RESEARCH PAPER

Evaluation of Antihyperglycemic Activity of Madhuca Longifolia Koenig Root Bark Extract

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ABSTRACT

The study aimed to evaluate the antihyperglycemic activity of methanolic extract of Madhuca longifolia Koenig in Streptozotocin (STZ)-induced diabetic rats at doses of 100, 200, and 400 mg/kg body weight. The Glucose oxidase–peroxidase (GOD–POD) method was employed to estimate blood glucose level. Treatment with the various concentration of extract showed a dose-dependent reduction in blood glucose levels. A dose dependent antihyperglycemic effect was observed with M. longifolia Koenig root bark extract. A mild but significant antihyperglycemic activity at the 8th hour was observed with dose.100 mg/kg b.w. A moderate antihyperglycemic effect with significant reduction of blood glucose levels at 8th, 12th, and 18th hours was observed with dose 200 mg/kg b.w. A rapid onset of action started from the 2nd hour and a pronounced, continuous effect up to 18 hours with 400 mg/kg b.w. Phenolic compounds, saponins, tannins, carbohydrates and glycosides were found through phytochemical screening while bioactive compounds such as triterpenoids and flavonoids were not prominently detected in the root bark extract of M.longifolia Koenig. These findings suggest that the root bark of M. longifolia Koenig possesses considerable potential, even in the absence of triterpenoids and flavonoids.

Keywords: *M. longifolia, Streptozotocin, antihyperglycemic, root bark extract, Glucose oxidase–peroxidase*

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by increased blood glucose level, caused by insufficient insulin production or ineffective insulin use. Hyperglycaemia is a condition where blood glucose levels are elevated, which is a common consequence of uncontrolled diabetes and, over time, leads to serious damage to many body systems, especially the nerves and blood vessels. Furthermore, it can cause severe complications like cardiovascular diseases, retinopathy, and renal failure [1]. It imposes a substantial economic burden on healthcare systems and affects the overall development of nations. According to International Diabetes Federation India has the second highest number of adults (20-79 years) with diabetes in the world [2]. Therefore, the discovery of safe, effective, and affordable antihyperglycemic agents remains a major research priority.

In present years, herbal medicines have gained a lot of attention for the treatment of diabetes due to their natural origin, low side effects, and affordability. A wide range of plant families such as, Leguminosae, Liliaceae, Lamiaceae, Cucurbitaceae, Asteraceae,Araliaceae, Moraceae, Rosaceae, and Euphorbiaceae have been reported antidiabetic properties [3].

The antihyperglycemic activity of medicinal plants is mediated by various mechanisms, such as by stimulating pancreatic β-cells, increasing insulin secretion, regenerating β-cells, increasing insulin sensitivity, and insulin-mimetic actions [4]. Many plant-derived chemical constituents have shown antidiabetic effects comparable to those of popular drugs like glibenclamide and tolbutamide. Though, many phytoconstituents remain poorly characterized, thereby necessitates further scientific validation.

Madhuca longifolia Koenig usually known as Mahua, belongs to family: *Sapotaceae*, is widely employed in traditional medicine for a range of therapeutic purposessuch as general weakness cough, bronchitis, and throat infections. Previous studies have reported that Chemical constituents of different parts such as Quercetin Myricetin, Flavan-3-ols possessed antioxidant and antihyperglycemic activities [5]. However, limited scientific data are available on the root bark. Therefore, the present study was undertaken to evaluate the antihyperglycemic potential of the root bark of *M. longifolia* Koenig.

MATERIALS AND METHODS

Plant Material

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The root bark of *Madhuca longifolia* Koenig was collected from the regions in and around forest areas of Maredimilli, East Godavari District, A.P. (India). The plant was authenticated and certified by Dr. M. Venkaiah, Associate Professor, Department of Botany, Andhra University, and Visakhapatnam. The voucher (TSN/DOP/ML/IND/2476) for the plants was deposited in the Department of Pharmacognosy and Phytochemistry, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

Preparation of root bark Extract

The root bark of *Madhuca longifolia* Koenig selected for the study was coarsely powdered using a mechanical grinder, passed through a 60–80 mesh sieve, and stored in airtight containers until further use. The powdered materials were then subjected to Soxhlet extraction using methanol as the solvent for 72 hours. The obtained extracts were subsequently concentrated under reduced pressure using a rotary evaporator, followed by complete drying.

