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Antidiabetic Effects of Ethanolic Extract of *Macrothelypteris torresiana*: *In Vitro* Enzyme Inhibition and *In Vivo* Evaluation in Streptozotocin-Induced Sprague-Dawley Rats

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Abstract

Background: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and associated long-term complications. Plant-based therapies possessing enzyme inhibitory and antioxidant properties have gained attention as complementary approaches for diabetes management. *Macrothelypteris torresiana* (*M. torresiana*) (Family: Thelypteridaceae) is a medicinal fern traditionally used in folklore medicine; however, its antidiabetic potential remains inadequately explored.

Methods: The present study evaluated the antidiabetic activity of the ethanolic extract of *M. torresiana* using both *in vitro* and *in vivo* approaches. *In vitro* assays included α-amylase and α-glucosidase inhibition, along with hydrogen peroxide (H_2O_2) radical scavenging activity. The extract exhibited IC_{50} values of 424.08 *µg/ml* (α-amylase), 238.30 *µg/ml* (α-glucosidase), and 662.16 *µg/ml* (H_2O_2 scavenging). *In vivo* studies were conducted in normal and streptozotocin-induced diabetic Sprague–Dawley rats treated with the extract for 28 days. Fasting blood glucose, serum insulin, glycated hemoglobin (HbA1c), lipid profile, and body weight were evaluated. One-way ANOVA and Dunnett's test were used as statistical analysis, and the findings were reported as mean ± SEM.

Results: The extract demonstrated dose-dependent inhibition of α-amylase and α-glucosidase enzymes and significant antioxidant activity *in vitro*. In diabetic rats, treatment significantly ($p<0.05$) reduced fasting blood glucose, HbA1c, total cholesterol, triglycerides, and LDL levels, while increasing serum insulin, HDL levels, and body weight compared to diabetic control. The observed effects were comparable to the standard drug Glibenclamide.

Conclusion: The findings of this study demonstrate that *M. torresiana* possesses significant antidiabetic activity, likely mediated through enzyme inhibition, antioxidant action, and improvement of metabolic disturbances. These results provide scientific support for the therapeutic potential of this fern as a natural candidate for the management of diabetes mellitus. However, further studies involving phytochemical standardization and molecular-level investigations are required to confirm its therapeutic potential.

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Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both, and is associated with long-term complications such as neuropathy, nephropathy, retinopathy, and cardiovascular diseases [1,2]. The increasing global prevalence of diabetes has created an urgent need for safer and more effective therapeutic strategies. One of the key approaches for managing postprandial hyperglycemia is the inhibition of carbohydrate-digesting enzymes, particularly α-amylase and α-glucosidase, which delay glucose absorption in the intestine [3-7]. However, currently available synthetic inhibitors such as acarbose are often associated with adverse gastroin-

testinal effects, prompting the search for alternative plant-based therapies.

Phytochemicals containing phenolic compounds and flavonoids are gaining much research interest for their various pharmacological actions, which include antioxidant and antidiabetic potentials. Phenolics play important roles in the treatment of diabetes through the inhibition of carbohydrate-metabolizing enzymes, stimulation of insulin production, increasing the sensitivity to insulin, and reducing oxidative stress. Flavonoids, a prominent class of phenolics, have been found to be capable of preventing oxidative damage to β cells of the pancreas and controlling critical metabolic pathways associated with blood sugar levels. In line with these findings, many plant-based materials loaded with phenolics have been found to inhibit α-amylase and α-glucosidase with high efficiency.

Ferns are among the neglected medicinal plants with bioactive properties. Recent investigations suggest that some species of ferns contain potent antioxidants, anti-inflammatories, and antidiabetics, which are primarily due to the presence of phenolic and flavonoid compounds. For example, the extracts of edible and medicinal ferns like *Diplazium esculentum* and Pteris species are known for their enzyme inhibition and hypoglycemic effects on experimental animals. *Macrothelypteris torresiana* (*M. torresiana*) (Gaud.) Ching (Family: Thelypteridaceae), commonly known as the marine maiden fern, is a perennial terrestrial fern widely distributed in tropical and subtropical regions [8,9]. It is traditionally used in folklore medicine for inflammatory conditions, gastrointestinal disorders, fever, and skin diseases [8,10,11]. Phytochemical investigations have revealed the presence of phenolic compounds, flavonoids, sterols, and glycosides in *M. torresiana*, indicating potential antioxidant and metabolic regulatory effects [10-12]. To the best of our knowledge, no comprehensive study has evaluated both the *in vitro* enzyme inhibitory and *in vivo* antidiabetic potential of *M. torresiana*, indicating a clear research gap.

Even though many ferns have been known to have medicinal properties, the medicinal potential of *M. torresiana* as an antidiabetic agent still needs to be investigated further. The lack of knowledge on this aspect has led to choosing this fern as our study subject.

