Research Article

ANTIHYPERGLYCEMIC AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *MADHUCA LONGIFOLIA* BARK

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ABSTRACT

The aim of the present study was to explore the antihyperglycemic and antioxidant potential of ethanolic bark extract of *Madhuca longifolia* (ML) in healthy, glucose loaded and streptozotocin induced diabetic rats. All three animal groups were administered with the ethanolic extract of Madhuca longifolia at a dose of 100 and 200 mg/kg body weight (p.o.) and the standard drug glibenclamide at a dose of 500 µg/kg. Serum glucose level was determined on days 0, 7, 14 and 21 of treatment. The extract exhibited a dose dependent hypoglycemic activity in all three animal models as compared with the standard antidiabetic agent glibenclamide. The antioxidant activity of the bark was evaluated by free radical scavenging activity using 1, 1-diphenyl-2-picrylhydrazil (DPPH), reducing power assay and superoxide scavenging activity. The results of the assay were then compared with a natural antioxidant ascorbic acid (vitamin C). The hypoglycemia produced by the extract may be due to the increased glucose uptake at the tissue level and/or an increase in pancreatic β-cell function, or due to inhibition of intestinal glucose absorption and a good source of compounds with antioxidant properties. Finally the study indicated the ethanolic extract of Madhuca longifolia to be a potential antidiabetic and antioxidant properties and the extract also exhibited significant free radical scavenging activity and superoxide scavenging activity.

Keywords: DPPH, Hypoglycemic activity, Madhuca longifolia.

INTRODUCTION

Diabetes mellitus (DM) is characterized by abnormalities in carbohydrate, lipid, and lipoprotein metabolisms, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis[1,2]. Numerous studies have been demonstrated that oxidative stress, mediated mainly by hyperglycemia-induced generation of free radicals, contributes to the development and progression of diabetes and its complications[3,4,5]. Abnormally high levels of free radicals which cause membrane damage due to peroxidation of membrane lipids and protein glycation and the simultaneous decline of antioxidant defense mechanisms leads to cell and tissue damage[6]. Pancreatic β-cells are particularly susceptible to the deleterious effects of reactive oxygen species (ROS), because of their low expression of the antioxidant enzymes genes as compared to other tissues. Thus, the increase of ROS leads to damage of β-cells through the induction of apoptosis and suppression of insulin biosynthesis[7,8]. As a new strategy for alleviating the oxidative damage in diabetes, interest has grown in the usage of natural antioxidants. It has been postulated that many of the negative effect of oxidative stress are diminished upon supplementation with certain dietary antioxidants such as vitamin E, C and other non-nutrient antioxidant such as flavonoids[9]. On the other hand, many plant species are known in folk medicine of different cultures to be used for their hypoglycemic properties and therefore used for treatment of DM. Despite this, few traditional antidiabetic plants have received proper scientific screening. The World Health Organization (WHO) has recommended that this area warrants further evaluation.

Madhuca longifolia, synonym *M. indica,* belonging to the family Sapotaceae, is an important economic tree growing throughout India. Traditionally, *Madhuca longifolia* bark has been used against rheumatism, ulcers, bleeding and tonsillitis. The flowers, seeds and seed oil of Madhuca have great medicinal value. Externally, the seed oil massage is very effective to alleviate pain. In skin diseases, the juice of flowers is rubbed for oleation. The present study was undertaken to investigate possible hypoglycemic/antihyperglycemic and antioxidant effects of ethanolic extract of *Madhuca longifolia* in healthy and streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS

Plant material: The bark of *Madhuca longifolia* was collected from the forest of Mangoor, Khammam, Andhrapradesh, and authenticated by botanist N. Murthy, Department of Botany, Kakatiya University., Warangal.

Preparation of plant extract: The plant material was dried in shade and bark was ground in a blender and sifted through a wire screen (mesh size 2 mm. 2 mm). The material was extracted by soxhlation process by refluxing with ethanol for 6hr, and was evaporated to dryness at a temperature below 30°C. The extract obtained with ethanol was 25 g.

