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Research Article

Invivo and *Invitro* Anti-Diabetic Effects of *Madhuca indica* Roxb., in Alloxan-Induced Diabetic rats

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1. INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by high levels of blood glucose with disturbances of carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both. Insulin deficiency and/or insulin resistance is associated with the pathogenesis of diabetic dyslipidemia and micro/macrovascular complications (Akpan et al., 2007).Diabetes mellitus is possibly the world's largest growing metabolic disorder, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases (Baily and Flatt, 1986). In spite of the availability of various antihyperglycemic agents, diabetes and its secondary complications continue to be a major problem in the world population. Medicinal plants and their bioactive constituents are used for the treatment of diabetes throughout the world and popularized as neutraceutical. Many indigenous medicinal plants have been found to be useful to successfully manage diabetes (Subramoniam et al., 1996; Mukherjee et al., 1997). In addition, many of the currently available drugs have been derived directly or indirectly from plant source. Even the discovery of the widely used hypoglycemic drug metformin came from the traditional approach of using *Galega officinalis*(Akpan et al., 2007).

The commonly encountered acute and late diabetic complications are already responsible for major causes of morbidity, disability and premature deaths in Asian countries. The underlying causes attributed to hyperglycemia ultimately result in oxidative stress, alterations in enzyme activities, protein glycosylation and several structural changes (Akpan et al., 2007).

*Madhucaindica*belongs to family Sapotaceae, also called as *Bassia latifolia* Roxb. *Madhuca latifolia* Roxb.Commonly called as in Hindi: Mahua Mohwa and in Kannada: Ippe.

According to Ethano Medical Uses, *Madhuca latifolia* bark has been used against diabetes, rheumatism, ulcers, bleeding and tonsillitis. The flowers, seeds and seed oil of madhuka 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. It is also beneficial as a nasya (nasal drops) in diseases of the head due to pitta, like sinusitis.

The saponins of *Madhuca latifolia* possess anti-inflammatory activity.Triterpenic saponins isolated from seeds of *Madhuca indica* exhibited inhibitory effect against two phyto-parasitic nematodes. The components of *Madhuca longifolia* Madhucosides A and B, protobassic acid glycosides posses inhibitory effect on free radical release from phagocytes.The fruits of *Madhuca indica* was reported to contain a number of triterpenoids including α- and β-amyrin acetates, 3β-monocaprylic ester of erythrodiol, 3β-capryloxy oleanolic acid and an acetate The other constituents isolated and characterized are n-hexacosanol, β-glucoside of β-sitosterol and free β-sitosterol. The nut-shell contains β- -glucoside of β-sitosterol, quercetin and dihydroquercetin.Myricetin and myricetin-3-O- rhamnoside have been isolated from the leaves of *Madhuca indica*. Madhucosides A and B, protobassic acid glycosides have also been isolated from *Madhuca indica* barks (Saha Sand Walia S., 2010).

2. MATERIALS AND METHODS

2.1. Chemicals

Alloxan was purchased from Hi-media, Mumbai. Carboxy methyl cellulose was procured from SD fine chemicals, Mumbai. All chemicals used were of analytical grade.

2.2. Plant material and extraction

The bark of *Madhucaindica*was collected in and around Siddarabetta region, Tumkur dist, Karnataka. The plants were identified and authenticated by Professor K. Siddappa, HOD, Department of Botany, Sree Siddaganga College of Science and a voucher specimen of the plants were kept in the college herbarium.

The shade-dried, coarsely powdered bark (2 kg) was extracted exhaustively with ethanol in a Soxhlet for 4-5 hand the extract thus obtained wasconcentrated to a small volume under vacuum using a rotary evaporator (Buchi rotavapor, Switzerland). Then extract was evaporated to dryness in a vacuum desiccator (J.R. Industrial Corporation, Mumbai, India) and stored in air tight container. The percentage yield of the extract (MIEE) was found to be 4.55% w/w. phytoconstituents.The shade dried, coarsely powdered plant material after extracted with ethanol was subjected extract with distilled water and concentrated same as above mentioned in ethanolic extraction. The percentage yield of the extract (MIAE) was found to be 2.35% w/w. The extracts thus obtained were kept in a glass container.

