-induced diabetic rats by enhancing endogenous antioxidant defences and suppressing lipid peroxidation. The study provides experimental evidence supporting the therapeutic potential of Torilis leptophylla as a natural source of antioxidant phytochemicals for the management of diabetes-associated oxidative stress. Further isolation, structural characterization, and mechanistic investigations of the active constituents are warranted to facilitate the development of novel phytopharmaceutical interventions.

Keywords: Torilis leptophylla; oxidative stress; diabetes mellitus; streptozotocin; antioxidant activity; superoxide dismutase; reduced glutathione; malondialdehyde.

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1. INTRODUCTION

1.1 Diabetes Mellitus: A Global Health Challenge

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from impaired insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide and has become a major public health concern due to its increasing incidence, long-term complications, and substantial socioeconomic burden. According to the International Diabetes Federation, over 537 million adults were living with diabetes in 2021, and this number is expected to rise significantly in the coming decades. The growing prevalence of diabetes has been attributed to population aging, urbanization, sedentary lifestyles, obesity, unhealthy dietary habits, and genetic predisposition.

Diabetes is primarily classified into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). Type 1 diabetes results from autoimmune destruction of pancreatic β-cells, leading to absolute insulin deficiency, whereas type 2 diabetes is characterized by insulin resistance accompanied by progressive β-cell dysfunction. Type 2 diabetes accounts for nearly 90–95% of all reported cases and is frequently associated with obesity and metabolic syndrome. Regardless of the underlying cause, chronic hyperglycaemia disrupts carbohydrate, lipid, and protein metabolism, ultimately affecting the normal physiological functions of multiple organs.

Persistent elevation of blood glucose levels contributes to the development of both microvascular and macrovascular complications. Microvascular complications include diabetic nephropathy, retinopathy, and neuropathy, while macrovascular complications encompass coronary artery disease, stroke, and peripheral vascular disease. These complications significantly increase morbidity and mortality among diabetic patients and remain the leading causes of disability and reduced quality of life. Consequently, effective management of diabetes requires not only glycemic control but also prevention of the pathological mechanisms responsible for tissue damage.

Although several synthetic antidiabetic drugs are available for clinical management, their long-term use is often associated with adverse effects such as hypoglycaemia, gastrointestinal disturbances, weight gain, and reduced therapeutic efficacy. Moreover, many conventional therapies primarily target blood glucose regulation without adequately addressing the underlying cellular damage responsible for disease progression. Therefore, increasing attention has been directed toward identifying natural therapeutic agents capable of providing multiple pharmacological benefits.

Medicinal plants have emerged as promising alternatives because they contain diverse bioactive phytochemicals, including flavonoids, phenolics, alkaloids, and glycosides, which exhibit antihyperglycemic, antioxidant, and anti-inflammatory properties. Among these, *Torilis leptophylla* has gained scientific interest due to its rich phytochemical profile and reported pharmacological activities. However, its antioxidant mechanisms and role in protecting against diabetes-induced oxidative stress remain inadequately explored. Therefore, investigating the antioxidant potential of *Torilis leptophylla* may contribute to the development of safer and more effective plant-based therapeutic strategies for diabetes mellitus.

1.2 Oxidative Stress and Its Role in Diabetes Mellitus

Oxidative stress is a major pathogenic factor in the development and progression of diabetes mellitus and its associated complications. It results from an imbalance between the excessive production of reactive oxygen species (ROS) and the body's antioxidant defence mechanisms. Under diabetic conditions, persistent hyperglycaemia increases the generation of ROS through mitochondrial dysfunction, activation of the polyol pathway, formation of advanced glycation end products (AGEs), and protein kinase C activation. These processes collectively promote oxidative damage to cellular lipids, proteins, and DNA, thereby impairing normal cellular function.

Pancreatic β-cells are particularly susceptible to oxidative injury because they possess relatively low levels of endogenous antioxidant enzymes. Excessive ROS can damage β-cell membranes and mitochondria, resulting in reduced insulin secretion and progressive β-cell dysfunction. In addition, oxidative stress contributes to insulin resistance in peripheral tissues, thereby worsening hyperglycaemia and accelerating the progression of diabetic complications such as nephropathy, neuropathy, retinopathy, and cardiovascular disease.

The endogenous antioxidant defence system plays a crucial role in protecting cells against oxidative damage. Superoxide dismutase (SOD) catalyses the conversion of superoxide radicals into less harmful molecules, while reduced glutathione (GSH) maintains intracellular redox balance by neutralizing free radicals. Conversely, malondialdehyde (MDA), a product of lipid peroxidation, serves as a reliable biomarker of oxidative stress and membrane damage. Therefore, assessment of SOD, GSH, and MDA is widely used to evaluate oxidative stress and the antioxidant efficacy of therapeutic agents in experimental diabetes.

Natural antioxidants derived from medicinal plants have gained considerable attention because they can scavenge free radicals, enhance endogenous antioxidant defences,

and reduce oxidative damage. These properties make antioxidant-rich plants, including *Torilis leptophylla*, promising candidates for the management of diabetes and its oxidative stress-mediated complications.