Therefore, the present study investigates the antidiabetic potential of the ethanolic extract of *M. torresiana* using both *in vitro* and *in vivo* models. Specifically, the study evaluates α-amylase and α-glucosidase inhibitory activities and antioxidant potential, and further assesses efficacy in streptozotocin (STZ)-induced diabetic Sprague–Dawley rats. This study provides scientific validation for the traditional use of the plant and explores its potential as a natural therapeutic agent for diabetes management.

Materials and Methods

Collection of M. torresiana

Fresh leaves of *M. torresiana* (Gaud.) Ching were collected during the appropriate growing season from Vijayawada, Andhra Pradesh, India (16.474296° N, 80.710114° E), as confirmed using GPS map camera data. The plant material was authenticated by Dr. P. Satyanarayana Raju, Taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, India. A voucher specimen was deposited for future reference (Figure 1).

Preparation of ethanolic extract of M. torresiana

The collected leaves were shade-dried and pulverized into coarse powder. A weighed quantity (100 *g*) was subjected to Soxhlet extraction using ethanol (500 *ml*) for 6-8 *hr*. Ethanol was selected as the extraction solvent due to its efficiency in extracting a wide range of bioactive compounds, particularly phenolics and flavonoids, as reported in previous phytochemical studies. The extract was filtered and concentrated under reduced pressure to obtain a dried extract (Ethanolic Extract of *M. torresiana*). The dried extract was stored at 4°*C* in an airtight container to prevent degradation of thermolabile phytoconstituents and minimize oxidative changes until further use. The percentage yield of the ethanolic extract was calculated and found to be approximately 29.52% w/w.

Preliminary phytochemical screening

Preliminary phytochemical screening of Ethanolic Extract of *M. torresiana* (EEMT) was carried out using standard qualitative tests to identify major classes of phytoconstituents, including alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, proteins, and carbohydrates [10,13]. The results of phytochemical analysis are presented in the results section. The current study did not quantitatively estimate the total phenolic and flavonoid content. Fourier Transform Infrared Spectroscopy (FTIR) analysis, however, verified the existence of functional groups that match to flavonoids and phenolics. Future research will take into account additional quantitative and chromatographic analyses.

In vitro analysis of *M. torresiana for anti-diabetic activity*

EEMT was evaluated at concentrations ranging

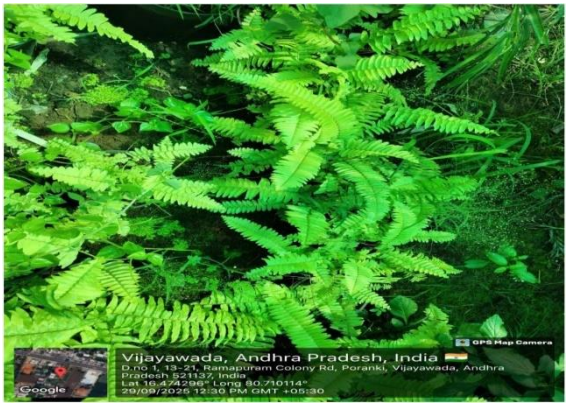

Figure 1. *Macrothelypteris torresiana*.

from 100 to 1000 *µg/ml* for all *in vitro* assays.

In vitro α-amylase inhibitory assay

α-Amylase inhibitory activity of the EEMT was evaluated using a spectrophotometric method [4,5]. The enzyme was incubated with various concentrations of the extract in phosphate buffer (pH=6.9) at 37°*C*, followed by the addition of soluble starch as the substrate. The reaction was terminated using Dinitro Salicylic Acid (DNS) reagent, and colour development was achieved by heating. After cooling, absorbance was measured at 540 *nm*. Acarbose was used as the reference standard. Percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

In vitro α-glucosidase inhibitory assay

The α-glucosidase inhibitory activity of EEMT was assessed using a spectrophotometric assay. The enzyme was incubated with varying concentrations of the extract in phosphate buffer (pH=6.8), followed by the addition of p-nitrophenyl-α-D-glucopyranoside as the substrate. The reaction was terminated using an alkaline solution, and the absorbance of the liberated p-nitrophenol was measured at 405 *nm*. Acarbose served as the standard inhibitor. Percentage inhibition was calculated as described above [3,6].

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Hydrogen peroxide (H_2O_2) radical scavenging assay

H_2O_2 radical scavenging activity of EEMT was evaluated by mixing different concentrations of the extract with H_2O_2 prepared in phosphate buffer (pH=7.4). The reaction mixture was incubated at room temperature, and absorbance was measured at 230 *nm* [14,15]. Ascorbic acid was used as the reference antioxidant. Scavenging activity was calculated using the following equation:

$$\% \text{ Scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Animals

For the experiment, adult male Sprague-Dawley rats weighing between 180 and 220 *g* were used. The animals were kept in a controlled environment with a 12-hour light-dark cycle, a temperature of 22±2°*C*, and a relative humidity of 55-65%. For the duration of the trial, drinking water and standard laboratory feed were freely available. The animals were given seven days to get used to the lab environment before the trial began. The experimental protocol was carried out in compliance with CCSEA rules and authorized by the Institutional Animal Ethics Committee (IAEC Approval No: 17/IAEC/CLPT/2024-25).