2.3. Animals

Wistar rats of either sex weighing 150-250 g were used for the study. The animals were obtained from the inbred animal colony of central animal house, Sree Siddaganga College of Pharmacy, Tumkur. The animals were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and 12-h light-dark cycles. The animals were randomized into experimental and control groups and housed three in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free assessed to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Sree Siddganga College of Pharmacy, Tumkur, Karnataka (SSCPT/IAEC.clear/80/09-10).According to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4. Determination of acute Toxicity

Acute toxicity study was carried out for MIEE and MIAE extracts using female albino mice (20-30 g) those maintained under standard husbandry conditions. The maximum upper limit dose 3000 mg/kg of above mentioned extracts were administered orally to three female mice. The animals were observed continuously for one hour, then frequently for four hours and later at the end of 24 h. After administration of the extracts, the animals were observed for behavioral changes. Further, animals were observed daily for 15 days, and mortality was recorded.

2.5. Effect of MIEE and MIAE in standardized Invivo diabetic model

2.5.1.Alpha- amylase inhibition assay(Sheikh et al., 2008).

Alpha-amylase activity was carried out by starch-iodine method. 10 µL of α-amylase solution (0.025 mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, 100 µL of starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 mL of 1% iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) X100/ (B-C), where, A= absorbance of the sample, B= absorbance of blank (without α-amylase), and C= absorbance of control (without starch).