1.3 Medicinal Plants as Natural Antioxidants

Medicinal plants have been extensively investigated as potential therapeutic agents for diabetes because they contain a wide range of bioactive phytochemicals with antioxidant and antihyperglycemic properties. Natural compounds such as flavonoids, phenolic acids, alkaloids, glycosides, and terpenoids are capable of scavenging reactive oxygen species, enhancing endogenous antioxidant enzyme activity, and reducing lipid peroxidation. Unlike many synthetic drugs that primarily focus on glycemic control, plant-derived compounds often exhibit multiple pharmacological effects, including antioxidant, anti-inflammatory, and cytoprotective activities.

Numerous experimental studies have demonstrated that phytochemical-rich medicinal plants improve oxidative stress biomarkers by increasing superoxide dismutase (SOD) and reduced glutathione (GSH) levels while decreasing malondialdehyde (MDA) concentrations. These findings suggest that natural antioxidants may help protect pancreatic β-cells, improve insulin sensitivity, and reduce the progression of diabetes-associated complications. Consequently, medicinal plants continue to serve as an important source for the discovery of novel, safe, and effective antioxidant therapies for diabetes mellitus.

1.4 *Torilis leptophylla*: Phytochemical and Pharmacological Potential

Torilis leptophylla (Apiaceae) is an annual herbaceous plant widely distributed in Asia, the Mediterranean region, and parts of Europe. Traditionally, it has been used in folk medicine for the treatment of inflammatory disorders, gastrointestinal ailments, wounds, and infectious diseases. In recent years, the plant has gained considerable scientific attention due to its rich phytochemical composition and diverse pharmacological properties.

Phytochemical investigations have revealed that *T. leptophylla* contains several biologically active constituents, including flavonoids, phenolic compounds, alkaloids, glycosides, tannins, and terpenoids. These secondary metabolites are known to possess strong antioxidant properties by scavenging free radicals, inhibiting lipid peroxidation, and enhancing endogenous antioxidant defence mechanisms. Previous experimental studies have also reported that extracts of *T. leptophylla* exhibit antioxidant, anti-inflammatory, antimicrobial, anti-ulcer, neuroprotective, and anti-arthritic activities, supporting its therapeutic potential in oxidative stress-related disorders.

Despite these promising pharmacological effects, scientific evidence regarding the antioxidant mechanisms of *T. leptophylla* in diabetes mellitus remains limited. In particular, its influence on oxidative stress biomarkers such as superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) has not been comprehensively investigated. Therefore, evaluating the antioxidant potential of *T. leptophylla* in streptozotocin-induced diabetic rats may provide valuable insights into its protective mechanisms and support its development as a potential phototherapeutic agent for the management of diabetes-associated oxidative stress.

1.5 Research Gap and Aim of the Study

Although numerous medicinal plants have been investigated for their antidiabetic properties, only a limited number have been systematically evaluated for their ability to modulate oxidative stress associated with diabetes mellitus. *Torilis leptophylla* has been reported to possess antioxidant, anti-inflammatory, antimicrobial, and other pharmacological activities owing to its rich phytochemical composition. However, comprehensive studies investigating its antioxidant mechanisms in experimental diabetes, particularly its effects on endogenous antioxidant biomarkers, remain scarce. Moreover, the relationship between its phytochemical constituents and their role in protecting against oxidative stress-induced cellular damage has not been fully elucidated.

The present study was therefore designed to investigate the antioxidant potential of the ethanolic extract of *Torilis leptophylla* in streptozotocin-induced diabetic rats. The study focused on evaluating its ability to modulate key oxidative stress biomarkers, including superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA), alongside assessment of glycemic control. Furthermore, bioactivity-guided fractionation and thin-layer chromatographic analysis were performed to identify the active fraction responsible for the observed antioxidant effects. The findings of this investigation are expected to provide scientific evidence supporting the therapeutic potential of *T. leptophylla* as a natural antioxidant and contribute to the development of plant-based interventions for the management of diabetes-associated oxidative stress.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

The whole plant of *Torilis leptophylla* was collected from the local region of Chittorgarh, Rajasthan, India, during its flowering season. The collected plant material was thoroughly washed with distilled water to remove adhering soil and other foreign matter, followed by shade drying at room temperature for 10–15 days. The dried material was then pulverized using a mechanical grinder

to obtain a coarse powder and stored in airtight containers until further use.

The plant specimen was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the institutional herbarium for future reference. Authentication ensured the botanical identity and purity of the plant material used in the present investigation.

2.2 Preparation of Plant Extracts

The powdered plant material was subjected to successive solvent extraction using solvents of increasing polarity, namely petroleum ether, chloroform, ethanol, and distilled water. Extraction was carried out using a Soxhlet apparatus, and each solvent was allowed to cycle until complete exhaustion of the plant material. The obtained extracts were concentrated under reduced pressure using a rotary evaporator and further dried to obtain solvent-free residues.

The percentage yield of each extract was calculated based on the weight of the dried extract relative to the initial weight of the powdered plant material. Among the different extracts, the ethanolic extract exhibited the highest extraction yield and was selected for subsequent phytochemical screening and pharmacological evaluation because of its higher content of bioactive phytoconstituents.

2.3 Preliminary Phytochemical Screening

The various extracts of *T. leptophylla* were subjected to qualitative phytochemical screening using standard pharmacognostic procedures. The extracts were tested for the presence of major classes of secondary metabolites, including alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, carbohydrates, proteins, and phenolic compounds. The ethanolic extract demonstrated the richest phytochemical profile and was therefore selected for further biological investigations.