Acute toxicity study

Acute oral toxicity of EEMT was evaluated using the fixed dose method in accordance with OECD guideline No. 423 and CCSEA guidelines [16,17]. The extract was administered orally at graded dose levels, and the animals were observed continuously for the first 24 *hr* and daily for 14 days for any signs of toxicity, behavioural changes, or mortality. No mortality or significant adverse effects were observed up to the highest tested dose, indicating that the extract is relatively safe. Based on these findings, doses of 200 and 400 *mg/kg* body weight were selected for subsequent antidiabetic studies.

Evaluation of normoglycemic rats

Normoglycemic rats were divided into four groups (n=6). The control group received the vehicle, the standard group received Glibenclamide (5 *mg/kg*, P.O.), and the test groups received EEMT at doses of 200 and 400 *mg/kg*, respectively. Blood glucose levels were measured at specified time intervals using a glucometer to assess the effect of the extract on normal glycemic status.

Evaluation of anti-diabetic activity

Induction of diabetes: Diabetes was induced in overnight-fasted rats by a single intraperitoneal injection of STZ (55 *mg/kg*), freshly prepared in cold citrate buffer (0.1 *M*, pH=4.5) [18,19]. Fasting blood glucose levels were measured after 72 *hr*. Rats with fasting blood glucose levels > 240 *mg/dL* were considered diabetic.

Experimental design and treatment protocol

As shown below, the rats were split into five groups at random, each with six individuals (n=6). The animals were divided into experimental groups at random, although the current study did not use blinding.

Group I – Normal Control: Normal rats received the vehicle alone (distilled water, 10 *ml/kg* body weight, P.O.).

Group II – Diabetic Control: Group II – Diabetic Control: Diabetes was induced by a single Intraperitoneal (IP) injection of streptozotocin (55 *mg/kg*). The diabetic rats subsequently received distilled water (10 *ml/kg*, P.O.) as the vehicle throughout the experimental period.

Group III – Standard Drug Treated: Diabetic rats were treated orally with Glibenclamide (5 *mg/kg*/day, P.O.)

Group IV – Diabetic treated with EEMT: (200 *mg/kg*/day, P.O.)

Group V Diabetic rats treated with EEMT: (400 *mg/kg*/day, P.O.)

The treatment was continued for a period of 28 days. Fasting blood glucose levels and body weight of the animals were recorded on day 0, day 7, day 14, day 21, and 28th day of the study. Blood samples were taken on day 28 of the experiment in order to evaluate several parameters.

Effect of ethanolic extract on fasting blood glucose (mg/dL) in STZ-induced diabetic rats: The animals were randomly divided into five groups, each containing six rats (n=6). Group I served as the normal control and received the vehicle alone, while Group II served as the diabetic control and received the vehicle. Group III

was treated with the standard drug Glibenclamide (5 *mg/kg*, P.O.) [20,21]. Groups IV and V received the EEMT orally at doses of 200 and 400 *mg/kg* body weight, respectively. Diabetes was confirmed 72 *hr* after STZ administration by measuring fasting blood glucose levels [22,23]. Glucometer was used to measure the amount of glucose in blood samples drawn from the tail vein at 0, 1, 2, 3, and 4 *hr* [24]. Rats were classified as diabetics and enrolled in the study if their fasting blood glucose levels consistently exceeded 240 (*mg/dL*) [25].

Assessment of body weight and lipid profile

Body weight of all animals was measured at baseline and at weekly intervals throughout the study using a digital weighing balance. After 28 days of treatment, rats were fasted overnight, and blood samples were collected for biochemical analysis. Serum was separated by centrifugation and used for the estimation of lipid profile parameters, including total cholesterol, triglycerides, and high-density lipoproteins, using standard diagnostic kits [24,26,27].

Levels of Very Low-Density Lipoproteins (VLDL) were calculated using the formula VLDL=TG/5, and Low-Density Lipoproteins (LDL) were calculated using the Friedewald equation LDL=TC−(HDL+VLDL) [15]. These parameters were evaluated to assess the effect of the EEMT on diabetes-associated dyslipidemia.

Estimation of serum insulin

For the estimation of serum insulin, blood samples were collected and allowed to clot, followed by centrifugation to obtain serum. Serum insulin concentrations were determined using a commercially available ELISA-based immunoassay kit according to the manufacturer's instructions, and absorbance was measured using a spectrophotometric method [28].

Estimation of glycated hemoglobin

After completion of the 28-day experimental period, the rats were fasted overnight (12 *hr* fasting) and anaesthetized using an approved method. Blood samples were collected under light anaesthesia for the estimation of glycosylated hemoglobin (HbA1c). HbA1c levels were estimated using a standard immunoturbidimetric method according to the manufacturer's instructions [28].