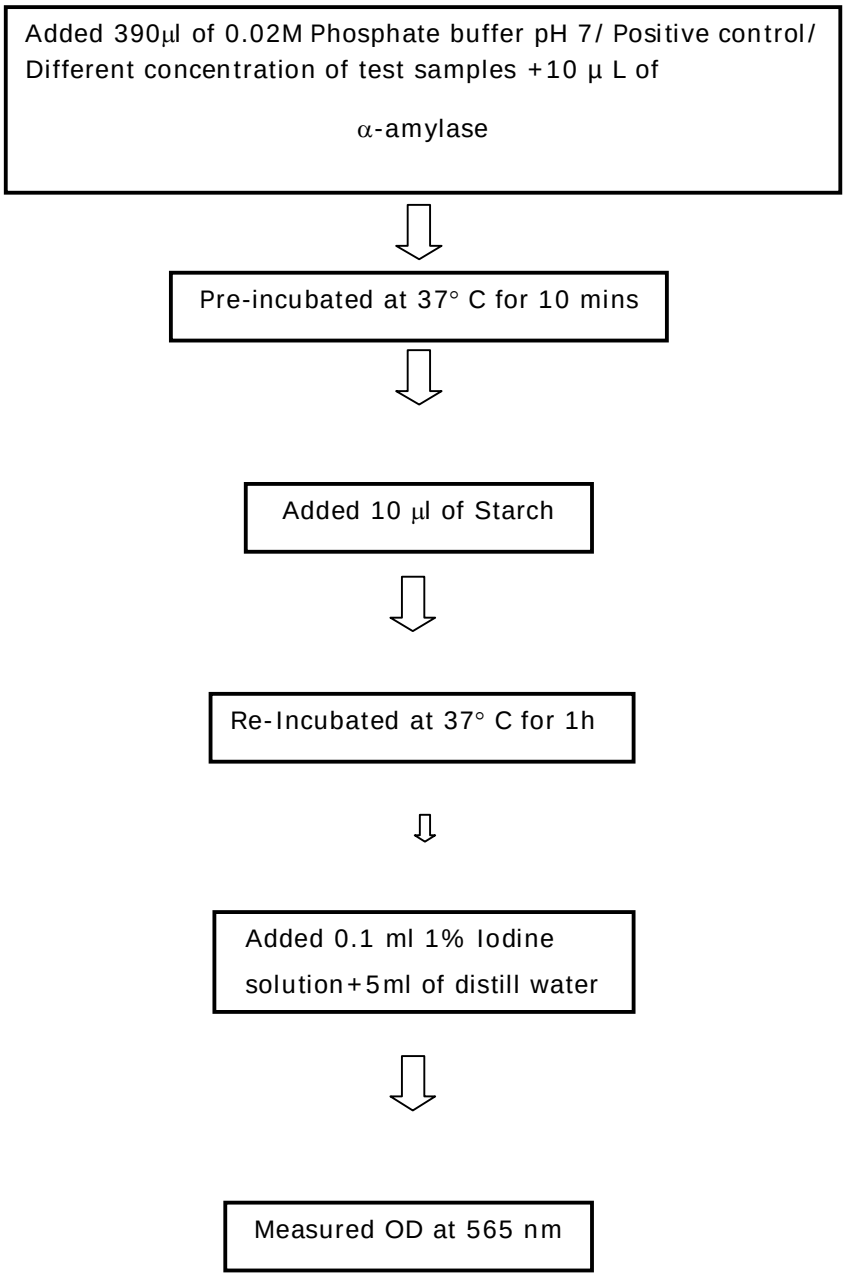
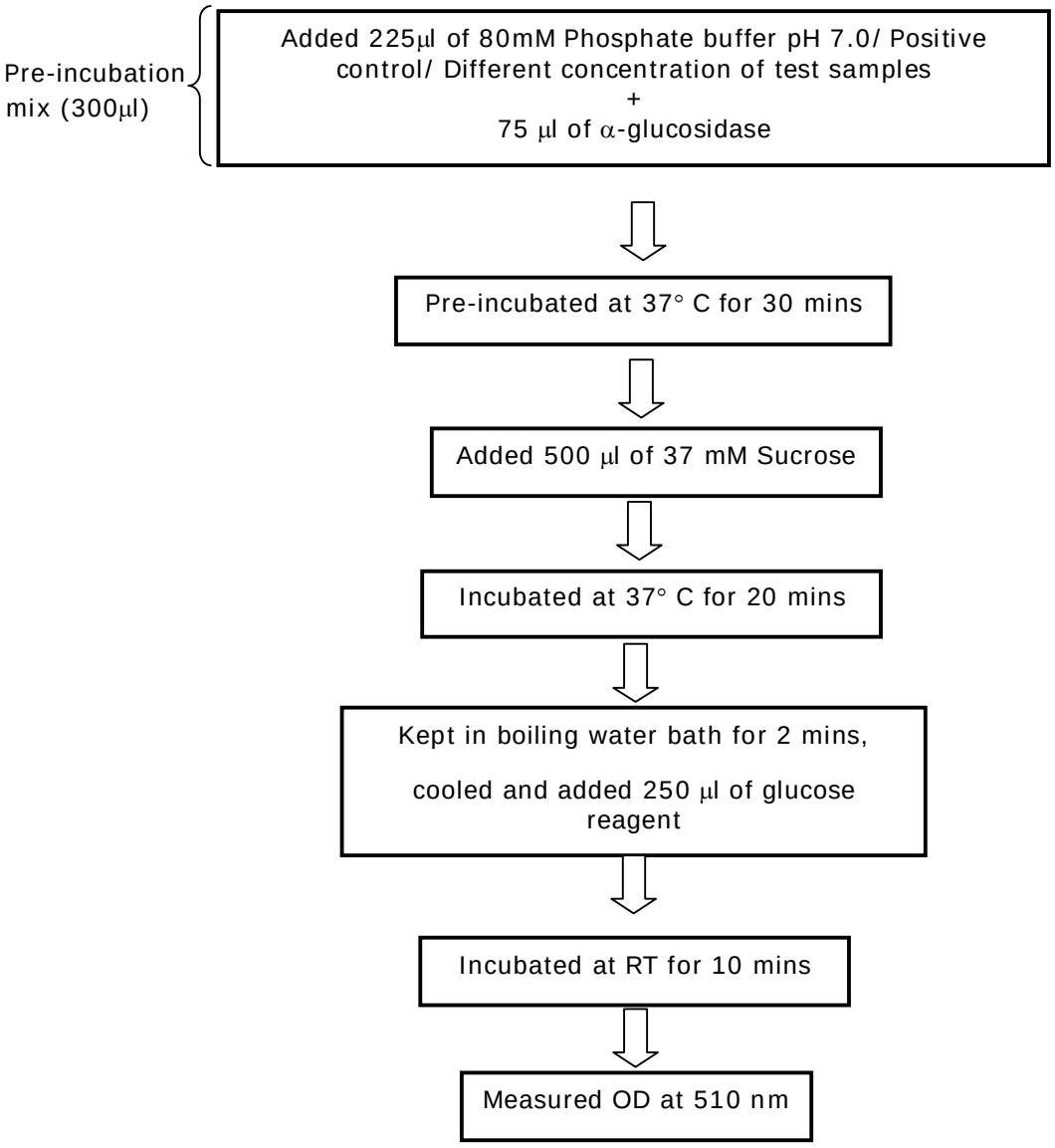


Chart. 1: Schematic flow chart of α-amylase enzyme inhibition assay procedure

2.5.2. Alpha-glucosidase inhibition assay(Matsui et al., 2007)

Alpha-glucosidase activity can be measured in-vitro by determination of the reducing sugar (glucose) arising from hydrolysis of sucrose by α-glucosidase enzyme, isolated from small intestine of rat.