2.4 Experimental Animals

Healthy adult Wistar albino rats of either sex, weighing 180–220 g, were procured from a CPCSEA-approved animal breeding facility. The animals were housed in polypropylene cages under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, and a 12 h light/12 h dark cycle) with free access to a standard pellet diet and water ad libitum. Prior to the commencement of the experiment, the animals were acclimatized to the laboratory environment for one week. All experimental procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and the study protocol was approved by the Institutional Animal Ethics Committee (IAEC).

2.5 Acute Oral Toxicity Study

The acute oral toxicity study of the ethanolic extract of *Torilis leptophylla* was performed according to the Organisation for Economic Co-operation and Development (OECD) Guideline 423. Female Wistar rats were administered the extract orally at the prescribed dose levels and observed continuously during the initial 4 h, followed by periodic observations for 14 days for any signs of toxicity, behavioural changes, morbidity, or mortality. The absence of mortality and significant toxic manifestations indicated that the extract was safe at the tested dose, and experimental doses of 100, 200, and 400 mg/kg body weight were selected for subsequent pharmacological evaluation.

2.6 Induction of Experimental Diabetes

Experimental diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) at a dose of 45 mg/kg body weight dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). Following STZ administration, animals were provided with 5% glucose solution for 24 h to prevent initial hypoglycaemia. After 72 h, fasting blood glucose levels were determined using a glucometer, and animals exhibiting fasting blood glucose levels greater than 250 mg/dL were considered diabetic and included in the study.

2.7 Experimental Design

The diabetic animals were randomly divided into six groups (n = 6). Group I served as the normal control, while Group II served as the diabetic control. Group III received metformin (100 mg/kg) as the standard drug. Groups IV, V, and VI received the ethanolic extract of *Torilis leptophylla* at doses of 100, 200, and 400 mg/kg body weight, respectively, for 21 consecutive days. At the end of the treatment period, fasting blood glucose was estimated, and blood and tissue samples were collected for the determination of HbA1c and oxidative stress biomarkers.

2.8 Biochemical Analysis

At the end of the 21-day treatment period, blood samples were collected for the estimation of glycated haemoglobin (HbA1c), and liver tissue homogenates were prepared for the assessment of oxidative stress biomarkers. Superoxide dismutase (SOD) activity was determined to evaluate enzymatic antioxidant defence, while reduced glutathione (GSH) levels were measured as an indicator of intracellular antioxidant capacity. Lipid peroxidation was assessed by estimating malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) method. Standard spectrophotometric procedures were employed for all biochemical estimations.

2.9 Bioactivity-Guided Fractionation and Thin-Layer Chromatography

The ethanolic extract, which demonstrated the highest biological activity, was subjected to bioactivity-guided fractionation using silica gel column chromatography. Elution was performed with solvent systems of increasing polarity to obtain different fractions. The collected fractions were monitored and pooled based on their chromatographic characteristics. The active fraction was further analysed by thin-layer chromatography (TLC) using an optimized solvent system. The developed chromatograms were visualized under ultraviolet light and after derivatization with suitable detecting reagents, and the retention factor (Rf) values were calculated to characterize the phytochemical profile of the active fraction.

2.10 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM) for six animals in each group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by an appropriate post hoc multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Percentage Yield and Phytochemical Screening

Successive solvent extraction of the powdered whole plant of *Torilis leptophylla* was carried out using petroleum ether, chloroform, ethanol, and distilled water. The extraction yield varied among the solvents depending on their polarity. The ethanolic extract exhibited the highest percentage yield, followed by the aqueous, chloroform, and petroleum ether extracts, indicating that ethanol was the most effective solvent for extracting polar bioactive constituents from the plant material.

Qualitative phytochemical screening revealed the presence of several important secondary metabolites, including alkaloids, flavonoids, glycosides, phenolic compounds, tannins, and terpenoids. Among all the extracts, the ethanolic extract demonstrated the richest phytochemical profile with a higher abundance of flavonoids, phenolics, and glycosides, which are widely recognized for their antioxidant properties. The petroleum ether extract showed comparatively fewer phytoconstituents, while the chloroform and aqueous extracts exhibited moderate phytochemical diversity.

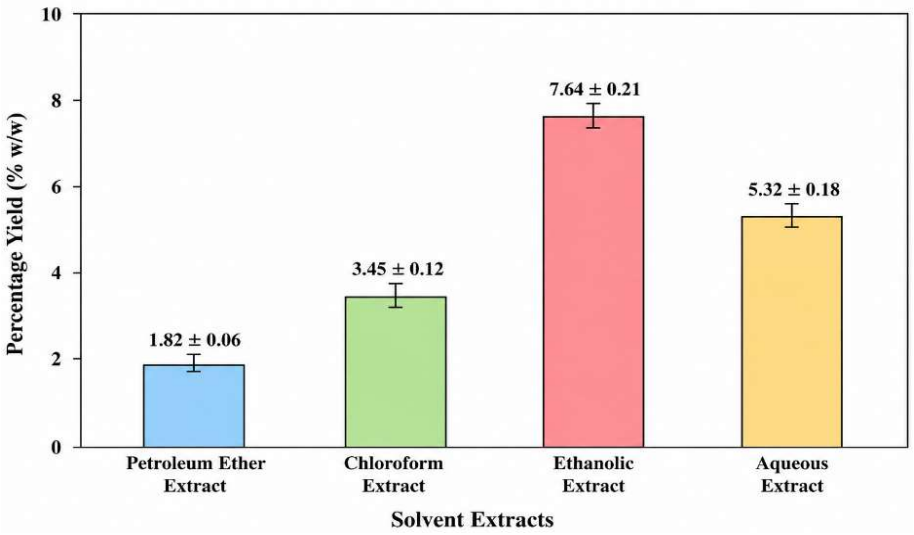
The higher extraction yield together with the presence of antioxidant-rich phytochemicals suggested that the ethanolic extract possessed greater pharmacological potential than the other solvent extracts. Therefore, it was selected for further evaluation of antioxidant activity, biochemical analysis, and bioactivity-guided fractionation.

