

Antidiabetic Effect of *Memecylon Talbotianum* Leaf Methanol Active Fraction in Male Wistar Rats

Bharathi, T.R., Noor Mohamed Jameel, Behrouz Jalali Ghassam, Shrisha Naik Bajpe, Prakash HS*

Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore, INDIA.

ABSTRACT

Introduction: *Memecylon talbotianum* Brandis, (Melastomataceae) is an indigenous medicinal plant used in ethno-medicine including Ayurveda. However, the potential of this plant in controlling diabetes has not yet been evaluated. The role of *M. talbotianum* leaf extract on streptozotocin (STZ, 2-deoxy-2-(3- (methyl-3-nitrosoureido)-d-glucopyranose- induced diabetes in male Wistar rats is investigated. **Methods:** The leaf extract was fractionated, its phytoconstituents were evaluated using HPLC and rats (6 animals in each group) were treated orally with *M. talbotianum* leaf methanol active fraction (MTLMAF) (100, 200 and 1000 mg/kg body weight) for 28 days with a single dose with metformin as a positive control. Biochemical parameters, histopathological and immunostaining studies were performed.

Results: Administration of MTLMAF, significantly decreased the glucose level after four weeks (312 ± 14 mg/dL in diabetic animals compared to 134 ± 13 mg/dL in MTLMAF treated animals) and controlled the levels of TG (155 ± 63.55 units/dL in diabetic and 100.66 ± 13.31 units/dL in treated animals), LDL, HDL, VLDL and endogenous antioxidants (SOD, catalase and GSH). Histopathological studies substantiated the protective role of MTLMAF. MTLMAF also alleviated liver and pancreatic damage in STZ-

diabetic rats. HPLC analysis revealed that rutin, quercetin and protocatechuic acid were the major components present in *M. talbotianum*. The presence of these compounds in *M. talbotianum* is reported for the first time here and these compounds may be responsible for antidiabetic properties.

Conclusion: Our study shows that MTLMAF could protect rats against streptozotocin induced diabetes, improves lipid profile, reduce the risk of oxidative stress and, ameliorate liver and pancreatic damage.

Keywords: HPLC, STZ-diabetes, *M. talbotianum*, MTLMAF, Antidiabetic.

Correspondence:

Prof. H. S. Prakash,

Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore – 570 006, INDIA.

Phone numbers: 9986288837

Faxsimile numbers: 0821-2419877.

E-mail: hasiprakash@gmail.com; bharathi99tr@gmail.com

DOI: 10.5530/pc.2017.3.17.

INTRODUCTION

Medicinal plants play an appreciable role in the development of modern herbal medicines where many diseases such as cancer, liver diseases and arthritis find no complete cure in allopathy. The bioactive compounds of medicinal plants are used as antidiabetic, chemotherapeutic, anti-inflammatory and anti-arthritis agents where no satisfactory cure is available in modern medicines. Although different classes of drugs are available to control diabetes, still it is a challenging task to develop a better molecule which is devoid of undesirable adverse effects compared to existing drugs. Therefore, the search for more effective and safer hypoglycemic agents is important area of active research and the efficacy of antidiabetic medicinal plants has been increasingly documented.¹

Memecylon (Melastomataceae) belongs to the genus of shrubs and trees which consists of about 150 species, mainly in the old world tropics, used in Ayurveda and Siddha systems of medicine for the treatment of leucorrhoea, polyuria, diabetes, bacterial infections, and viral diseases including herpes and chickenpox.² The phytoconstituents of the aerial plant parts including β-amyrin, sitosterol, oleanolic acid, ursolic acid and umbellactone were shown to have various pharmacological activities.³⁻⁵

Memecylon talbotianum Brandis, is one of the endemic plants of Western Ghats of India which is distributed in moist deciduous to evergreen forests of Karnataka, Tamil Nadu, Sri Lanka and Malaya and this plant is used as a folk medicine, although in recent years, the anti-inflammatory, antidiabetic, antioxidant and antimicrobial activities of other species of the genus *Memecylon* have been reported.⁶⁻⁷ So far no scientific report is available to support the antidiabetic activity of *M. talbotianum*.