2.6. Effect of MIEE and MIAE in standardized alloxan-induced diabetic rat model

2.6.1. Induction of Diabetes mellitus

An i.p. injection of alloxan (100 mg/kg body weight) was administered, later (20 mg/kg body weight) was administered in the next day to five animals per group, in a volume of 4 ml/kg after overnight fasting for 12 h.After five day post alloxan administration, SG levels were estimated by the enzymatic glucose oxidase method. Rats showing SG level > 250 mg/dl were considered as diabetic and included in the study.

2.6.2. Experimental design Single-dose one-day study

The experimental rats were divided into five groups of six rats each treated asGroup 1:Normal control (NC) received 1% CMC; Group 2:Diabetic control (DC) received 1% CMC; Group 3:DC rats treated with MIEE (100 mg/kg, p.o.); Group 4:DC rats treated with MIEE (300 mg/kg, p.o.);Group 5:DC rats treated with MIAE (100 mg/kg, p.o.); Group 6:DC rats treated with MIAE (300 mg/kg, p.o.); Group 7:DC rats treated with glibenclamide [GLB] (10 mg/kg, p.o.)

Blood samples were collected at 0, 2, 4 and 6 h after extract/GLB administration. SG was estimated by the enzymatic glucose oxidase method. Percentage reduction in glycemia was calculated with respect to the initial (0 h) level

according to: Percentage reduction in glycemia = [(Gi – Gt)/Gi] x 100; Where Gi is initial glycemia and Gt is glycemia at 2, 4, and 6h (Veerapur et al., 2010).

2.6.3. Experimental design for Multiple-dose 15 days study

The above groups of animals were further treated with respective doses of MIEE , MIAE and GLB for 15 days in order to evaluate the chronic effect of extract/GLB treatment on hyperglycemia. Whereas, GLB (0.5 mg/kg, p.o./day) was administered for eight weeks. Percentage reduction in glycemia was calculated with respect to the initial (0 day) level according to percentage reduction in glycemia = [(Gi – Gt)/Gi] x 100; Where Gi is initial glycemia and Gt is the glycemia value at 15 day (Veerapur et al., 2010).

2.6.4. Oral glucose tolerance test (OGTT)

On 10 day, glucose tolerance of various groups was estimated by a simple OGTT. Glucose (2 g/kg) was administered to 12 h fasted rats and blood samples were collected from the retro-orbital plexus at 0 (before glucose load), 30, 60 and 120 min after glucose administration. SG was estimated by the enzymatic glucose oxidase method. The results were expressed as integrated area under curve for glucose ($AUC_{glucose}$), which was calculated by trapezoid rule. (Vishwakarma et al., 2003).

2.6.7. Estimation of biochemical parameters

At the end of the treatment schedule, blood samples were collected from retro-orbital plexus. Serum was separated and triglyceride (STG), total cholesterol (STC) and HDL-cholesterol (HDL-c)were analyzed by semi-autoanalyser (Qualigen, Mumbai) using diagnostic reagent kit (ERBA diagnostics Mannheim GMBH, Germany

VLDL-cholesterol (VLDL-c) and LDL-cholesterol (LDL-c) in serum were calculated as per Friedewald's equation (Friedewald et al., 1972).

The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were also calculated.

$$VLDL - c = \frac{Triglyceri\ de}{5}$$

$$LDL - c = Total\ cholestero\ l - \frac{Triglyceri\ de}{5} - HDL - c$$

2.6. Statistical analysis

The data were expressed as mean ± S.E.M. Statistical comparisons were performed by One-way ANOVA followed by Tukey's post hoc test using GraphPad Prism Version 5.0 (San Diego, CA).