Experimental Animals

Healthy adult Wistar albino rats of either sex weighing about 150–200 g were purchased from Mahaveer Enterprises Pvt. Ltd., Hyderabad, India. The animals were maintained under standard laboratory conditions (temperature 25 ± 2°C; relative humidity 50 ± 15%; 12 h light/dark cycle) in animal house of department of Pharmacology AU College of Pharmaceutical Sciences, Andhra University. They were housed in polypropylene cages and fed with standard pellet diet and water.

Phytochemical Screening:

The methanolic extract of *Madhuca longifolia* Koenig was subjected to Phytochemical Screening according to the procedures of Inkoto et al., 2018**[6].**

Chemicals and Drugs

All chemicals used in the study were of analytical grade. Streptozotocin (STZ) (Sigma-Aldrich), Glibenclamide, a standard antidiabetic drug, was obtained from Orchid Pharmaceuticals Pvt. Ltd. Blood glucose levels were estimated using a commercially available glucose estimation kit supplied by Span Diagnostics Ltd.

Estimation of Blood Glucose

Blood glucose levels were measured using an auto analyser (Screen Master 3000) and a glucometer. A cooling centrifuge (Remi) was used for serum separation. The estimation of blood glucose was carried out by the glucose oxidase–peroxidase (GOD–POD) method **[7]**. based on Trinder's reaction, in which glucose is oxidized to gluconic acid and hydrogen peroxide. The formed hydrogen peroxide was then reacted with chromogenic agents to produce a coloured compound, and the intensity of the produced colour is directly proportional to the concentration of glucose present in the sample.

Acute Toxicity Studies

Acute toxicity studies were carried out according to OECD guidelines **[8]**. The methanolic extract of *Madhuca longifolia* Koenig root bark at a dose of 2000 mg/kg body weight was orally administered to rats.

Induction of Diabetes

Experimental diabetes was induced in overnight-fasted rats by a single intraperitoneal injection of streptozotocin (60 mg/kg body weight) dissolved in freshly prepared citrate buffer (pH 4.5) **[9]**. After 72 hours, blood glucose levels were measured, and rats with glucose levels between 185–460 mg/dL were considered diabetic and included in the study.

Evaluation of Extracts for Antihyperglycemic Activity and Statistical Analysis

The methanolic root bark of *Madhuca longifolia* Koenig extracts of was evaluated for antihyperglycemic activity in Streptozotocin (STZ)-induced diabetic rats at doses of 100, 200, and 400 mg/kg body weight. Animals were fasted for 12 hours prior to drug administration. The same treatment procedure was followed for both the vehicle (control group) and the standard drug (Glibenclamide 0.45 mg/kg b.w.). Blood samples were collected from the retro-orbital plexus, and serum was separated. Blood glucose levels were measured at 0, 2, 4, 8, 12, 18, and 24 hours, and the percentage reduction in glucose levels was calculated for each group. The results were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Student's *t*-test to assess the significance of differences between groups.

The study animal rats were divided into of five rats (*n* = 6) as follows:

Group 1: Vehicle control (1% CMC suspension)

Group 2: Received extract of *M. longifolia* (100 mg/kg)

Group 3: Received extract of *M. longifolia* (200 mg/kg)

Group 4: Received extract of *M. longifolia* (400 mg/kg)

Group 5: Standard (Glibenclamide 0.45mg/kg)

RESULTS

Phytochemical Screening:

Phytochemical Screening revealed the presence of saponins, phenolic compounds, tannins, carbohydrates and glycosides in the *Madhuca longifolia* Koenig methanolic extract. The Liebermann–Burchard test was negative, indicating the absence of steroidal and triterpenoid compounds. Flavonoids were also absent (Table 1).

Table 1. Qualitative Chemical tests on extracts of *M.longifolia*

NAME OF THE TEST	Methanolic extract of *M.longifolia*
i) Steroids: a. Salkowski Test: b. Liebermann Buchard's Test	Negative
ii)Triterpenes:	Negative

a) Salkowski Test: b) Liebermann Buchard's Test: c) Ischugajju Test: d) Brickorn and Brinar Test:	
iii) Saponins: a) Foam Test: b) Haemolysis Test:	Positive
iv) Alkaloids: a. Mayer's Test: b. Dragendroff's Test: c. Wagner's Test: d. Hager's Test:	Negative
v) Carbohydrates: a. Molisch's Test: b. Fehling's Test: c. Benedict's Test: d. Barfoed's Test	Positive
vi) Flavonoids: a. Shinoda Test: b. b) Ferric chloride Test: c. Lead Acetate Test: d. Zinc- HCl reduction test:	Negative
vii) Tannins: a. Ferric chloride Test: b. Gelatin Test:	Positive
viii) Glycosides: a. Borntrager's Test b. Baljet Test: c. Legal Test: **d.** Keller-Killiani Test:	Positive

Acute Toxicity test

Then the animals were observed for mortality and behavioural changes for 7 days. No signs of toxicity or mortality were observed, which indicating the safety of the extract at the tested dose.