The present study investigates *in vitro* and *in vivo* antidiabetic activity of methanol active fractions of *M. talbotianum* leaves against streptozotocin-induced diabetes mellitus in male Wistar rats. The phytoconstituents present in *M. talbotianum* was characterized by high-performance liquid chromatography (HPLC) analysis. The possible mechanisms involved in the antidiabetic activity of *M. talbotianum* were also investigated.

MATERIALS AND METHODS

Chemicals

Streptozotocin, metformin 500 mg tablets, thiobarbituric acid, glutathione oxidase, glutathione reductase, pyrogallol, Triton-X 100, bovine serum albumin, propidium iodide, α- amylase, α- glucosidase, acarbose and HPLC standards were obtained from Sigma Aldrich. All other solvents and chemicals were purchased from Merck Biosciences. Rabbit polyclonal IgG, Insulin (H-86), rabbit anti-mouse insulin antibody, Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody were obtained from Santa Cruz Biotechnology Co. Limited.

Plant Materials

Fresh aerial parts of *M. talbotianum* collected from the Western Ghats of India, during June 2015 were authenticated by a taxonomist, Prof Sampath Kumara K.K., at University of Mysore (UOM). The voucher specimen (No. # IOE LP0002) were deposited at the Department of Biotechnology, UOM. The leaves of the plant were washed under running tap water to remove adhering contaminants, air dried in shade and pulverized in a mechanical grinder to obtain coarse powder.

Preparation of extracts and partial purification

Ground leaf powder (250 g) was extracted sequentially using 500 mL of non-polar and polar solvents in increasing polarity hexane < ethyl acetate < methanol <water using Soxhlet apparatus by continuous hot percolation (boiling point, 52°C to 62°C) until the solvent became colorless. The resultant solvent extracts were concentrated in a SpeedVac (Savant SPD 2010, Thermo Scientific, Germany) under reduced pressure. The yield from these solvents were - hexane (5.37 g); ethyl acetate (6.2 g); methanol (8.23 g) and water (2.32 g) from 250 g dry weight of leaf. Only methanol extract was selected for further purification as it exhibited highest antidiabetic activity. Partial purification was carried out using silica gel column chromatography 6 g of methanol extract were

mixed with 3 g of silica gel as stationary phase loaded on to the column. A glass column (50 cm×2 cm dia.) was equilibrated systematically by passing ethyl acetate repetitively. The methanol extract was loaded on to the packed glass column. Flow rate was set to 10 mL/min. The column was eluted with ethyl acetate-methanol mobile phase with the following increasing polarity: 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. Six fractions were collected from the methanol extract to obtain active fractions. The effluents were collected in a flask and allowed to dry. The yields of six fractions were 0.126, 0.236, 0.322, 0.233, 0.323, and 0.422 g. *In vitro* (bioassays) α-amylase and α-glucosidase inhibitory activities were carried out using all fractions and the active fraction was identified and named as *M. talbotianum* leaf methanol active fraction (MTLMAF).

Analysis of phytoconstituents using High Performance Liquid Chromatography (HPLC)

HPLC separation of MTLMAF was carried out as described Ruangchakpet and Sajaanantakul⁸. A thermo hypersil gold (4.6 mm × 150 cm, 5 µm) column/PDA maintained at 35°C with different detection times was used in the study. The mobile phase A used was water containing 2.5% formic acid and mobile phase B used was acetonitrile; water and acetic acid in the ratio of 45:54:1; flow rate was 1mL/min; injection volume 10 µL. The retention time for each eluted component in the analyzed sample was compared with the following standards: rutin, protocatechuic acid, quercetin, synapic acid, cinnamic acid, chlorogenic acid and synergetic acid.

***In vitro* antidiabetic studies**

Inhibition of α-amylase and α-glucosidase

Screening of crude extracts and eluted methanol fractions for pancreatic α-amylase (PPA) and α-glucosidase inhibition was carried out according to previous methods⁹⁻¹⁰.