Table 1. Percentage Yield and Major Phytochemical Constituents of Different Extracts of *Torilis leptophylla*.

Phytochemical Constituents	Petroleum Ether Extract (% Yield: 1.82 ± 0.06)	Chloroform Extract (% Yield: 3.45 ± 0.12)	Ethanolic Extract (% Yield: 7.64 ± 0.21)	Aqueous Extract (% Yield: 5.32 ± 0.18)
Alkaloids	+	++	+++	++
Flavonoids	–	++	+++	++
Glycosides	–	+	+++	++
Phenolic Compounds	+	++	+++	++
Tannins	–	+	+++	++
Terpenoids	+	++	+++	+
Saponins	–	+	++	++
Carbohydrates	–	+	++	+++
Proteins	–	+	++	++
Amino Acids	–	+	++	++

Values are expressed as mean ± SEM ($n = 3$). +++ : Abundant presence; ++ : Moderate presence; + : Low presence; – : Absent.

Figure 1. Percentage Yield of Different Solvent Extracts of *Torilis leptophylla*.



3.2 Acute Oral Toxicity Study

The acute oral toxicity study of the ethanolic extract of *Torilis leptophylla* was carried out in accordance with OECD Guideline 423 to evaluate its safety profile. Throughout the 14-day observation period, no mortality or treatment-related signs of toxicity were observed in the experimental animals. The rats remained healthy and exhibited normal behavioural patterns, including locomotor activity, food and water intake, grooming, and body weight gain. Furthermore, no visible abnormalities such as tremors, convulsions, salivation, respiratory distress, or changes in skin and fur texture were recorded.

The absence of toxic manifestations indicated that the ethanolic extract was well tolerated at the tested dose levels and possessed a wide margin of safety. Based on these observations, three dose levels (100, 200, and 400 mg/kg body weight) were selected for the evaluation of antioxidant and antidiabetic activities in streptozotocin-induced diabetic rats. These findings confirmed the suitability of the extract for subsequent pharmacological investigations.

3.3 Effect of *Torilis leptophylla* on Glycemic Parameters

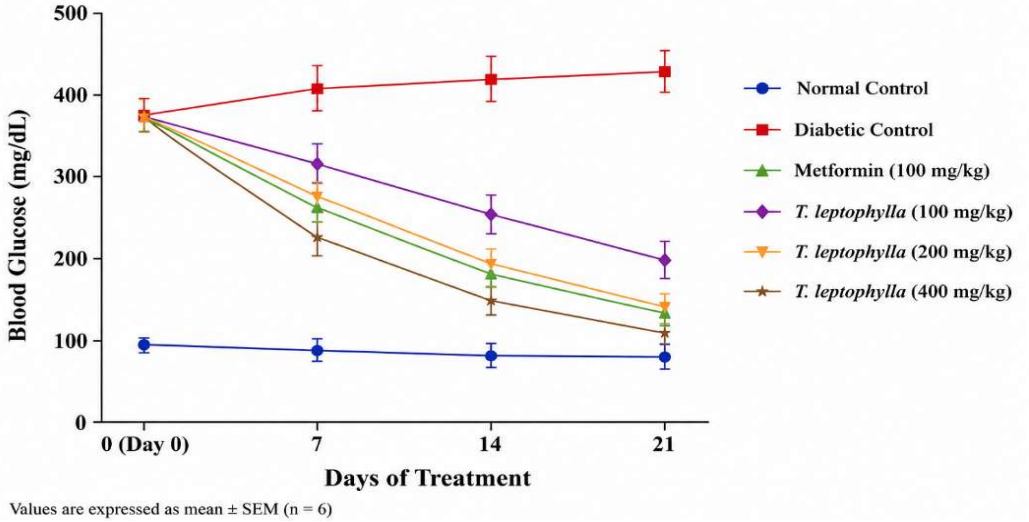
Administration of streptozotocin (STZ) produced a marked elevation in fasting blood glucose levels in the diabetic control group compared with the normal control group, confirming successful induction of experimental diabetes. Oral administration of the ethanolic extract of *Torilis leptophylla* for 21 consecutive days resulted in a significant dose-dependent reduction in fasting blood glucose levels. Among the tested doses, the 400 mg/kg treatment exhibited the greatest antihyperglycemic effect and demonstrated glucose-lowering activity comparable to that of the standard drug, metformin.