3. RESULTS

3.1. Acute oral toxicity studies

Animals showed good tolerance to single doses of MIEE and MIAE in doses as high as 2 g/kg and were non-lethal. Therefore 100 and 300 mg/kg of MIEE and MIAE were selected for the present study.Further, administration of both the doses of MIEE and MIAEfor fifteen-days did not produce any noticeable signs of toxicity (behavioral changes).

3.2. Effect of MIEE and MIAE in standardized invitro diabetic rats

3.2.1. Alpha- amylase inhibition assay

The extracts exhibited IC_{50} less than 100 µg/mL will be considered active in comparison with Acarbose standard. Madhuca indica aqueous extract (MIAE), Madhuca indica ethanolic extract (MIEE),showed enzyme inhibition activity with IC_{50} 19.00,64.00, 64µg/mL respectively.

3.2.2. Alpha-glucosidase inhibition assay

The extracts exhibited IC_{50} less than 100 µg/mL will be considered active in comparison with Acarbose standard. Madhuca indica aqueous extract (MIAE), Madhuca indica ethanolic extract (MIEE),does not show enzyme inhibition.

3.3. Effect of MIEE and MIAE in standardized alloxan-induced diabetic rats

3.3.1. Single-dose one-day study

A single dose of MIEE (100 and 300 mg/kg) to the diabetic rats not showed any significant changes in SG levels when compared diabetic control rats. Administration of MIAE at the dose of 100 and 300 mg/kg exhibited significant ($P<0.05$ and $P<0.001$) reduction in SG levels at 4^{th} and 6^{th} hours of administration when compared to basal levels (0 h). MIAE 300 mg/kg treatment showed better activity at 6^{th} h of administration compared to lower dose. Whereas, administration of GLB showed significant ($P<0.05$; $P<0.001$) reduction is SG

levels at all intervals of post GLB treatment when compared to their basal levels (Table.1).
The maximum percent reduction in SG levels was found in MIAE 300 mg/kg (at 6th h) treated animals were 27.96%. Treatment with MIAE and GLB showed significant (P<0.05; P<0.01; P<0.001) reduction in SG levels at different time intervals when compared to diabetic control (Fig. 1). These results suggest that MIAE have better anti-hyperglycemic activity when compared to MIEE in this model.

3.3.2. Multiple-dose 15 day study

Multiple doses adminstration of MIEE at both the doses for 15 days not produced any significant reduction in SG levels when compared to basal values (0 day) (Table 2). Whereas MIAE at the doses of 100 and 300 mg/kg for 15 days showed significant reduction in SG levels on 1st, 7th 15th days of administration. The % reduction in glycemia was found to be 20.30%, 25.80% after administration of 10th and 15th days of MIAE 100 mg/kg and 22.12%, 44.60% and 49.78% 7th, 10th and 15th days of MIAE 300 mg/kg treatment when compared to diabetic control and the effect was comparable with GLB. These values suggested that aqueous extract of Madhuca indica(MIAE) showed good antidiabetic activity than ethanolic extract (MIEE) after multiple dose treatment for 15 days in diabetic rats.

3.3.3. Oral glucose tolerance test (OGTT)

Intra gastric administration of glucose (2 g/kg) did not produced significant change in SG level of normal control rats and AUC for the 120 min interval was not altered. The diabetic rats exhibited significant elevation in fasting SG (at 0h) and showed significant impairment in glucose tolerance to exogenously administered glucose compared to normal rats (Fig. 1 A). Treatment with different dose of MIEE (100 & 300 mg/kg), MIAE (100 and 300 mg/kg) and GLB (10 mg/kg) significantly (P<0.001) improved the glucose tolerance (Fig 1A). Further, treatment of MIEE and MIAE exhibited significant (P<0.001) reduction in SG level over the period of 120 min compared to diabetic control group (Fig 1A).
Integrated areas under the glucose curve over 120 min (AUC) of diabetic group was significantly higher (P<0.001) compared to normal control. Treatment with MIEE, MIAE and GLB produced a significantly (P<0.001) decreased AUC compared to diabetic control (Fig1B). Furthermore, estimation of AUC values indicated that, treatment with different doses of MIEE (100 and 300 mg/kg),47.16%, 57.38% respectively, MIAE (100 and 300 mg/kg)53.74%, 65% respectively and GLB (10 mg/kg) 57.20% decrease in SG levels compared to diabetic rats (Fig 1B). In this model both the extract at both the dose levels exhibits hypoglycemic property to exogenously administered glucose and to reduce the blood sugar level in diabetic rats.