***In vivo* studies**

Experimental Animals

The study was carried out on healthy adult male Wistar rats in the weight range of (180-200 g), selected from an inbred group housed in designed cages and maintained under standard conditions of temperature (23 ± 1°C) and humidity (55-60%) with a 12--h light/dark cycles for a minimum of one week before use. Rats were put into 6 groups each group with 6 rats on the basis of initial weight and kept in separate cages; all rats consumed rodent diet and tap water was provided *ad libitum*. Clinical monitoring of the animals was performed weekly to evaluate body weight and blood glucose and insulin levels. All animals were cared for according to the guiding principle in the care and use of animals in Central Animal House Facility, DOS in Zoology, UOM. The animal care and experimental procedures performed were in compliance with the Regulations for Animal Research and Animal Ethical Committee of the UOM (Animal Order No: UOM/IAEC/07/2013).

Experimental induction of diabetes and treatment

Diabetes was induced in male Wistar rats by intraperitoneal administration of STZ (single dose of 60 mg/kg body weight) dissolved in freshly prepared 0.01 M citrate buffer pH 4.5. After 48 h rats with marked hyperglycemia (fasting blood glucose >250 mg/dL) were selected and used for the study. All animals were cared as per the guidelines of Institute Animal Ethics committee¹¹. For animal treatments, extracts were reconstituted in water and animals were force fed orally by using 25-27 gauge feeding needles.

Selection of dose of the extract

LD50 was determined as per the Organisation for Economic Co-operation and Development (OECD) guidelines for fixing the dose for biological

evaluation. The LD50 of the extracts falls under category 5 values with no death and no signs of acute toxicity at dose of 2000 mg/kg. The biological evaluation of the extracts was carried out at dose levels of 100 and 200 mg/kg body weight.

Experimental design

The rats were divided into six groups, each having six animals; each group was given the following treatment:

Group I : Normal control rats

Group II : STZ-induced diabetic control rats.

Group III : Diabetic rats with MTLMAF (100 mg/kg)

Group IV : Diabetic rats with MTLMAF (200 mg/kg)

Group V : Control rats with only MTLMAF (1000 mg/kg)

Group VI : Diabetic rats with Standard Metformin (100 mg/kg)

Body weight and Blood glucose estimation

Blood samples were collected from tip of rat tail and blood glucose levels were estimated on 1, 7, 14, 21 and 28 days of treatment using GlucocardTM 01-mini Blood Glucose Meter. Body weight was measured initially and during treatment period. Glucose tolerance test was performed as described by Matteucci and Giampietro¹².

Analysis of biochemical parameters and serum enzymes

On completion of the treatment period, rats were anesthetized by inhalation and blood was drawn by tail vein puncture and cardiac puncture. The whole blood was centrifuged at 2000 × g at 4°C for 10 min. Serum samples were collected and stored at -20°C for further use. The levels of triglycerides, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) in the serum samples were measured using commercial assay kits as obtained from Agappe Diagnostics as per the manufacturer's protocol using a semi autoanalyser (Mispa Excel Chemical Analyser). Results are expressed as units/dL.

Analysis of antioxidant enzymes

The liver was excised, weighed and homogenized (10% w/v) in ice-cold 50 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 4000 × g at 4°C for 10 min and the supernatant obtained was stored at -20°C. The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) were measured from the supernatant. SOD activity was measured using pyrogallol (2 mM) auto-oxidation in Tris buffer¹³. CAT activity was assayed by using H₂O₂ as the substrate in phosphate buffer¹⁴ and GSH was estimated using Ellman's reagent¹⁵.

Estimation of protein

Protein was determined by the method of Lowry¹⁶ using bovine serum albumin as standard, at 660 nm.

Histopathology of liver and pancreatic tissues

For the histopathological studies, pancreas and liver tissue samples were collected and fixed in 10% buffered formalin, embedded in paraffin wax. Paraffin embedded tissue sections (4 µm each) were deparaffinized, rehydrated, and subjected to hematoxylin and eosin staining and examined microscopically at 1×400 magnification¹⁷.