A progressive decline in blood glucose concentration was observed throughout the treatment period in extract-treated groups, indicating sustained improvement in glycemic control. The reduction was more pronounced in the medium- and high-dose groups, suggesting a dose-dependent pharmacological response. In contrast, diabetic control animals maintained persistently elevated blood glucose levels throughout the study period.

Assessment of glycated haemoglobin (HbA1c), an established indicator of long-term glycemic control, further supported these findings. The diabetic control group exhibited significantly increased HbA1c levels compared with the normal control group, reflecting prolonged hyperglycaemia. Treatment with the ethanolic extract significantly reduced HbA1c levels in all treatment groups, with the maximum reduction observed at 400 mg/kg. The improvement in HbA1c indicated effective regulation of chronic blood glucose levels following administration of *T. leptophylla*.

The observed antihyperglycemic activity may be attributed to the presence of antioxidant phytoconstituents such as flavonoids, phenolic compounds, and glycosides, which are known to improve insulin sensitivity, protect pancreatic β-cells from oxidative injury, and enhance glucose utilization. These findings demonstrate that the ethanolic extract effectively ameliorated hyperglycaemia in streptozotocin-induced diabetic rats and provided the basis for further evaluation of its antioxidant effects.

Figure 2. Effect of *Torilis leptophylla* on Blood Glucose Levels During the Treatment Period.



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

Table 2. Effect of *Torilis leptophylla* on Blood Glucose and HbA1c Levels in STZ-Induced Diabetic Rats.

Groups (*n* = 6)	Fasting Blood Glucose (mg/dL)				HbA1c (%) (Day 21)
	Day 0	Day 7	Day 14	Day 21	
Normal Control	96.25 ± 6.14	90.18 ± 5.21	84.32 ± 4.62	81.47 ± 4.13	4.62 ± 0.28
Diabetic Control	374.86 ± 15.32[a]	405.21 ± 16.48[a]	418.67 ± 17.21[a]	428.36 ± 18.14[a]	10.85 ± 0.52[a]
Metformin (100 mg/kg)	372.54 ± 14.85	260.38 ± 12.45[b]	179.64 ± 10.23[b]	132.18 ± 8.76[b]	6.15 ± 0.31[b]
T. leptophylla (100 mg/kg)	370.21 ± 13.92	316.25 ± 14.21[b]	249.37 ± 12.89[b]	198.34 ± 11.23[b]	7.82 ± 0.41[b]
T. leptophylla (200 mg/kg)	371.08 ± 15.11	276.38 ± 11.87[b]	191.62 ± 9.64[b]	142.36 ± 9.12[b]	6.48 ± 0.34[b]
T. leptophylla (400 mg/kg)	373.14 ± 14.03	224.57 ± 10.32[b]	147.28 ± 8.21[b]	110.42 ± 7.35[b]	5.21 ± 0.27[b]

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

[a] $p < 0.001$ vs. Normal Control (one-way ANOVA followed by Tukey's *post hoc* test)

[b] $p < 0.001$ vs. Diabetic Control (one-way ANOVA followed by Tukey's *post hoc* test)

3.4 Effect of *Torilis leptophylla* on Oxidative Stress Biomarkers

The antioxidant activity of the ethanolic extract of *Torilis leptophylla* was evaluated by estimating the levels of superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) in streptozotocin-induced diabetic rats. Induction of diabetes resulted in a marked disturbance of the endogenous antioxidant defence system, as evidenced by a significant decrease in SOD and GSH levels along with a substantial increase in MDA concentration in the diabetic control group compared with the normal control group. These findings confirmed the development of oxidative stress following streptozotocin administration.

Treatment with the ethanolic extract of *T. leptophylla* for 21 days significantly restored the antioxidant status of diabetic animals in a dose-dependent manner. Animals receiving the extract exhibited a progressive increase in SOD activity compared with the diabetic control group, indicating enhanced enzymatic antioxidant defence against reactive oxygen species. The highest dose (400 mg/kg) demonstrated the greatest improvement and produced antioxidant activity comparable to that observed with metformin-treated animals.