Statistical analysis

All *in vitro* experiments were performed in triplicate (n=3), and results were expressed as mean±SEM. Percentage inhibition and scavenging activities were calculated, and IC_{50} values were determined from concentration–response curves by linear interpolation. All *in vivo* data were expressed as mean±SEM (n=6). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A value of $p<0.05$ was considered significant. GraphPad Prism software (version 10.0.6) was used for statistical analysis.

Results

All data are expressed as mean±SEM, and statistical significance was regarded at $p<0.05$.

Preliminary phytochemical screening

The qualitative phytochemical analysis of EEMT revealed the presence of carbohydrates, proteins, phenolics, flavonoids, steroids, and saponins, while alkaloids, cardiac glycosides, and triterpenoids were absent (Table 1).

Acute toxicity study

During the acute oral toxicity evaluation, the EEMT did not produce any mortality or observable signs of toxicity in experimental animals up to the highest tested dose. The extract was found to be safe at the tested dose level, indicating a wide margin of safety. Based on these observations, 200 *mg/kg* body weight ($1/10^{th}$) and 400 *mg/kg* body weight (approximately $1/5^{th}$) of the highest safe dose were selected as the low and high doses, respectively, for subsequent *in vivo* antidiabetic studies.

α-Amylase inhibitory activity M. torresiana (EEMT)

The EEMT produced a concentration-dependent inhibition of α-amylase activity over the range of 100-1000 *µg/ml*. The percentage inhibition increased from 28.26±0.28% at 100 *µg/ml* to 74.53±0.33% at 1000 *µg/ml*, with an IC_{50} value of 424.08 *µg/ml*, indicating moderate inhibitory activity. The standard drug acarbose showed comparatively higher inhibition, producing 37.29±0.42% inhibition at 100 *µg/ml* and 86.79± 0.31% inhibition at 1000 *µg/ml*, with an IC_{50} value of 280.33 *µg/ml*. Although the extract was less potent than acarbose, the marked inhibition of α-amylase suggests its potential contribution to antidiabetic activity. The decrease in absorbance with increasing extract concentration reflects reduced enzyme activity, resulting in higher percentage inhibition (Table 2 and Figure 3).

α-glucosidase inhibition by M. torresiana (EEMT)

The EEMT showed a concentration-dependent inhibition of α-glucosidase activity between 100 and 1000

Table 1. Qualitative phytochemical screening of ethanolic extract of *M. torresiana (EEMT)*

SI. No.	Phytoconstituents	Inference
1	Alkaloids	–
2	Carbohydrates	+
3	Cardiac glycosides	–
4	Proteins and amino acids	+
5	Tannins and phenolics	+
6	Steroids and sterols	+
7	Triterpenoids	–
8	Saponins	+
9	Flavonoids	+

Figure 2. Preparation of ethanolic extract of *M. torresiana*.

Table 2. α-Amylase Inhibition by *M. torresiana (EEMT)*

Sample	Concentration (*µg/ml*)	% Inhibition	IC $_{50}$ *µg/ml*
M. torresiana (Test)	100	28.26±0.28	424.08 *µg/ml*
	200	36.14±0.11	
	400	49.27±0.23	
	800	61.34±0.34	
	1000	74.53±0.33	
Acarbose (standard)	100	37.29±0.42	280.33 *µg/ml*
	200	44.41±0.35	
	400	58.32±0.32	
	800	71.12±0.19	
	1000	86.79±0.31	

Values are expressed as mean±SEM (n=3). IC$_{50}$ values were calculated by linear interpolation from the concentration–response curve.

µg/ml. The inhibitory effect increased from 38.57± 0.62% at 100 *µg/ml* to 91.17±0.38% at 1000 *µg/ml*, with an IC$_{50}$ value of 238.30 *µg/ml*, indicating strong α-glucosidase inhibitory potential. The standard drug acarbose exhibited higher inhibition, showing 44.09± 0.73% inhibition at 100 *µg/ml* and 96.07±0.88% at 1000 *µg/ml*, with an IC$_{50}$ value of 145.01 *µg/ml*. Although the extract was less potent than acarbose, the marked inhibition of α-glucosidase suggests its potential role in reducing intestinal glucose breakdown and absorption (Table 3 and Figure 4).

H_2O_2 radical scavenging activity of M. torresiana (EEMT)

The EEMT demonstrated a concentration-dependent H_2O_2 radical scavenging activity over the range of 100-1000 *µg/ml*. The scavenging effect increased from 23.10±0.29% at 100 *µg/ml* to 67.03±0.27% at 1000 *µg/ml*, with an IC$_{50}$ value of 662.16 *µg/ml*, indicating moderate antioxidant activity. The standard antioxidant ascorbic acid exhibited stronger scavenging activity, showing 47.79±0.41% inhibition at 100 *µg/ml* and 92.03±0.21% at 1000 *µg/ml*, with an IC$_{50}$ value of 121.01 *µg/ml*. Although less potent than ascorbic acid, the observed radical scavenging activity supports the antioxidant potential of *M. torresiana*, which may contribute to its antidiabetic effects (Table 4 and Figure 5).