Immunostaining studies of pancreatic tissues

Immunohistochemistry was performed on formalin fixed, paraffin-embedded hepatic and pancreatic tissues. Pancreatic samples were immunostained with rabbit polyclonal IgG Insulin (H-86) according to

manufacturer's instructions. For immunostaining studies, the pancreatic tissues were fixed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.3) for 1 h at 4°C, immersed in 0.1 M phosphate buffered saline (PBS) at room temperature, washed with PBS, allowed to permeabilize with 0.1% Triton-X 100 and again washed with PBS. The primary antibody rabbit anti-mouse insulin antibody (1:50) in PBS + 30 mg/mL BSA+ 0.1% Triton X-100 were added, incubated for 90 min and washed 3 times consequently with 0.1% Triton X-100 in PBS. Secondary antibody horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (1:200 was added at proper dilutions in PBS + BSA) washed 3 times with 0.2% Triton X-100. The tissue sections were incubated for 60 min at room temperature, and nuclei were stained with propidium iodide (PI-1:1000) for one min and washed with PBS, observed and analysed using confocal laser scanning imaging facility (LSM 710, Carl Zeiss, Germany) equipped with an excitation filter 515-560 and magnification at 20x¹⁸.

Statistical analysis

The experiment was carried out with three replications. Data are expressed as mean ± standard deviation (SD) (n = 6). Differences between treatment means were compared for significance using Kruskal-Wallis analysis of variance at P≤0.05.

RESULTS

In vitro antidiabetic studies

Inhibition of α-amylase and α-glucosidase

The IC₅₀ values for α-amylase of crude methanol, ethyl acetate and water extracts including standard acarbose were found to be 160, 130, 122, 120 µg at 240 µg/reaction respectively (Fig 1A), and the IC₅₀ values for α-glucosidase were found to be 9.1, 7.9 and 7.0 µg for methanol and ethyl acetate extracts, including standard acarbose at 15 µg/reaction (Fig 1B). Further methanol extract which has shown the lowest IC₅₀ value was partially purified using silica gel column chromatography to obtain active fraction and fractions were further eluted. Among these eluted fractions, the fraction ethyl acetate-methanol ratio of 20:80 showed the highest α-amylase and α-glucosidase inhibition with IC₅₀ values of 180 and 10 µg (Fig 1A & 1B) respectively. Hence this fraction was selected as active fraction named as *M. talbotianum* leaf methanol active fraction (MTLMAF) and used in all *in vivo* studies.

Analysis of phytoconstituents using high performance liquid chromatography (HPLC)

The phytochemical analysis of MTLMAF by HPLC revealed the presence of several phenolic constituents and flavonoids. The constituents included protocatechic acid, syneric acid, ferulic acid, sinapic acid, rutin, chromoic acid, quercetin and cinnamic acid (Fig 2).

In vivo studies

Body weight and Blood glucose estimation

The initial and final (28th day) body weights of rats are summarized in Fig 3A & 3B. During the course of experiment, the body weight of normal control animals significantly increased (3.2% over their initial readings) after 28 days of treatment, whereas a significant (P<0.05) decrease (5.3%) was observed in diabetic control rats. Administration of MTLMAF or the standard metformin to diabetic rats reversed this weight loss. Significant (P<0.05) increases in the final body weight of these animals was observed. As shown in Fig 3A, the level of glucose increased by three folds on injection of STZ. On oral administration of MTLMAF at the concentration of 100 and 200 mg/kg/day to diabetic rats, the glucose level was reduced by 50% in the first week of treatment,

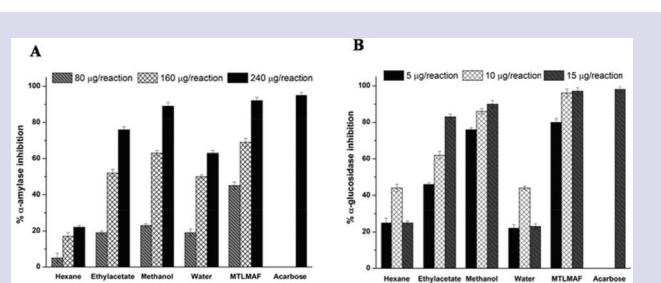


Figure 1: *In vitro* antidiabetic property represented through inhibition of α-amylase and α-glucosidase enzyme by different solvent extracts of *M. talbotianum*.