Antihyperglycemic Activity of *Madhuca longifolia* Root Bark Extract

The methanolic extract of *Madhuca longifolia root bark* was tested for antihyperglycemic effects in streptozotocin (STZ)-induced diabetic rats at doses of 100, 200, and 400 mg/kg body weight. The changes in blood glucose levels at various time intervals are shown in Table 2 and Figure 1. The vehicle-treated control group (1% CMC) has not shown any significant variation in blood glucose levels throughout the experimental period, indicating the stability of the diabetic condition.

Table No 2. Effect of methanol extract of *Madhuca longifolia* root barkon blood glucose levels (mg/dl) in STZ induced diabetic rats

Group (n=6)	Treatment mg/kg b.w.	Time in hours						
		0	2	4	8	12	18	24
1	Control	352.26± 14.13	343.53± 12.32	343.00± 14.68	341.09± 12.03	335.06± 11.37	341.05± 11.95	339.63± 10.76
2	Glibenclamide (0.45 mg/kg b.w.)	353.29 ±12.13	261.20± 8.52**	201.93± 5.24***	274.68± 15.41*	302.52 ±3.47*	323.76± 5.00	333.85± 7.09
3	ML 100	339.7± 12.4	333.87± 7.19	321.92 ± 5.58	302.37± 4.75*	304.94± 6.73	309.69± 4.37	313.70± 5.94
4	ML200	343.24± 11.42	333.51± 9.13	324.02± 4.46	301.79± 4.97*	282.16 ±7.44**	310.42± 5.68*	324.08± 10.64
5	ML400	355.29 ±8.09	328.15± 6.65*	295.04± 9.15**	261.13± 6.97***	291.59± 5.50**	318.50± 5.13*	344.54± 3.91

N.S: No significant difference as compared to zero hr (P>0.05); *: significant decrease as compared to Zero hr (P< 0.05); **: More significant decrease as compared to zero hr (P<0.01); ***: Highly significant decrease as compared to zero hr (P< 0.001).

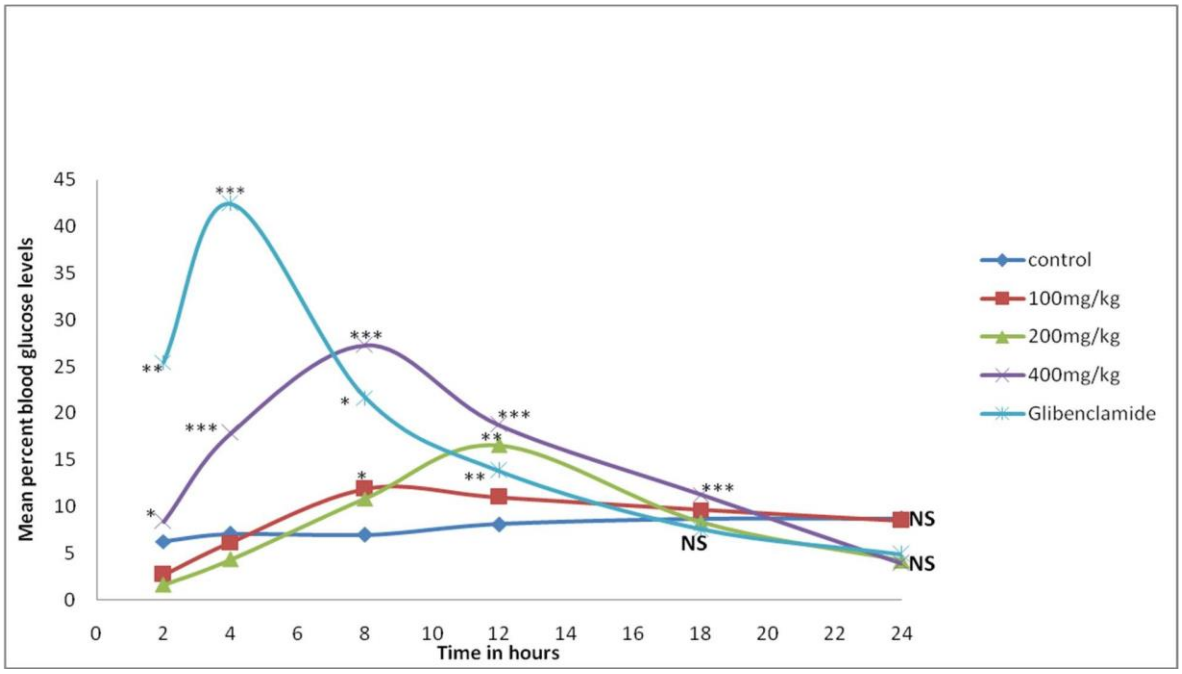

Figure 1. Effect of methanolic extract of *M. longifolia* root bark on percent decrease blood glucose levels in STZ induced diabetic rats