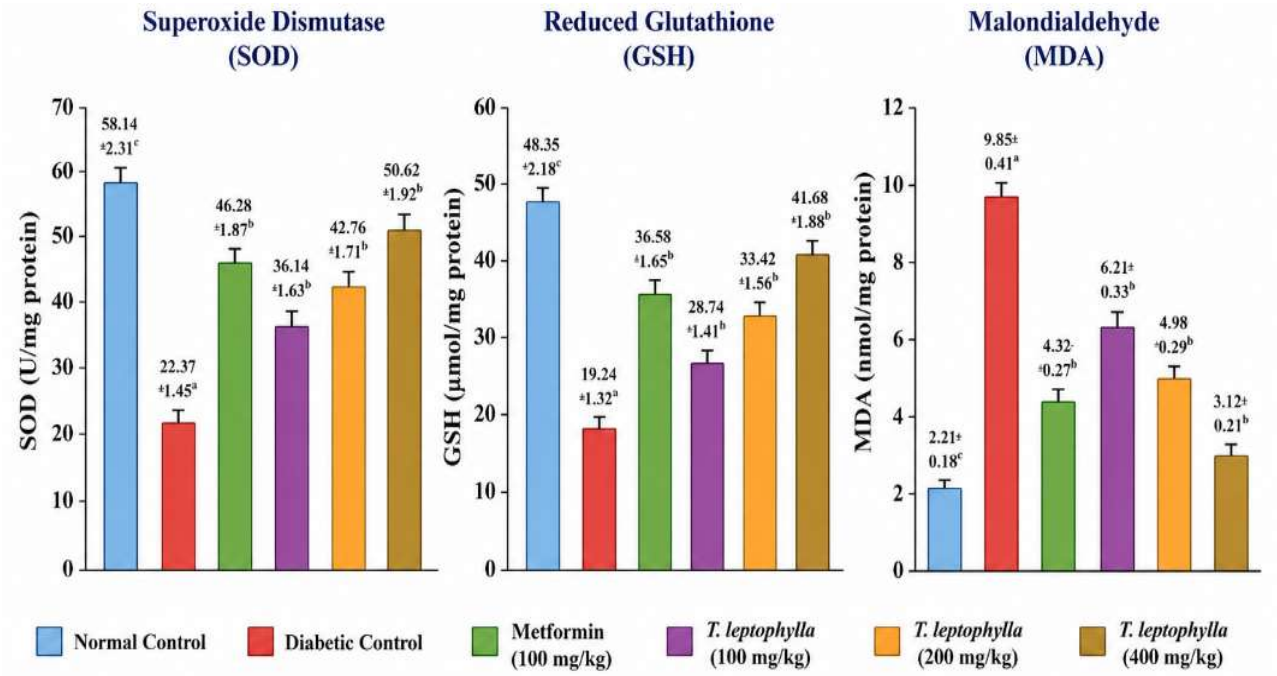
Similarly, reduced glutathione (GSH) levels were significantly elevated in extract-treated groups compared with diabetic controls. Restoration of intracellular GSH

suggested improved cellular redox balance and enhanced detoxification of reactive oxygen species generated during hyperglycaemic conditions. The increase in GSH was more pronounced in the medium- and high-dose treatment groups, demonstrating the dose-dependent antioxidant potential of the extract.

In contrast, malondialdehyde (MDA), a well-established marker of lipid peroxidation, was significantly elevated in diabetic control animals, indicating increased oxidative damage to cellular membranes. Administration of the ethanolic extract markedly reduced MDA levels in all treatment groups, with the maximum reduction observed at the highest dose. The decline in MDA concentration reflected inhibition of lipid peroxidation and effective protection against oxidative membrane damage.

The simultaneous enhancement of SOD and GSH together with suppression of MDA indicates that *T. leptophylla* effectively strengthened the endogenous antioxidant defence system while minimizing oxidative stress-induced cellular injury. These antioxidant effects are likely attributable to the presence of flavonoids, phenolic compounds, and other bioactive phytoconstituents identified during phytochemical screening. Collectively, these findings demonstrate that the ethanolic extract possesses significant antioxidant activity and contributes to the protection of pancreatic tissues against oxidative damage associated with experimental diabetes.

Figure 3. Effect of *Torilis leptophylla* on Oxidative Stress Biomarkers (SOD, GSH, and MDA) in STZ-Induced Diabetic Rats.



Values are expressed as mean ± SEM ($n = 6$).
[a] $p < 0.001$ vs. Normal Control; [b] $p < 0.001$ vs. Diabetic Control; [c] $p < 0.001$ vs. Diabetic Control.
One-way ANOVA followed by Tukey's post *hoc* test.

Table 3. Effect of *Torilis leptophylla* on Oxidative Stress Biomarkers in STZ-Induced Diabetic Rats.

Groups ($n = 6$)	SOD (U/mg protein)	GSH (μmol/mg protein)	MDA (nmol/mg protein)
Normal Control	58.14 ± 2.31[c]	48.35 ± 2.18[c]	2.21 ± 0.18[c]
Diabetic Control	22.37 ± 1.45[a]	19.24 ± 1.32[a]	9.85 ± 0.41[a]
Metformin (100 mg/kg)	46.28 ± 1.87[b]	36.58 ± 1.65[b]	4.32 ± 0.27[b]
***T. leptophylla* (100 mg/kg)**	36.14 ± 1.63[b]	26.74 ± 1.41[b]	6.21 ± 0.33[b]
***T. leptophylla* (200 mg/kg)**	42.76 ± 1.71[b]	33.42 ± 1.56[b]	4.98 ± 0.29[b]
***T. leptophylla* (400 mg/kg)**	50.62 ± 1.92[b]	41.68 ± 1.88[b]	3.12 ± 0.21[b]

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

[a] $p < 0.001$ vs. Normal Control (one-way ANOVA followed by Tukey's post *hoc* test)

[b] $p < 0.001$ vs. Diabetic Control (one-way ANOVA followed by Tukey's post *hoc* test)

[c] $p < 0.001$ vs. Diabetic Control (one-way ANOVA followed by Tukey's post *hoc* test)