3.4.7. Estimation of Lipid parameter

STG, STC, VLDL-c and LDL-c levels were significantly (P<0.001) increased whereas HDL-c was (P<0.05) decreased in diabetic rats compared to normal rats. (Table 3). The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were significantly (P<0.001) elevated in the diabetic group. Both the dose of MIEE and MIAE exhibited significant reduction (P<0.001) in all tested lipid parameters and restoring them to near-normal values (Table 4) except HDL-c. HDL-c was significantly (P<0.05; P<0.01) increased.

4. DISCUSSION

The treatment goal of diabetic patients is to maintain near normal levels of glycemic control, in both fasting and post-prandial conditions.Many natural sources have been investigated with respect to suppression of glucose production from the carbohydrates in the gut or glucose absorption from the intestine.[9] Alpha-amylase catalyses the hydrolysis of alpha-1,4-glycosidic linkages of starch, glycogen and various oligosaccharides.Alpha-glucosidase further breaks down the disaccharides to simple sugars, readily available for intestinal absorption. The inhibition of their activity in the digestive tract of humans is considered to be effective tool to control diabetes. In addition, these effects may leads to diminished absorption of monosaccharides. The major outcome of this study reveals that both the extracts of Madhuca indica, have exhibited potent inhibition of alpha-amylase enzyme activity.
Proper standardization and validation of diabetic model is very essential to know whether experimental animals are in type I or type II diabetic or in both conditions. Carefully selection of experimental parameters is handy to know the same. Such experiments will help to elucidate the possible mode of action of test compound.
Administration of MIAE exhibited significant reduction in serum glucose levels in both single-dose one day and multiple-dose 15 day study in alloxan-induced diabetic studies. Whereas MIAE showed significant reduction in serum glucose levels in single-dose one day study. These experimental protocols substantiate the antidiabetic activity of title plant.
Exogenously administered glucose (2 g/kg) to diabetic animals exhibited higher glucose levels with increased AUC. These data suggested that these diabetic rats resembling type-I or severe diabetic conditions in which a maximum pancreatic β-cell damage occurred. Treatment of MIEE and MIAE exhibited improved glucose tolerance and also increases in insulin levels in response to

exogenously administered glucose. The efficacy of these extracts were comparable to standard glibenclamide, and could be mediated by improving the glycemic control mechanisms and insulin secretion from remnant pancreatic β-cells and/or extra pancreatic pathways may be in act.

DM is often linked with altered lipid metabolism. It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal condition (Diwanjee et al., 2009). The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma (Ananthan et al., 2004). It has been demonstrated that insulin deficiency in diabetes leads to a variety of disruption in metabolic and regulatory processes, which in turn lead to accumulation of lipids (Goldberg, 1981). In the present study, the above-mentioned changes in the lipid profile of diabetic animals were well documented. Treatment with the MIEE and MIAE resulted in significant attenuation in serum TG, TC, VLDL-c and LDL-c. These effects might partly be due to the insulin stimulatoryeffect of MIEE and MIAE for low secretion of cholesterol biosynthesis enzymes.