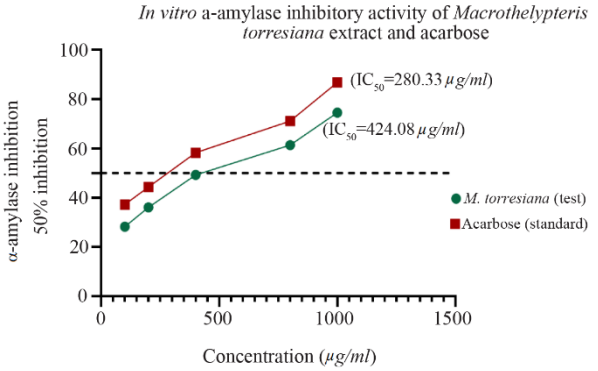

Figure 3. *In vitro* α-amylase inhibitory activity of *M. torresiana* (EEMT). IC$_{50}$ values were calculated by linear interpolation from the concentration–response curve.
Values are expressed as mean±SEM (n=3). IC$_{50}$ values were calculated by linear interpolation from the concentration–response curve.

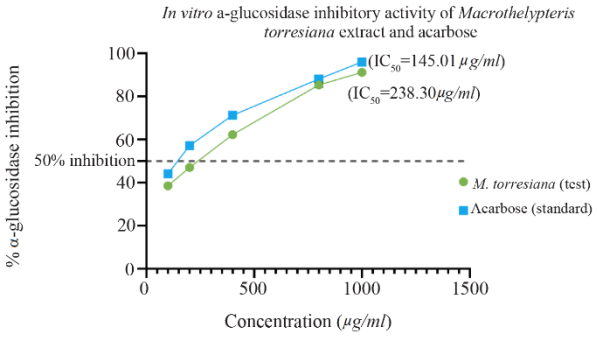

Figure 4. *In vitro* α-glucosidase inhibitory activity of *M. torresiana* (EEMT). IC$_{50}$ values were calculated by linear interpolation from the concentration–response curve.
Values are expressed as mean±SEM (n=3). IC$_{50}$ values were calculated by linear interpolation from the concentration–response curve.

Table 3. α-glucosidase inhibition by *M. torresiana (EEMT)*

Sample	Concentration (*µg/ml*)	% Inhibition	IC $_{50}$ *µg/ml*
M. torresiana (test)	100	38.57±0.62	238.30 *µg/ml*
	200	47.08±0.48	
	400	62.33±0.53	
	800	85.39±1.08	
	1000	91.17±0.38	
Acarbose (standard)	100	44.09±0.73	145.01 *µg/ml*
	200	57.22±1.14	
	400	71.31±0.91	
	800	88.16±1.05	
	1000	96.07±0.88	

Values are expressed as mean±SEM (n=3). IC$_{50}$ values were calculated by linear interpolation from the concentration–response curve.

In vivo studies

Effect of EEMT extract in normoglycemic rats: The results of the study demonstrated that treatment with a single dosage of the ethanolic extract of *M. torresiana* did not generate any significant change in fasting blood glucose (*mg/dL*) levels in normoglycemic rats (Table 5).

Effect of EEMT on fasting blood glucose (mg/dL) levels in STZ-induced diabetic rats: The results of the investiga

Table 4. H_2O_2 Radical Scavenging Activity of *M. torresiana (EEMT)*

Sample	Concentration (*µg/ml*)	% Inhibition	IC $_{50}$ *µg/ml*
M. torresiana (test)	100	23.10±0.29	662.16 *µg/ml*
	200	36.13±0.09	
	400	42.05±0.16	
	800	54.18±0.31	
	1000	67.03±0.27	
Ascorbic acid (standard)	100	47.79±0.41	121.01 *µg/ml*
	200	58.31±0.11	
	400	69.72±0.27	
	800	84.42±0.07	
	1000	92.03±0.21	

Values are expressed as mean±SEM (n=3). IC_{50} values were calculated by linear interpolation from the concentration–response curve.

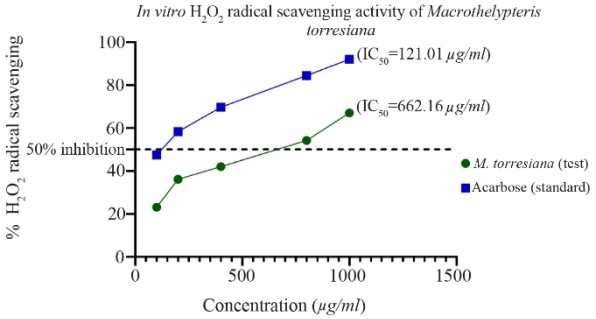

Figure 5. *In vitro* hydrogen peroxide (H_2O_2) radical scavenging activity of *M. torresiana* (EEMT). IC_{50} values were calculated by linear interpolation from the concentration–response curve.
Values are expressed as mean±SEM (n=3). IC_{50} values were calculated by linear interpolation from the concentration–response curve.

tion indicated that the EEMT caused a considerable hypoglycemic impact in STZ-induced diabetic rats, but no noteworthy changes were found in normoglycemic animals. Blood glucose concentrations measured at the end of 28 days of administration of EEMT at low (200 *mg/kg*) and high (400 *mg/kg*) doses were significantly reduced ($p<0.01$) relative to the control group of diabetic rats. The effect on blood glucose by EEMT was similar to that of Glibenclamide (5 *mg/kg*), an antidiabetic drug, showing the antidiabetic activity of *M. torresiana* ($p<0.05$) (Table 6 and Figure 6).