Administration of the methanolic extract *Madhuca longifolia root bark* produced a dose-dependent antihyperglycemic effect. Group 2 (100 mg/kg) has exhibited a mild but significant reduction ($P < 0.05$) only at the 8th hour, with glucose levels decreasing from 339.7 ± 12.4 to 302.37 ± 4.75 mg/dL. In terms of percentage, 11.90 ± 3.44% blood glucose reduction was observed. Group 3 (200 mg/kg) has shown a more noticeable effect, showing significant reductions at the 8th and 18th hours ($P < 0.05$) and at the 12th hour ($P < 0.01$), with levels declining from 343.24 ± 11.42 to 301.79 ± 4.97, 282.16 ± 7.44, and 310.42 ± 5.68 mg/dL, respectively. The percentage decreases of blood glucose were 10.85 ± 2.73%, 16.58 ± 3.47%, and 8.30 ± 2.91% at 8, 12, and 18 hours, respectively.

The Group 4 (400 mg/kg dose) has shown and exhibited a marked and constant greater significance effect, with significant reductions observed from the 2nd hour onward ($P < 0.05$), at the 4th and 8th hours ($P < 0.01$), and a highly significant reduction at the 8th hour ($P < 0.001$). Blood glucose levels decreased from 355.29 ± 8.09 to 261.13 ± 6.97 mg/dL at peak effect (8th hour). The percentage blood glucose reductions were 8.40 ± 2.94%, 17.86 ± 1.78%, 27.26 ± 1.58%, 18.74 ± 2.16%, and 11.26 ± 0.85% at 2, 4, 8, 12, and 18 hours, respectively shown n Figure 2 and Table 3. Overall, the methanolic extract of *Madhuca longifolia root bark* showed rapid onset and sustained activity up to 12–18 hours, with a maximum antihyperglycemic effect at 8–12 hours.

Table 3. Effect of methanol extracts of *Madhuca longifolia* root bark on percent decrease blood glucose levels in STZ induced diabetic rats

Group (n=6)	**Treatment mg/kg b.w.**	**Time in hours**					
		2	4	8	12	18	24
1	Control	2.30±2.02	2.48±2.83	2.90±2.61	4.54±2.92	2.91±2.55	3.29±2.34
2	Glibenclamide (0.45 mg/kg b.w)	25.47±4.23***	42.41±2.94***	21.65±5.62*	13.81±3.41**	7.59±4.60	4.87±4.18
3	ML 100	2.73±3.79	6.13±4.15	11.90±3.44*	10.99±4.46	9.66±4.02	8.51±4.08
4	ML 200	1.63±2.78	4.32±2.56	10.85±2.73*	16.58±3.47**	8.30±2.91*	4.18±4.37
5	ML 400	8.40±2.94*	17.86±1.78***	27.26±1.58***	18.74±2.16***	11.26±0.85***	3.91±1.87