3.5 Bioactivity-Guided Fractionation and Thin-Layer Chromatographic Analysis

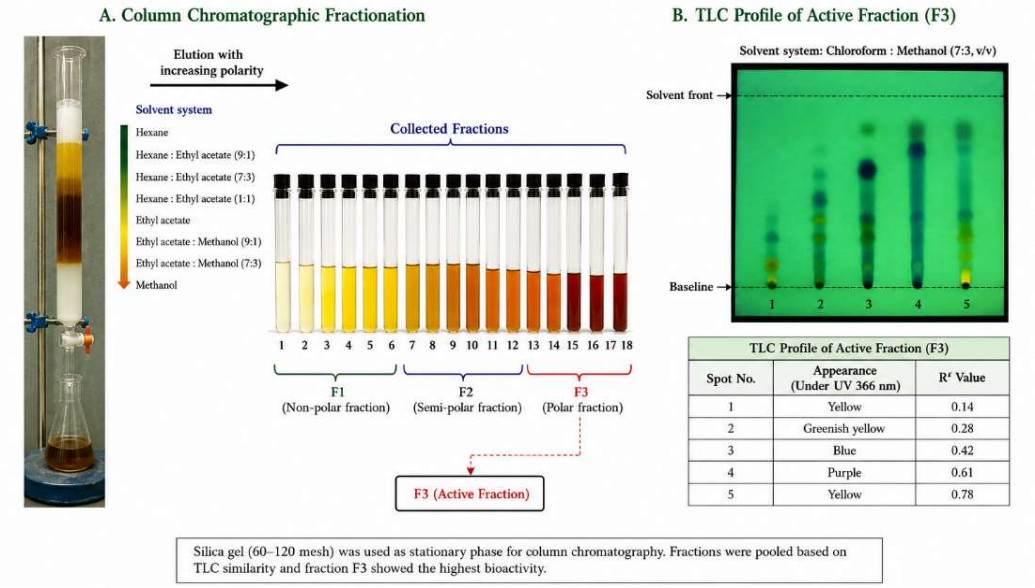
Based on the promising antioxidant and antihyperglycemic activities observed with the ethanolic extract of *Torilis leptophylla*, the extract was subjected to bioactivity-guided fractionation using silica gel column chromatography. Elution with solvent systems of increasing polarity yielded three major fractions (F1–F3). The fractions were monitored throughout the separation process, pooled according to their chromatographic similarities, and concentrated for further evaluation.

Among the isolated fractions, fraction F3 exhibited the highest biological activity and was therefore selected for chromatographic characterization. Thin-layer chromatography (TLC) of the active fraction produced well-resolved spots with distinct retention factor (Rf) values, indicating the presence of multiple phytochemical constituents. The chromatographic profile suggested that the active fraction was enriched with polar phytoconstituents, particularly flavonoids and phenolic compounds, which are well known for their free radical scavenging and antioxidant activities.

The enrichment of antioxidant phytochemicals in fraction F3 supports the biochemical findings obtained in the experimental study. The superior antioxidant activity observed in extract-treated animals may therefore be attributed to the synergistic action of these bioactive constituents, which likely contribute to the restoration of endogenous antioxidant defences and protection against oxidative stress-induced cellular damage.

The chromatographic findings further validate the selection of the ethanolic extract as the most pharmacologically active extract and provide a scientific basis for future isolation and structural characterization of individual bioactive compounds responsible for the observed antioxidant effects.

Figure 4. Column Chromatographic Fractionation and TLC Profile of the Active Fraction (F3) of *Torilis leptophylla*.



4. DISCUSSION

4.1 Antidiabetic Potential of *Torilis leptophylla*

The present study demonstrated that the ethanolic extract of *Torilis leptophylla* possesses significant antidiabetic activity in streptozotocin-induced diabetic rats. Administration of streptozotocin successfully induced persistent hyperglycaemia, as evidenced by elevated fasting blood glucose and HbA1c levels in the diabetic control group. These findings are consistent with the well-established diabetogenic action of streptozotocin, which selectively destroys pancreatic β-cells through oxidative stress-mediated mechanisms, resulting in insulin deficiency and impaired glucose homeostasis.

Oral treatment with the ethanolic extract of *T. leptophylla* produced a significant and dose-dependent reduction in fasting blood glucose levels throughout the experimental period. The high-dose treatment (400 mg/kg) showed the greatest improvement and exhibited an antihyperglycemic effect comparable to that of metformin. Furthermore, the significant reduction in HbA1c levels indicates sustained improvement in long-term glycemic control rather than a transient decrease in blood glucose concentration. Since HbA1c reflects the average blood glucose level over an extended period, its reduction suggests that the extract effectively maintained glucose homeostasis during the treatment period.

The observed antihyperglycemic activity may be attributed to the synergistic action of various phytoconstituents identified during preliminary phytochemical screening, particularly flavonoids, phenolic compounds, and glycosides. These bioactive compounds have been reported to improve insulin sensitivity, enhance peripheral glucose utilization, inhibit intestinal glucose absorption, and protect pancreatic β-cells from oxidative injury. Their combined pharmacological actions may account for the significant improvement in glycemic parameters observed in the present investigation.

The present findings are in agreement with previous experimental studies reporting the antidiabetic activity of medicinal plants rich in flavonoids and phenolic compounds. Several investigators have demonstrated that natural antioxidants improve glucose metabolism by preserving β-cell integrity and reducing oxidative stress-induced cellular damage. Similar observations have also been reported for *Torilis leptophylla*, where its phytochemical constituents exhibited notable antioxidant and pharmacological activities. Therefore, the significant reduction in blood glucose and HbA1c observed in this study supports the therapeutic potential of *T. leptophylla* as a promising natural source of antidiabetic bioactive compounds.