Animals: Experiments were performed with Albino rats procured from Mahaveera Enterprises (Hyderabad, A.P.,

India), weighing between 180 to 210gms. The animals were housed in colony cages (four per cage) under conditions of standard lighting, temperature (22±1°c) and humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. During this period, they had free access to food and water. The experiments were planned after the approval of Institutional Animal Ethical Committee (IEAC), Vaagdevi College of Pharmacy, Warangal, and A.P., India.

Acute toxicity and selection of doses: The acute toxicity studies were carried out in adult female albino rats weighing 150-200 g, by up and down method as *per* OECD 425 guidelines (OECD). Overnight fasted animals received test drug at a dose of 2000 mg/kg body weight orally. Then the animals were observed continuously once in half an hour for the next 4 hours and then after 24 hours for general behavioral, neurologic and autonomic profiles and to find out mortality. The extract was found safe to up to a dose of 2000 mg/kg body weight.

Oral glucose tolerance test: The oral glucose tolerance test was performed in overnight fasted normal animals. Rats divided into four groups (n=6) were administered 2% gum acacia solution, ethanolic extract 100 mg/kg, 200 mg/kg and glibenclamide (500 µg/kg). Glucose (2 g/kg) was fed 30 min after the administration of ethanolic extract. Blood was withdrawn from the retro-orbital sinus at 0, 30, 60, 90 and 120 min of ethanolic extract administration. Fasting serum glucose levels were estimated by the GOD-POD method.

Normoglycemic study: For normoglycemic study, rats were divided into five groups (n=6) and administered 2% gum acacia solution, ethanolic extract 100 mg/kg, 200 mg/kg and glibenclamide (500 µg/kg) (7). Blood glucose levels were estimated on days 0, 4, 8 and 12.

Induction of experimental diabetes

Diabetes was induced by administering intraperitoneal injection of a freshly prepared STZ solution (60 mg/kg of body weight) in 0.1M cold citrate buffer to the overnight fasted rats. Because of the STZ instability in aqueous media, the solution is made using cold citrate buffer (pH 4.5) immediately before administration. Animals with blood glucose values above 250 mg/dL on day 3 of STZ injection were considered as diabetic rats. The treatment was started after day 5 of diabetes induction and was considered as day 1 of treatment.

Experimental design: The animals were divided into five groups of six animals, as follows: group I, normal healthy control; group II, diabetic control (STZ 60 mg/kg i.p.); group III, diabetic + ethanolic extract (100 mg/kg body weight, orally); group IV, diabetic + ethanolic extract (200 mg/kg body weight, orally); and group VI, diabetic + glibenclamide (500 µg/kg body weight, orally).

Blood sampling and estimation: At the end of day 12, blood samples were collected from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hemocrit Capillaries, Mucaps). Blood was collected into fresh vials containing anticoagulant and separated in a centrifuge at 10000 rpm for 2 min. There are several methods for the estimation of blood glucose. In the present study the blood glucose level was determined by using commercially available glucometer kit based on the Glucose oxidase method.

Reducing power assay: Various concentrations of the extracts (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50^c for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 16µg/ml) was used as standard.

% Increase in reducing power = (Atest / Ablank)-1 X 100

A test is absorbance of test solution; Ablank is absorbance of blank. The antioxidant activity of ethanolic extract of bark of *Madhuca longifolia* expressed as IC50 and compared with standard.

DPPH free radical scavenging activity: The free radical scavenging activity was followed by the DPPH method. 0.1mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in ethanol at different concentration (1-100 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to100 µg/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation

DPPH Scavenged (%) = [(Acontrol- Atest) / Acontrol] × 100

Where Acontrol is the absorbance of the control reaction and Atest is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the ethanolic bark extract was expressed as IC50 and compared with standard. The IC50 value was defined as the concentration (in µg/ml) of extracts that scavenges the DPPH radicals by 50%.

Statistical analysis: Data were statistically evaluated by use of one-way ANOVA, followed by post hoc Scheffe's test using 7.5 version of the SPSS computer software. The values were considered significant at $P<0.05$.