Changes in body weight: On completion of 28 days of treatment, a marked rise in body weight was noted in the normal control, ethanolic extract treatment (EEMT), and drug treatment groups, while a decline in body weight was evident among diabetic control animals. These variations were statistically significant ($p< 0.05$) (Table 7 and Figure 7).

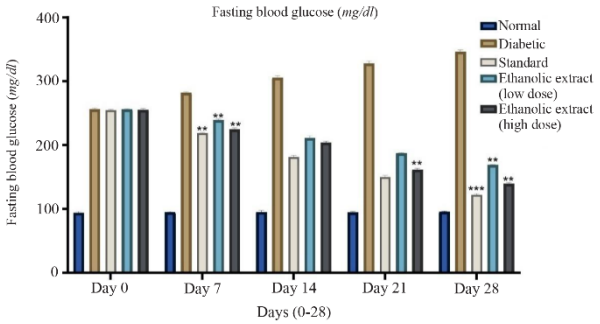

Figure 6. Effect of ethanolic extract of *M. torresiana* (EEMT) on fasting blood glucose levels in STZ-induced diabetic rats.
After 28 days of treatment, fasting blood glucose levels were significantly reduced in the EEMT-treated groups compared with the diabetic control group. The low-dose EEMT group showed a significant reduction (mean difference=90.97 *mg/dL*, 95%CI: 25.75-156.2, $p=0.0050$), while the high-dose EEMT group showed a greater reduction (mean difference=106.5 *mg/dL*, 95%CI: 41.30-171.7, $p=0.0012$). The standard drug Glibenclamide also significantly reduced blood glucose levels (mean difference = 118.2 *mg/dL*, 95%CI: 53.02-183.5, $p=0.0004$).
Values are expressed as mean±SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control group.

Changes in serum insulin and glycosylated hemoglobin (HbA1c): After 28 days of treatment, blood samples were collected and serum insulin and glycosylated hemoglobin (HbA1c) levels were estimated and compared among the normal control, diabetic control, standard drug–treated, and extract-treated groups. Serum insulin levels showed a significant increase, while HbA1c levels were significantly reduced in extract-treated groups compared to the diabetic control. The observed changes were statistically significant ($p<0.05$) (Table 8).

Lipid profile: The lipid profile of animals treated with the EEMT showed significant improvement compared with streptozotocin-induced diabetic control rats (Table 9, Figure 8). Treatment with EEMT resulted in a significant reduction ($p<0.01$) in total cholesterol (14.89 and 23.49%), LDL (15.44 and 26.69%), VLDL (15.77 and 27.15%), and triglycerides (17.11 and 32.22%) following administration of low (200 *mg/kg*) and high (400 *mg/kg*) doses, respectively. Additionally, HDL levels were significantly increased ($p<0.05$) by 23.84 and 39.21% in the extract-treated diabetic rats. The observed changes in lipid parameters were statistically significant. In contrast, untreated diabetic rats showed elevated levels of total cholesterol, LDL,

Table 5. Effect of ethanolic extract of *M. torresiana (EEMT)* in normoglycemic rats

Group treatment (n=6)	0 *hr*	1 *hr*	2 *hr*	3 *hr*	4 *hr*
Normal control	95.20±0.48	94.61±0.79	93.96±1.05	92.87± 1.17	92.18±0.69
Glibenclamide	95.17±0.90	92.28±0.46	89.26±0.71*	87.62±0.82*	85.4±0.93**
Ethanolic extract (low dose)	95.44±1.08	94.39±0.70	93.19±0.57	86.09±0.26	90.80±0.53*
Ethanolic extract (high dose)	95.79±0.92	93.68±0.98	91.88±0.71	88.18±0.87	88.92±0.42*

*p<0.05, **p<0.01, ***p<0.001, ***p<0.0001. Values are expressed as mean±SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test, with diabetic control as the reference group.

Table 6. Effect of ethanolic extract of *M. torresiana* (EEMT) on fasting blood glucose levels (*mg/dL*) in STZ-induced diabetic rats

Group treatment (n=6)	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control	93.80±2.04	94.66±1.20	95.10±2.32	94.29±1.80	95.31±1.02****
Diabetic-Control (STZ)	256.04±2.21	281.74±1.96	305.36±3.22	327.69±4.01	346.26± 2.83
Glibenclamide (5 *mg/kg*)	254.69±1.08	218.39±1.55**	181.26±2.39	149.71±2.79	121.83±2.01***
Ethanolic extract (low dose)	255.87±1.19	239.18±1.58**	211.04±3.11	187.23±0.68	168.90±0.56**
Ethanolic extract (high dose)	255.12±2.23	224.61±2.40**	203.82±2.07	161.5±2.05**	139.42±2.02**

*p<0.05, **p<0.01, ***p<0.001, ***p<0.0001. Values are expressed as mean±SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test, with diabetic control as the reference group.