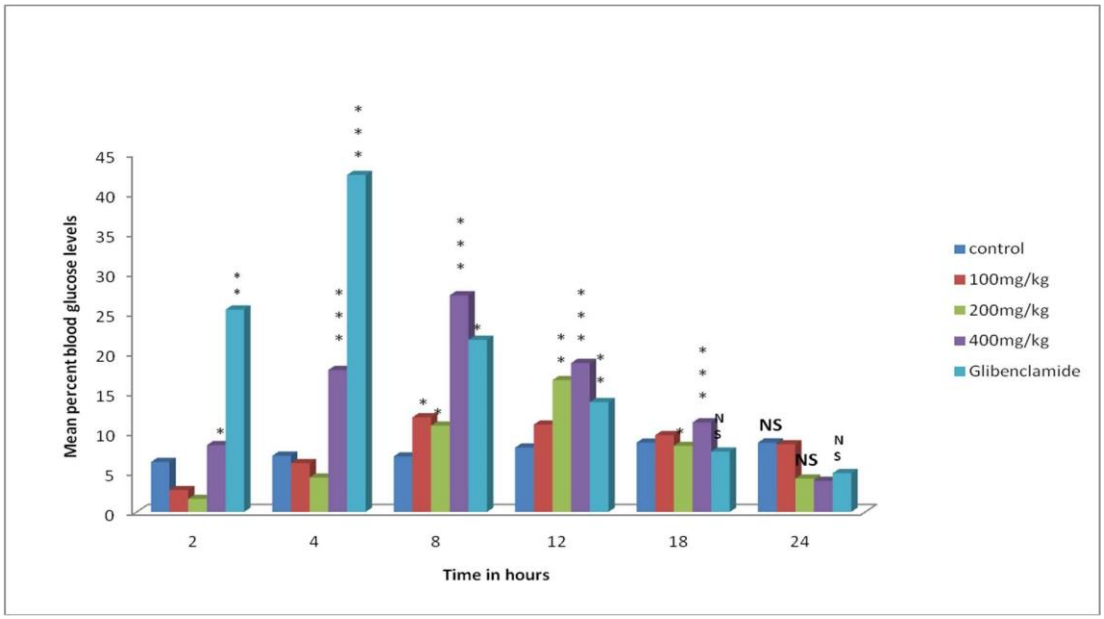

Figure 2. Bar diagram showing effect methanolic extract of *M. longifolia* root bark on percent decrease blood glucose levels in STZ induced diabetic rats

DISCUSSION

The methanolic extract of *M.longifolia* Koenig root bark showed the presence of saponins, tannin, phenolic compounds, carbohydrates and glycosides (Table No 1). According to scientific literature extract of *M.longifolia* contains Phytosterols (β-sitosterol, α-spinosterol) Flavonoids (quercetin, dihydroquercetin), Triterpenoids (lupeol acetate, α & β-amyrin acetates)[10] which are known to show antihyperglycemic activity through multiple mechanisms such as by enhancing insulin secretion, improving peripheral glucose uptake, and inhibiting carbohydrate-digesting enzymes. In contrast, the present investigation revealed that presence of saponins, phenolic compounds, tannins, carbohydrates, and glycosides in the root bark extract of *M. longifolia* Koenig.Even though triterpenoids and flavonoids, which are common antidiabetic compounds, were not found in the extract root bark methanolic extract of *M. longifolia* Koenig, it still showed strong antihyperglycemic activity. This effect may be due to the combined action of the identified phytoconstituents, especially saponins and phenolic compounds, which are known to lower blood glucose levels [11]. Tannins and glycosides may also help with delayed glucose absorption and better glycaemic control [12, 13].

The results of the study clearly showed that the methanolic extracts of *M. longifolia* Koenigroot bark at dose levels of 200 and 400 mg/kg b.w. produced a significant reduction of blood glucose level. Comparing the results of 100, 200, and 400 mg/kg b. w extracts of *M. longifolia* Koenigroot bark in diabetic rats, it was found that the extract at 400 mg/kg b.w showed a highly significant ($P < 0.001$) decrease in blood glucose levels when compared to the control and standard Glibenclamide.

Dahake et al. [13] and Seshgiri et al. [14] demonstrated that methanolic extracts of *M. longifolia* Koenig bark showed the significant antihyperglycemic activity. Furthermore, Prashanth et al. [15] reported that the ethanolic *M. longifolia* Koenig bark showed a dose-dependent antihyperglycemic activity in streptozotocin-induced diabetic rats. Ghosh et al.[16] also confirmed that the hydroethanolic leaves extract of *Madhuca longifolia* possesses significant antihyperglycemic activity in alloxan-induced diabetic models. Moreover, methanolic and ethanolic seed extracts of *M. longifolia* Koenig also found to exhibit antidiabetic effects, as reported by Seshgiri et al[14].Overall, scientific evidence suggests that various parts of *M. longifolia* Koenig such as leaves, stem bark and seeds, showed significant antihyperglycemic activity. Nevertheless, previous studies have not extensively evaluated the root bark for this activity. The present study specifically focuses on the root bark of *M.longifolia* Koenig and proves, for the first time, its significant antihyperglycemic effect.

CONCLUSION

The present study showed that the methanolic root bark extract of *M. longifolia* Koenig showed significant antihyperglycemic activity in STZ-induced diabetic rats. Furthermore, the extract showed its effect in dose-dependent manner, with the 400 mg/kg b.w. dose showing a maximum significant reduction in blood glucose levels comparable to the standard drug glibenclamide. Bioactive phytoconstituents such as triterpenoids, flavonoids, tannins, saponins, and phytosterols, which are known to exert antidiabetic effects through multiple mechanisms may be responsible for the reported antihyperglycemic activity.

Competing interests

The authors declare that they have no competing interests

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