RESULTS

Acute toxicity studies: Acute toxicity studies revealed the non-toxic nature of ethanolic extract at the two dose levels i.e. 1000mg/kg and 2000mg/kg were tested. There were no morphological changes like distress, hair loss, restlessness, convulsions, laxative effect, coma, weight

loss, etc. At the end of the treatment period, there was no lethality or toxic reaction at any of the doses selected.

Glucose tolerance test: In all groups except for glibenclamide, at 30 min of initiating glucose tolerance test, blood glucose concentration was higher than at zero time but decreased significantly from 30 min to 120 min and the results are shown in Table 1. Ethanolic extracts were enhancing glucose utilization, thus the blood glucose level was significantly decreased in glucose loaded rats.

Normoglycemic study: In normoglycemic rats, the doses of 100 and 200 mg/kg reduced hyperglycemia on days 4, 8 and 12 of treatment (results are shown in Table 2). A significant hypoglycemic activity was found on day 12 with 100 and 200 mg/kg doses.

STZ induced diabetes: After oral administration of 100 and 200 mg/kg of the ethanolic extract of *Madhuca longifolia*, a significant reduction was observed in the blood glucose level of STZ induced diabetic rats. A dose dependent effect was seen with doses of 100 and 200 mg/kg of ethanolic extract throughout the study period (results are shown in Table 3). Blood sugar level was also determined before and after glibenclamide treatment. Glibenclamide, a known hypoglycemic agent, reduced blood sugar level.

Reducing power assay: Reducing power assay is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe+3) to form potassium ferrocyanide (Fe+2), which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm.The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table 4 shows the reducing power of ethanolic bark extract of *Madhuca longifolia*. From figure 1 it was found that the reducing power of the extract increased with the increase in concentrations. The EC50 value of the extract was found to be 66.49µg/ml and 60.19µg/ml for ethanolic bark extract of *Madhuca longifolia* and ascorbic acid respectively. Reducing power capabilities of extract was found to be closer to ascorbic acid.

DPPH free radical scavenging activity: The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Table 5 shows the percentage of DPPH radical scavenged by ascorbic acid and ethanolic extract of bark at various concentrations (µg/ml)**.** Figure 2 illustrates a decrease in the concentration of DPPH radical due to the scavenging ability of the soluble constituents in the ethanolic extract of leaves of *Madhuca longifolia* and the standard ascorbic acid, as a reference compound, presented the highest activity at all concentrations. The IC50 values were found to be 58.13µg/ml and 47.56µg/ml for ethanolic bark extract of *Madhuca longifolia* and ascorbic acid respectively.