4.2 Modulation of Oxidative Stress Biomarkers by *Torilis leptophylla*

Oxidative stress plays a pivotal role in the progression of diabetes mellitus by promoting excessive production of reactive oxygen species (ROS), which damage cellular lipids, proteins, and DNA. In the present study, streptozotocin-induced diabetic rats exhibited significantly reduced levels of superoxide dismutase (SOD) and reduced glutathione (GSH), along with elevated malondialdehyde (MDA), confirming the presence of severe oxidative stress.

Treatment with the ethanolic extract of *Torilis leptophylla* significantly restored the antioxidant defence system in a dose-dependent manner. Increased SOD activity indicated improved enzymatic scavenging of free radicals, while elevated GSH levels suggested restoration of intracellular redox balance and enhanced protection against oxidative injury. At the same time, the significant reduction in MDA levels demonstrated inhibition of lipid peroxidation and reduced oxidative damage to cellular membranes.

The antioxidant activity of *T. leptophylla* can be attributed to its rich phytochemical composition, particularly flavonoids and phenolic compounds identified during preliminary phytochemical screening. These bioactive constituents are known to neutralize reactive oxygen species, enhance endogenous antioxidant enzyme activity, and protect pancreatic β-cells from oxidative damage. Such mechanisms may have contributed to the observed improvement in glycemic control and antioxidant status.

The present findings are consistent with previous studies reporting that flavonoid-rich medicinal plants effectively improve oxidative stress biomarkers in experimental diabetes. Therefore, restoration of SOD and GSH together with reduction of MDA demonstrates that *Torilis leptophylla* exerts a protective antioxidant effect, which is likely an important mechanism underlying its antidiabetic activity.

4.3 Phytochemical Contribution to the Antioxidant Activity

The significant antioxidant activity observed in the present study is likely associated with the diverse phytochemical constituents present in the ethanolic extract of *Torilis leptophylla*. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolic compounds, alkaloids, and glycosides, all of which have been extensively reported to possess strong antioxidant and free radical scavenging properties. These phytoconstituents may act synergistically to reduce oxidative stress by neutralizing reactive oxygen species, inhibiting lipid peroxidation, and enhancing endogenous antioxidant defences.

Bioactivity-guided fractionation further demonstrated that fraction F3 exhibited the greatest biological activity, suggesting enrichment of the active phytochemicals in this fraction. Thin-layer chromatographic analysis also revealed distinct phytochemical bands, supporting the presence of multiple bioactive compounds. The higher antioxidant potential of F3 may therefore be attributed to the concentration of polar constituents, particularly flavonoids and phenolic compounds, which are recognized for their ability to protect cells against oxidative damage.

These findings indicate that the antioxidant effects of *T. leptophylla* are closely related to its phytochemical composition. Further isolation and structural characterization of the active compounds present in fraction F3 may facilitate the identification of novel antioxidant molecules with potential therapeutic application in diabetes mellitus.

4.4 Study Limitations and Future Perspectives

The present study demonstrated the antioxidant potential of the ethanolic extract of *Torilis leptophylla* in streptozotocin-induced diabetic rats; however, certain limitations should be acknowledged. The investigation was limited to an experimental animal model and focused primarily on biochemical antioxidant markers. Although bioactivity-guided fractionation identified the active fraction (F3), the individual phytoconstituents responsible for the observed biological activity were not isolated or structurally characterized. In addition, the molecular mechanisms underlying the antioxidant action of the extract were not investigated.

Future studies should focus on the purification and characterization of the active compounds using advanced analytical techniques such as HPLC, LC-MS/MS, and NMR spectroscopy. Further investigations involving molecular docking, gene and protein expression analyses, and long-term toxicity studies are also required to elucidate the precise mechanisms of action. Moreover, well-designed preclinical and clinical studies are necessary to confirm the safety, efficacy, and therapeutic potential of *Torilis leptophylla* as a natural antioxidant agent for the management of diabetes mellitus and its associated complications.

5. CONCLUSION

The present study demonstrated that the ethanolic extract of *Torilis leptophylla* possesses significant antioxidant and antidiabetic activities in streptozotocin-induced diabetic rats. Treatment with the extract effectively reduced fasting blood glucose and HbA1c levels while significantly restoring the endogenous antioxidant defence system through increased superoxide dismutase (SOD) and reduced glutathione (GSH) levels, accompanied by a reduction in malondialdehyde (MDA), a key marker of lipid peroxidation. These findings indicate that the therapeutic effects of *T. leptophylla* are mediated not only through improved glycemic control but also through attenuation of oxidative stress.

Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, alkaloids, and glycosides, which are likely responsible for the observed biological activities. Furthermore, bioactivity-guided fractionation followed by thin-layer chromatographic analysis identified fraction F3 as the most active fraction, suggesting that it contains concentrated antioxidant phytoconstituents responsible for the protective effects observed in this study.

Overall, the findings provide scientific evidence supporting the traditional medicinal use of *Torilis leptophylla* and highlight its potential as a natural source of antioxidant compounds for the management of diabetes mellitus and its oxidative stress-mediated complications. Further studies involving isolation of active phytoconstituents, molecular mechanism studies, and clinical evaluation are warranted to validate its therapeutic potential and facilitate the development of novel plant-based antidiabetic formulations.

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