Figure 1: Inhibition of α-amylase (concentration per total reaction mixture 250 µL) and α-glucosidase (concentration per total reaction mixture 100 µL) of various solvent extracts from *Memecylon talbotianum*. Acarbose was used as a positive control. Results are expressed as percentage of the control. Each value is expressed as Mean ± SD, n=3, p<0.05 compared to positive control.

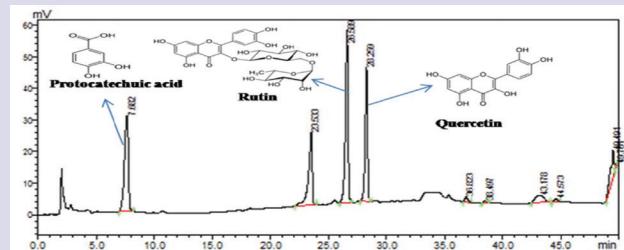


Figure 2: Analysis of phytoconstituents of MTLMAF based on their retention time comparison with standard phytoconstituents

Figure 2: HPLC profile of MTLMAF. Thermo hypersil gold (4.6 mm x 150 cm, 5µm,) column maintained at 35°C was used in the study. The binary mobile phase consisted of 2.5% formic acid (A) and acetonitrile; water and acetic acid (45:54:1) (B). The gradient elution was as follows: 18% B for 9 min, 18-50% B over 20 min, 50% B for 16 min. A 10 µL aliquot of sample solution was injected into the column. The flow rate was maintained at 1 mL / min. Chromatographic data were recorded by PDA at different detection times. The presence of bioactives was detected based on their retention time.

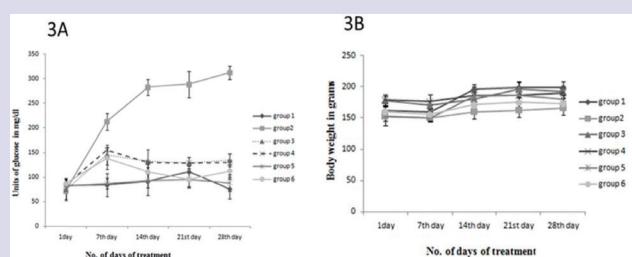


Figure 3: Effect of MTLMAF on glucose level in Streptozotocin-induced diabetic male wistar rats.

Figure 3A & 3B: Values are expressed as Mean ± SD (n=6). Gp I - Normal rats, Gp II -STZ-induced diabetic control rats, Gp III - Diabetic rats with MTLMAF (100 mg/kg), Gp IV - Diabetic rats with MTLMAF (200 mg/kg), Gp V - Control rats with only MTLMAF (1000 mg/kg), Gp VI - Diabetic rats with Standard Metformin 100 mg/kg.

Table 1: Effect of MTLMAF on biochemical parameters in Streptozotocin-induced male Wistar diabetic rats