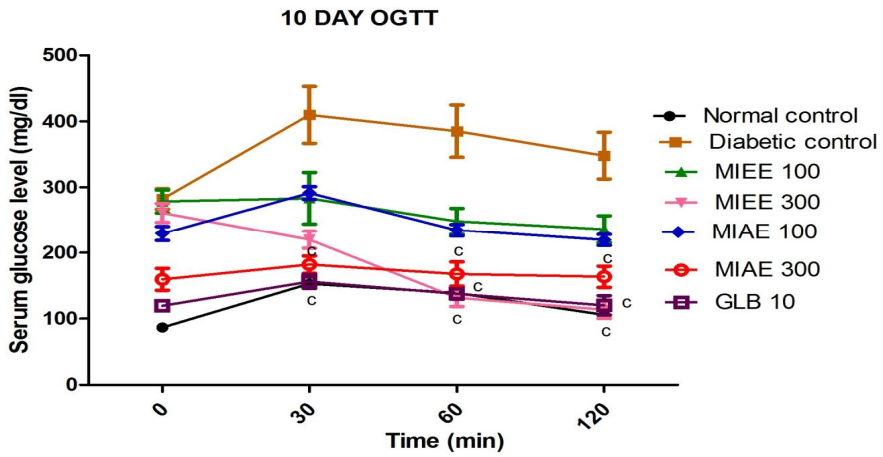
5. CONCLUSION

The present study reports for the first time to our knowledge that *Madhuca indica*aqueous extract possesses more antidiabetic activity than ethanol extract of *Madhuca indica.*Furthermore, it could also result from synergizing action of a combination of other biomarkers acting by interaction with multiple targets of diabetes. Taken together, the present study provides the scientific evidence to justify the traditional value of the title plant.

Table 1: Effect of MIEE and MIAE on SG levels in Alloxan-induced diabetic rats [Single-dose one-day study]

Treatment [dose/kg b.w]	SG levels [mg/dl]			
	0h	2h	4h	6h
Normal control	90.04±4.52	89.14±4.73 (0.99)	87.94±4.24 (2.33)	87.96±3.34 (2.31)
Diabetic Control (DC)	311.30±8.66	310.68±11.65 (0.19)	301.86±11.58 (3.03)	300.38±8.09 (3.50)
DC+MIEE [100 mg/kg]	297.90±23.41	284.92±19.56 (4.37)	291.44±26.88 (2.16)	284.84±27.61 (4.38)
DC+MIEE [300 mg/kg]	292.40±25.03	277.28±28.75 (5.17)	270.28±27.09 (7.56)	267.10±26.18 (8.65)
DC+MIAE [100 mg/kg]	303.10±11.93	288.36±9.54 (4.72)	254.12±11.67 [a] (16.24)	243.80±12.80 [b] (19.56)
DC+MIAE [300 mg/kg]	303.38±21.86	286.04±19.49 (5.71)	263.58±14.51 [a] (12.58)	215.66±14.36 [b] (27.96)
DC+GLB [10 mg/kg]	330.72±31.98	268.70±29.92 [a] (19.04)	214.52±16.46 [c] (34.48)	143.18±13.25 [c] (54.42)

Each value represents Mean ± S.E.M., n=6. Values in parentheses indicate Percent reduction in glycemia and [c]*P*<0.001, [b]*P*<0.01 and [a]*P*<0.05 compared to basal values [0 hr] of the same group. One-way ANOVA followed by Tukey's post test.



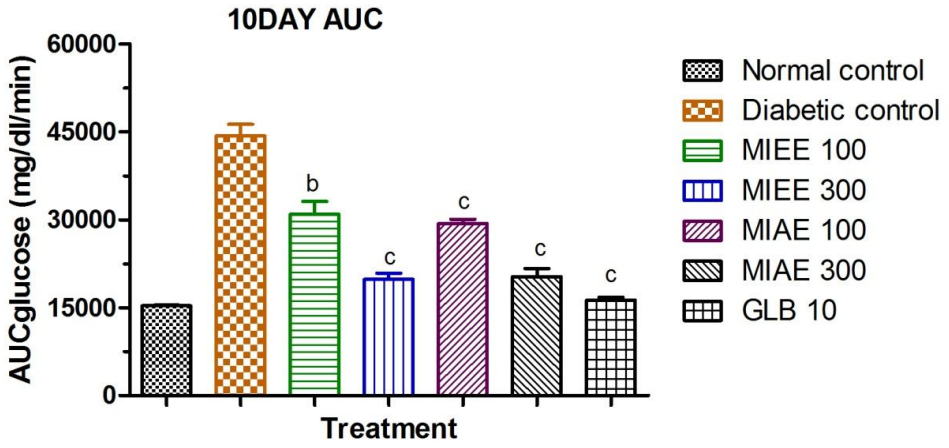


Fig. 1: Effect of MIEE and MIAE on glucose tolerance in fasted diabetic rats. [A] SG levels were measured prior to, and after p.o. administration of glucose alone (2 g/kg body weight), or in combination with MIEE, MIAE or GLB. [B] Area under curve for glucose (AUCglucose) values for 0-120 min post glucose load. Data represent the mean S.E.M., for 6 rats $^{c}P < 0.001$ as compared with normal rats (one way ANOVA followed by Tukey's post-test).