Table 7. Changes in body weight (*g*) at (days)

Group treatment (n=6)	Day 0	Day 14	Day 28
Normal control	211.67±1.03	214.8±1.06	229.7±1.9***
Diabetic control	182.46±1.64	182.1±1.80	164.6±2.0
Glibenclamide	208.92±1.25**	209.6±1.07**	221.8±1.8**
Ethanolic extract (low dose)	189.12±0.06**	201.4±0.89**	209.3±2.1*
Ethanolic extract (high dose)	203.74±1.33**	206.9±2.78**	216.7±1.9**

*p<0.05, **p<0.01. Values are expressed as mean±SEM (n=6). Statistical comparison was performed against the diabetic control group using one-way ANOVA followed by Dunnett's multiple comparison test.

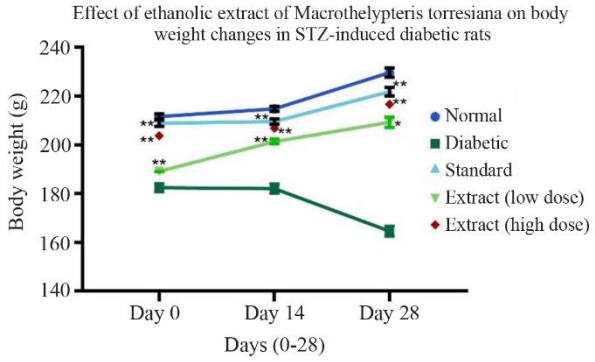

Figure 7. Effect of ethanolic extract of *M. torresiana* (EEMT) on body weight changes in STZ-induced diabetic rats.
After 28 days of treatment, body weight was significantly improved in the EEMT-treated groups compared with the diabetic control group. The low-dose EEMT group showed a significant improvement (mean difference=-23.55 *g*, 95%CI: -44.63 to -2.48, p=0.0287), while the high-dose EEMT group demonstrated a greater improvement (mean difference=-32.73 *g*, 95%CI: -53.80 to -11.65, p=0.0039). The standard Glibenclamide-treated group also showed a significant improvement in body weight (mean difference=-37.05 *g*, 95%CI: -58.13 to -15.98, p=0.0016).
Values are expressed as mean±SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control group.

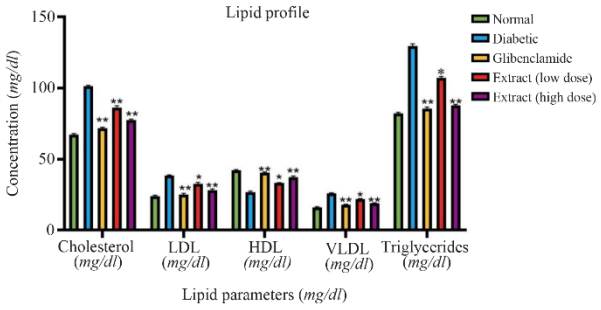

Figure 8. Effect of ethanolic extract of *M. torresiana* (EEMT) on lipid profile in STZ-induced diabetic rats.
Values are expressed as mean±SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control group.

VLDL, and triglycerides along with a marked reduction in HDL levels.

Discussion

The current study was carried out to examine the possible antidiabetic properties of the EEMT *via in vitro* and *in vivo* experiments. Acute toxicity testing showed that the extract was non-toxic at these dose levels, suggesting high safety, which is in line with other studies conducted on plant extracts for antidiabetic purposes [11,18].

Phytochemical analysis performed in the current study indicated that EEMT contained phenolics, flavonoids, saponins, and steroids, all of which can make an important contribution to the antidiabetic properties of the extract. The beneficial effects of phenolics have been widely studied, with these compounds being known to exhibit antihyperglycemic activity through several mechanisms, such as inhibition of carbohydrate digestion enzymes, promotion of insulin release, and sensitization of tissues to insulin [7,18]. Flavonoids, in turn, are capable of protecting pancreatic beta cells from oxidative stress and affecting metabolic processes related to glucose [13,29].

The *in vitro* studies demonstrated that EEMT exhibited concentration-dependent inhibition of α-amylase

Table 8. Effect of ethanolic extract of *M. torresiana (EEMT)* on serum parameters after 28 days

Group treatment (n=6)	Serum-Insulin (*µIU/ml*)	Glycosylated-Hemoglobin (%*HbA1c)*
Normal control	14.9±1.18	6.17±0.28
Diabetic control	4.20±0.74	11.38±0.90
Glibenclamide	13.4±0.21**	8.29±0.97**
Ethanolic extract (low dose)	8.96±0.19**	7.27±0.47**
Ethanolic extract (high dose)	11.21±0.17**	6.93±0.41**

*p<0.05, **p<0.01. Values are expressed as mean±SEM (n=6). Statistical comparison was performed against the diabetic control group using one-way ANOVA followed by Dunnett's multiple comparison test.