Profiles	Control	Diabetic control	Diabetic + 100mg MTLMAF	Diabetic + 200mg MTLMAF	Non-diabetic + only MTLMAF 1000mg	Diabetic + Metformin 100mg
Serum cholesterol ^b	68.00 ± 7.11	84.33 ± 8.60 [#]	94.21 ± 5.50 [*]	86.04 ± 10.01 [*]	79.01 ± 10.04 [*]	77.66 ± 13.08 [*]
Serum triglycerides ^b	71.25 ± 24.33	155.01 ± 63.55 [#]	100.33 ± 9.71 [*]	100.66 ± 13.31 [*]	77.33 ± 14.01 [*]	91.01 ± 36.03 [*]
HDL cholesterol ^b	38.25 ± 1.25	17.66 ± 1.15 [#]	28.66 ± 1.15 [*]	24.01 ± 8.01 [*]	41.66 ± 3.05 [*]	26.33 ± 6.65 [*]
LDL cholesterol ^b	42.01 ± 2.16	86.06 ± 13.37 [#]	80.86 ± 1.22 [*]	71.06 ± 4.71 [*]	49.86 ± 5.31 [*]	77.13 ± 14.25 [*]
VLDL cholesterol ^b	14.65 ± 4.49	29.01 ± 12.71 [#]	20.06 ± 1.94 [*]	21.13 ± 2.66 [*]	18.08 ± 3.03 [*]	24.02 ± 7.27 [*]
CHOL:HDLCHOL ^b	2.75 ± 0.20	3.63 ± 0.46 [#]	2.08 ± 0.31 [*]	3.43 ± 0.57 [*]	2.53 ± 0.37 [*]	2.02 ± 3.98 [*]
LDL:HDL ratio ^b	1.10 ± 0.12	4.90 ± 0.92 [#]	2.49 ± 0.23 [*]	3.36 ± 0.47 [*]	1.19 ± 0.26 [*]	2.92 ± 0.68 [*]
Blood Urea ^b	15.2 5± 4.34	19.01 ± 9.01 [#]	18.01 ± 1.03 [*]	18.08 ± 2.02 [*]	16.07 ± 1.08 [*]	19.01 ± 6.08 [*]
Creatinine ^b	0.62 ± 0.06	0.68 ± 0.08 [#]	0.64 ± 0.15 [*]	0.69 ± 0.06 [*]	0.60 ± 0.06 [*]	0.68 ± 0.08 [*]
SGOT(AST) ^a	567.05 ± 65.01	602.33 ± 151.01 [#]	576.33 ± 40.09 [*]	549.33 ± 70.43 [*]	547.66 ± 21.36 [*]	497.66 ± 109.02 [*]
SGPT(ALP) ^a	187.00 ± 189.05	208.03 ± 17.01 [#]	79.01 ± 15.71 [*]	106.33 ± 33.85 [*]	176.33 ± 12.66 [*]	75.33 ± 15.63 [*]
AP ^a	321.25 ± 42.04	503.02 ± 106.78 [#]	430.33 ± 37.44 [*]	440.66 ± 17.07 [*]	440.01 ± 46.93 [*]	477.66 ± 190.43 [*]
Total protein	7.00 ± 0.45	8.43 ± 0.63 [#]	7.22 ± 0.24 [*]	7.05 ± 0.12 [*]	6.80 ± 0.21 [*]	7.11 ± 0.36 [*]

Table 1: Values are expressed as Mean ± SD (n=6 for each group). # denotes P<0.05 when compared Normal control group; * denotes P<0.05 when compared to diabetic control group. MTLMAF – *M. talbotianum* leaf methanol active fraction. 'a' – values expressed as units of activity /mg protein. 'b' – values are expressed as units /dL

Table 2: Effect of MTLMAF on enzymatic and non-enzymatic antioxidants in the liver tissues of Streptozotocin-induced diabetic male Wistar rats

Groups	Catalase ^a	SOD ^a	GSH ^b
Control	32.7 ± 1.4	24.7 ± 1.2	40.1 ± 1.9
Diabetic control	22.4 ± 1.8 [#]	17.8 ± 0.9 [#]	18.2 ± 0.9 [#]
Diabetic +100mg MTLMAF	26.8 ± 1.6 [*]	21.7 ± 1.7 [*]	32.1 ± 0.9 [*]
Diabetic + 200mg MTLMAF	29.7 ± 0.8 [*]	23.8 ± 1.2 [*]	38.9 ± 0.6 [*]
Non diabetic +1000mg MTLMAF	30.7 ± 1.4 [*]	24.0 ± 0.9 [*]	42.1± 0.9 [*]
Diabetic + Metformin 100mg	36.4 ± 1.8 [*]	25.8 ± 0.2 [*]	42.2 ± 2.3 [*]

Table 2: Values are expressed as Mean ± SD (n=6 for each group). # denotes P<0.05 when compared Normal control group; *denotes P<0.05 when compared to diabetic control group. MTLMAF – *M. talbotianum* leaf methanol active fraction. 'a' – values expressed as units of activity /mg protein. 'b' – values are expressed as units /dL

when compared to diabetic control animals. At the end of the treatment, this reduction reached to 78%.

The glucose tolerance test performed with 100 mg of MTLMAF treatment showed improved glycemic control after 30 min of treatment. There was about 72% reduction in glucose level after 150 min of treatment when compared to diabetic rats (data not shown).