Figure 1: Graph of % increase in reducing power

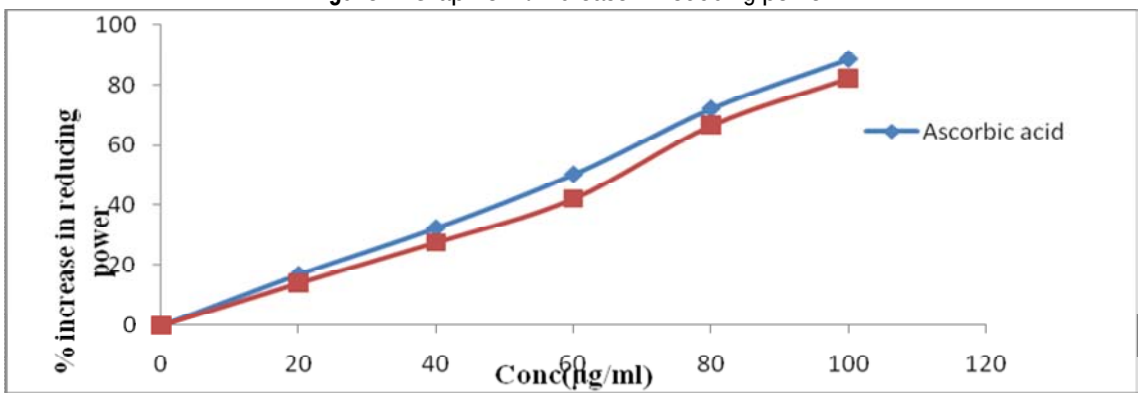

Figure 2: Graph of % DPPH free radical scavenging activity

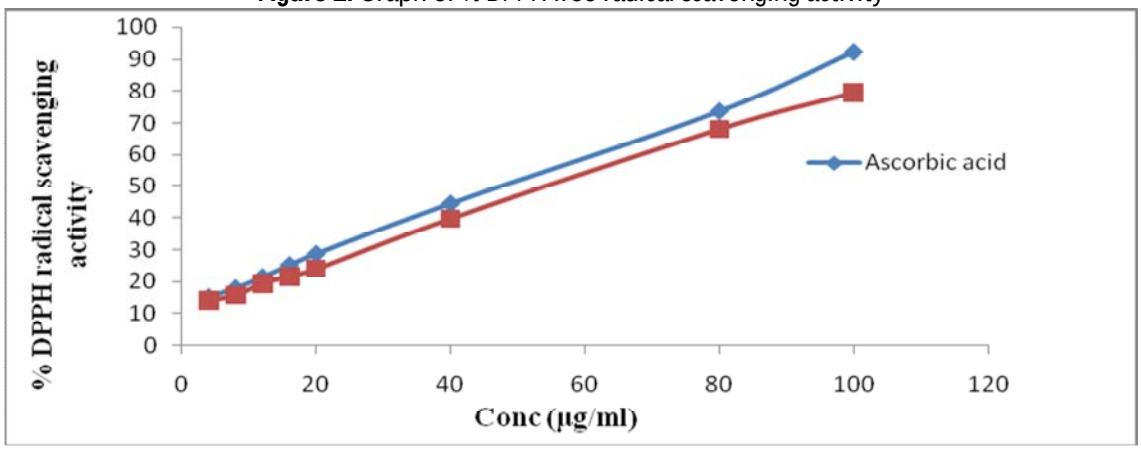

Table 1: Effect of ethanolic extract on serum glucose level (mg/dL) on glucose tolerance test in glucose loaded rats

Group	Treatment	0 min	30 min	60 min	90 min	120 min
1	Control (vehicle)	89.2±2.9	108.3±1.1	103.7± 2.1	100.2*±1.4	96.3*±1.6
2	Ethanolic extract (100 mg/kg)	80.4±1.1	92.7±2.1	86.7±3.1	83.6±1.8	79.8±1.3
3	Ethanolic extract (200 mg/kg)	89.3±1.7	97.4±2.2	90.5±2.5	84.2*±1.2	75.4*±1.3
4	Glibenclamide (500 µg/kg)	83.4±1.5	79.5±1.1	80.1±2.7	75.1*±2.1	73.6±2.9

Values are expressed as mean ± SEM; *statistically significant difference from the corresponding zero time value; *P*<0.05.

Table 2: Effect of ethanolic extracts on serum glucose level (mg/dL) in normal fasted animals

Group	Treatment	Day 0	Day 4	Day 8	Day 12
1	Control (vehicle)	78.4±3.3	76.1±36	79.4±2.2	76.3±2.4
2	Ethanolic extract (100 mg/kg)	82.1±2.6	80.3±3.3	79.8*±3.7	77.5*±1.3
3	Ethanolic extract (200 mg/kg)	74.4±2.3	70.6±2.4	67.3±3.6	61.8*±1.3
4	Glibenclamide (500 µg/kg)	80.1±2.3	73.2±3.9	66.7*±3.2	59.6*±1.7

Values are expressed as mean ± SEM; *statistically significant difference from the corresponding zero time value; *P*<0.05.

Table 3: Effect of ethanolic bark extract of *Madhuca longifolia* on serum glucose level (mg/dL) in streptozotocin induced diabetic rats

Group	Treatment	Week 0	Week 1	Week 2	Week 3
1	Normal control	128.86±1.71	141.32±2.03	130.13*±3.52	117.37±1.16
2	Diabetic control	190.81±2.68	309.55±4.48	380.05±5.13	410±2.06
3	Ethanolic extract (100 mg/kg)	390.13±3.08	332.39*±3.14	268.94±2.06	200.90±3.46
4	Ethanolic extract (200 mg/kg)	411.91±5.02	341.13±4.6	200.52*±1.33	141.37*±4.63
5	Glibenclamide (500 µg/kg)	385.5±2.02	302.8*±3.32	190.3±3.68	132.6±4.87

Values are expressed as mean ± SEM; *statistically significant difference from the corresponding zero time value; *P*<0.05.