Table 9. Effect of ethanolic extract of *M. torresiana (EEMT)* on serum lipid profile

Group	Cholesterol (*mg/dL*)	LDL (*mg/dL*)	HDL (*mg/dL*)	VLDL (*mg/dL*)	Triglycerides (*mg/dL*)
Normal control	67.18±0.77	23.89±0.7	42.17±0.46	16.12±0.44	82.15±0.78
Diabetic control (STZ)	101.33±0.53	38.39±0.49	26.80±0.69	25.93±0.36	129.37±1.74
Glibenclamide	71.69±0.71**	25.02±0.93**	40.27±0.73**	17.81±0.45**	85.46±1.16**
Ethanolic extract (low dose)	86.24±1.30*	32.46±1.14*	33.19±0.26*	21.84±0.43*	107.23±0.87*
Ethanolic extract (high dose)	77.52±0.60**	28.14±0.96**	37.31±0.76**	18.89±0.34**	87.68±0.82**

*p<0.05, **p<0.01. Values are expressed as mean±SEM (n=6). Statistical comparison was performed against the diabetic control group using one-way ANOVA followed by Dunnett's multiple comparison test.
Low-Density Lipoprotein; High-Density Lipoprotein (HDL); Very Low-Density Lipoproteins (VLDL).

and α-glucosidase enzymes. These findings are consistent with previous studies demonstrating that plant-derived phenolic compounds can effectively inhibit these enzymes, thereby reducing postprandial hyperglycemia [3-6]. The stronger inhibition of α-glucosidase compared to α-amylase observed in this study is particularly beneficial, as it may reduce gastrointestinal side effects commonly associated with strong α-amylase inhibition. Additionally, the observed hydrogen peroxide radical scavenging activity indicates the antioxidant potential of the extract, which is important since oxidative stress is a key factor in the progression of diabetes and its complications [14,15].

In the *in vivo* model, STZ-induced diabetes resulted in significant hyperglycemia, weight loss, and dyslipidemia, which are characteristic features of diabetic pathology. Treatment with EEMT significantly reduced fasting blood glucose levels, indicating improved glycemic control. This effect may be attributed to enhanced insulin secretion or improved peripheral glucose utilization, as supported by the observed increase in serum insulin levels and reduction in HbA1c levels. Similar antihyperglycemic effects have been reported for other plant extracts rich in phenolics and flavonoids [20,21].

Furthermore, EEMT treatment significantly improved lipid profile parameters by reducing total cholesterol, triglycerides, LDL, and VLDL levels, while increasing HDL levels. These findings are consistent with previous studies demonstrating the hypolipidemic effects of plant-derived bioactive compounds in diabetic models [8,30].

Despite the promising findings, the present study has certain limitations. The ethanolic extract was not subjected to quantitative phytochemical standardization, and Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were not determined. Furthermore, chromatographic profiling techniques such as High Performance Thin-Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS), or Gas Chromatography/Mass Spectrometry (GC-MS) were not performed to identify and characterize the bioactive constituents responsible for the observed antidiabetic activity. Although qualitative phytochemical screening confirmed the presence of phenolics and flavonoids, the exact active compounds remain unidentified. In addition, molecular and mechanistic investigations, including the evaluation of oxidative stress biomarkers, insulin signaling pathways, pancreatic histopathology, and glucose transporter-related mechanisms, were not conducted. The study also did not employ blinding during experimental assessment, which may introduce observer bias. Therefore, further studies involving phytochemical standardization, compound isolation, mechanistic validation, and clinical investigations are required.

Overall, the antidiabetic effects of EEMT observed in this study may be attributed to a combination of enzyme inhibition, antioxidant activity, and modulation of metabolic pathways, likely mediated by its phenolic and flavonoid content. Although the extract exhibited slightly lower potency compared to the standard drug Glibenclamide, its multi-targeted mechanism and natural origin highlight its potential as a safer alternative or complementary therapy for diabetes management.

Conclusion

In conclusion, it can be stated that the EEMT exhibits beneficial effects in controlling hyperglycemia through inhibition of carbohydrate-digesting enzymes and antioxidant activity *in vitro*, and by improving blood glucose levels and lipid profile in STZ-induced diabetic rats *in vivo*, while showing no significant ef-

fect in normoglycemic rats. Thus, the present findings scientifically support the antidiabetic potential of this underexplored fern. However, detailed phytochemical characterization, chromatographic standardization, and mechanistic studies are required before clinical translation of *M. torresiana* as an antidiabetic agent. These findings provide a basis for further pharmacological and clinical investigations.

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Conflict of Interest

Authors declare no conflict of interests.

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