Table 2: Effect of MIEE and MIAE on SG levels in alloxan-induced diabetic rats [Multiple-dose study]

Treatment [dose/kg b.w]	SG levels [mg/dl]			
	Day 0	Day 7	Day 10	Day 15
Normal Control	90.04±4.52	87.47±2.17 (2.85)	87.20±1.73 (3.15)	86.72±2.33 (3.68)
Diabetic Control (DC)	311.30±8.66	308.90±7.96 (0.72)	306.14±6.17 (1.67)	303.88±7.35 (3.99)
DC+ MIEE [100 mg/kg]	297.90±23.41	286.38±21.32 (3.74)	275.50±19.51 (7.28)	273.86±18.61 (7.73)
DC+ MIEE [300 mg/kg]	292.40±25.03	276.78±23.26 (5.30)	258.10±22.40 (11.73)	255.66±16.58 (11.71)
DC+MIAE [100 mg/kg]	303.10±11.93	262.74±14.99 (13.51)	241.86±14.67 [a] (20.30)	223.94±11.27 [a] (25.80)
DC+ MIAE [300 mg/kg]	303.38±21.86	233.20±11.21 [a] (22.12)	163.00±15.47 [c] (44.60)	148.54±17.65 [c] (49.78)
DC+GLB [10 mg/kg]	330.72±31.98	150.32±10.19 [c] (52.19)	141.64±14.44 [c] (54.62)	95.12±4.93 [c] (69.57)
Each value represents Mean ± S.E.M., n=6. Values in parentheses indicate Percent reduction in glycemia and $^{c}P<0.001$, $^{b}P<0.01$ and $^{a}P<0.05$ compared to basal values [0 hr] of the same group. One-way ANOVA followed by Tukey's post test.				

Table 3: Effect of different doses of MIEE and MIAE in lipid profile in alloxan-induced diabetic ratsrats model [multiple dose-fifteen day study]

Serum parameter	Normal Control	Diabetic Control	MIEE 100 mg/kg	MIEE 300 mg/kg	MIAE 100 mg/kg	MIAE 300 mg/kg	GLB 10 mg/kg
FBS	89.72±2.33	306.88±7.35 [c]	223.94±18.61 [d]	255.66±16.58	223.94±11.27 [d]	148.54±17.65 [d]	95.12±4.93 [f]
STC (mg/dl)	69.46±1.25	115.84±7.07 [c]	95.42±4.34 [e]	81.6±2.94 [e]	75±0.78 [e]	75.54±1.6 [e]	71.12±3.63 [f]
STG (mg/dl)	72.96±1.18	138.36±8.56 [c]	95.12±4.21 [d]	96.62±5.04 [e]	80.02±1.02 [d]	76±2.38 [f]	72±3.21 [f]
HDL-C (mg/dl)	26.46±0.82	16.26±0.72 [c]	18.56±0.25 [e]	19.44±1.01 [d]	24.7±1.73 [f]	23.08±0.46 [f]	24.52±0.90 [f]
VLDL-C (mg/dl)	14.59±0.24	27.67±0.77 [c]	19.02±0.84 [e]	19.32±1.01 [e]	16.00±0.20 [e]	15.2±0.48 [e]	14.4±0.29 [f]
LDL-C (mg/dl)	28.48±0.87	71.90±3.69 [c]	57.83±4.84 [d]	42.83±3.39 [d]	34.296±2.19 [e]	33.26±2.23 [f]	32.2±2.43 [f]
TC/HDL-c ratio	2.6±0.06	7.1±0.33 [c]	5.13±0.21 [d]	4.25±0.33 [e]	3.101±0.24 [e]	3.10±0.12 [e]	2.97±0.16 [f]
LDL-c/HDL-c ratio	1.07±0.04	4.44±0.30 [c]	3.1±0.25 [d]	2.24±0.26 [d]	1.43±0.19 [e]	1.4±0.12 [f]	1.33±0.14 [f]
Each value represent Mean± S.E.M (n=5). $^{c}P<0.001$ compared to normal control and $^{d}P<0.05$; $^{e}P<0.01$; $^{f}P<0.001$when compared withdiabetic control. One way ANOVA followed by Tukey's post test							

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