Table 4: Results of % increase in reducing power assay

Tested material	Conc (µg/ml)	% increase in reducing power (±SEM)	EC 50 (µg/ml)
Ascorbic acid	20	16.97 ± 0.02	60.19
	40	32.56± 0.02	
	60	50.34 ± 0.05	
	80	72.21 ± 0.06	
	100	88.45 ± 0.04	
Ethanolic extract	20	14.13 ± 0.03	68.49
	40	27.78 ± 0.04	
	60	42.36 ± 0.02	
	80	66.79 ± 0.05	
	100	82.31 ± 0.03	

Values are expressed as mean ± SEM; *statistically significant difference from the corresponding zero time value; *P*<0

Table 5: Results of % DPPH radical scavenging activity

Tested material	Conc. (µg/ml)	% DPPH radical Scavenged (±SEM)	IC50 (µg/ml)
Ascorbic acid	4	15.10 ± 0.03	47.56
	8	17.90 ± 0.03	
	12	21.34 ± 0.02	
	16	25.13 ± 0.05	
	20	28.67 ± 0.06	
	40	44.39 ± 0.05	
	80	73.64 ± 0.02	
	100	92.18 ± 0.03	
Ethanolic extract	4	13.98 ± 0.07	55.13
	8	15.67 ± 0.08	
	12	19.37 ± 0.05	
	16	21.56 ± 0.05	
	20	23.89 ± 0.03	
	40	39.67 ± 0.02	
	80	68.12 ± 0.04	
	100	79.57 ± 0.03	

DISCUSSION

A wide range of synthetic oral antidiabetic drugs such as sulfonylureas and biguanides have been used for 50 years now in the treatment of diabetes. However, they have not been of much benefit in controlling the complications of the disease. In the present study, the antihyperglycemic activity of ethanolic bark extract of *Madhuca longifolia* was assessed in normal and STZ induced diabetic rats. Oral administration of a single dose of ethanolic bark extract of *Madhuca longifolia* caused a significant decrease in serum glucose level in normal rats. A dose of 200 mg/kg of ethanolic extract produced maximum glucose lowering effect, whereas 100 mg/kg of ethanolic extract showed a significant hypoglycemic effect throughout the study period. In the oral glucose tolerance test, the *Madhuca longifolia* bark extract showed significant reduction of serum glucose levels and these effects were dose dependent. The extract of *Madhuca longifolia* bark displayed a significant hypoglycemic effect in normal rats. The main mechanism by which the extracts bring the hypoglycemic effects most probably involves stimulation of peripheral glucose consumption.

The significant hypoglycemic effects of *Madhuca longifolia* bark in diabetic rats indicate that this effect can be mediated by stimulation of glucose utilization by peripheral tissues. The results of the present study clearly indicated the ethanolic extract of *Madhuca longifolia* bark to have a hypoglycemic effect on STZ induced diabetic rats.

The reducing property of ethanolic bark extract of *Madhuca longifolia* implies that it is capable of donating hydrogen atom in a dose dependent manner. The high content of phenolic compounds in the extract may be a contributing factor towards antioxidant activity because the phenolic compounds are known to have direct antioxidant property due to the presence of hydroxyl groups, which can function as hydrogen donor. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Reducing power assay method is based on the principle that Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide7 (Fe^{2+}). Results show that the *Madhuca longifolia* possess reducing power capabilities and acts as a potent antioxidant. DPPH· is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity of antioxidants. From the results it can be stated that the *Madhuca longifolia* possess hydrogen donating capabilities and acts as an antioxidant.

CONCLUSION

This research provides information which could trigger further research in the direction of partial or full isolation and characterization of the constituents of bark extract of *Madhuca longifolia* in order to decipher the specific phytochemical constituent(s) responsible for the hypoglycemic activity and free radical scavenging activity of the plant. When this is done, extracts of *Madhuca longifolia* could find important application in phytotherapy.

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