Analysis of biochemical parameters and serum enzymes

The effect of MTLMAF on these lipids were estimated their concentrations in all our experimental groups. Administration of MTLMAF, significantly decreased the glucose level after four weeks (312 ± 14 mg/dL in diabetic animals to 134 ± 13 mg/dL in MTLMAF treated animals) and controlled the levels of TG (155 ± 63.55 units/dL in diabetic and 100.66 ± 13.31 units/dL in treated animals), LDL (86.06 ± 13.37 units/dL in diabetic and 71.06 ± 4.71 units/dL in treated animals), HDL (17.66 ± 1.15 units/dL in diabetic and 24.01 ± 8.01 units/dL in treated animals), VLDL (29.01 ± 12.71 units/dL in diabetic and 20.06 ± 1.94 units/dL in treated animals). In diabetic control group the concentrations of TC, LDL, VLDL, TG were found elevated whereas the concentration of HDL was decreased. Treatment with MTLMAF and metformin showed marked reversal of changes in the serum lipid parameters as compared

to diabetic rats. The activities of SGOT, SGPT and ALP enzymes were found elevated in Group II as compared to Group I treated rats. In Group III and Group IV animals which received with MTLMAF showed a marked decrease in the enzyme activities, whereas protein concentration was found enhanced (Table 1).

Analysis of antioxidant enzymes

The treatment of diabetic animals with MTLMAF (100-200 mg/kg) increased the activities of catalase (19-35%), SOD (21-33%) and glutathione (50-56%) when compared to untreated diabetic rats. Hence, MTLMAF effectively controlled the oxidative stress under disease conditions by increasing the activities of these antioxidants. The levels of enzymatic and non-enzymatic antioxidants in the liver tissues of various groups of experimental animals are presented in Table 2.

Histopathology of pancreatic and liver tissues

Histopathological studies of the pancreatic and liver tissues were performed and results are shown in Fig 4A & 4B. Group I rats showed normal architecture of the pancreas with dark, granulated beta cells in the islet of Langerhans. Induction of diabetes using streptozotocin resulted in damaged, shrunken islets. There is evidence of atrophic changes,

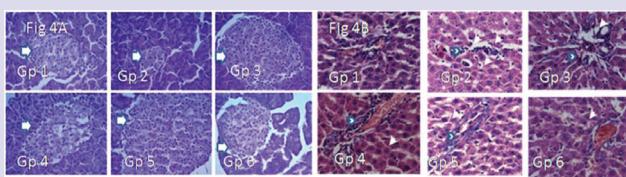


Figure 4: Histopathology of Streptozotocin-induced diabetic male wistar rat pancreatic and liver tissues

Figure 4A & 4B: Histopathology of rat pancreatic and liver tissue
Gp I - Normal rats, Gp II - STZ-induced diabetic control rats,
Gp III - Diabetic rats with MTLMAF (100 mg/kg), Gp IV - Diabetic rats with MTLMAF (200 mg/kg), Gp V - Control rats with only MTLMAF (1000 mg/kg), Gp VI - Diabetic rats with Standard Metformin 100 mg/kg as visualized by fluorescence microscopy.

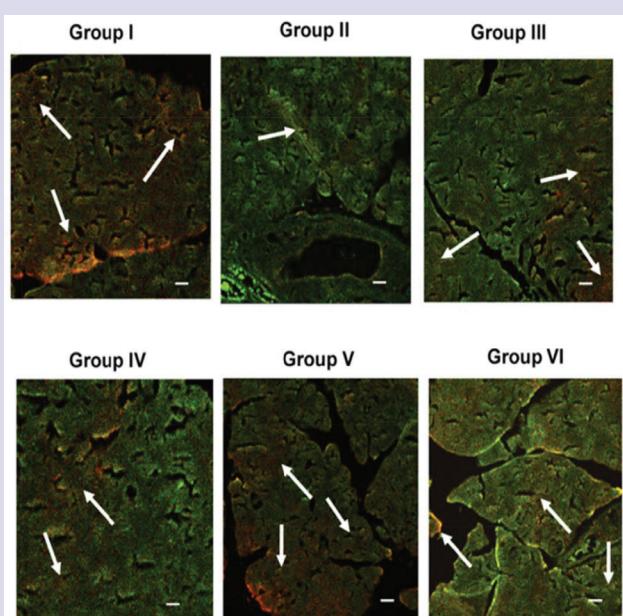


Figure 5: Pathological and Immunohistochemical staining of Streptozotocin-induced diabetic male wistar rat pancreatic tissues

Figure 5: Pathological and Immunohistochemical analysis of rat pancreas streptozotocin induced diabetic male Wistar rats were treated with MTLMAF the pancreas were isolated for anti-insulin immunostaining against insulin antibodies as visualized by the confocal microscopy equipped with an excitation filter 515-560 and magnification at 20x, scale bar: 10 µm. Gp I - Normal rats, Gp II STZ-induced diabetic control rats, Gp III - Diabetic rats with MTLMAF (100 mg/kg), Gp IV - Diabetic rats with MTLMAF (200 mg/kg), Gp V - Control rats with only MTLMAF (1000 mg/kg), Gp VI - Diabetic rats with Standard Metformin 100 mg/kg. An Arrow mark indicates co-localization.

vacuolation, infiltration of lymphocytes and damage in connective tissue parenchyma of islets in these diabetic rats. The abnormal histopathology of pancreas due to streptozotocin-induced diabetes was reversed in the MTLMAF and metformin treated diabetic animals. Treatment with MTLMAF in Group III animals resulted in appearance of less damaged islets cells when compared to untreated diabetic rats (Group II). The recovery in metformin-treated group was evident by the near normal

size islets and granulated beta cells (Fig 4A). The histopathological studies of the liver tissues were also performed (Fig 4B). In Group I animals the portal tracts were composed of portal vein radical, and bile duct radical. The central veins appeared normal. In diabetic Group II rats the hepatocytes showed global microvesicular steatosis, dilated bile duct with inflammatory cell infiltration in the portal area. In the nondiabetic rats treated with MTLMAF, the hepatocytes, portal tracts and central veins appeared normal. In Group III animals, the hepatocytes showed mild inflammation, central veins appeared normal and no steatosis was observed. However, diabetic metformin treated group showed maximum connective tissue restoration (Fig 4B).

Immunostaining studies of pancreatic tissues

Weak insulin immunoreactivity was detected in the pancreas of STZ treated rats compared to control group. Whereas in the tissues of MTLMAF treated group, significant increased immunoreactivity of insulin was observed as evidenced by co-localization (Fig 5).

DISCUSSION

Streptozotocin induces necrosis of beta cells resulting in diabetes mellitus¹⁹ and used to induce experimental diabetes in animals. The long term effects of diabetes include damage, dysfunction and failure of various organs²⁰. According to WHO reports, there are 347 million people worldwide suffering from diabetes and deaths may double between 2005 and 2030²¹. The management of diabetes without any side effects is still a challenge to the medical profession. Further, after the recommendations made by WHO on diabetes mellitus²², investigation on hypoglycemic agents from medicinal plants has received greater attention. More than 800 plants are reported to be in use as traditional remedies for diabetes²³.

The study was aimed to investigate the therapeutic potential of *M. talbotianum* with respect to diabetes by employing *in vitro* and *in vivo* studies. Carbohydrate hydrolyzing enzymes α-amylase and α-glucosidase are responsible for glucose generation from diet. α-Amylase hydrolyzes α-1,4-glycosidic bonds and splits up starch components into smaller oligosaccharides and disaccharides, such as maltose. The α-glucosidases hydrolyze disaccharides to monosaccharides. Inhibition of these two enzymes is important for management of diabetes. Extracts of green tea have α-amylase inhibitory activity²⁴ and a number of plant extracts have been shown to exert antidiabetic property through α-glucosidase inhibition. The methanol extract of *M. talbotianum* showing the highest IC50 value was partially purified and named as *M. talbotianum* leaf methanol active fraction (MTLMAF) and used in all *in vivo* studies in this study.

The HPLC analysis of MTLMAF revealed the presence of phenolic and flavonoid constituents. These constituents are proven to have antioxidant and antidiabetic properties²⁵. Among these protocatechuic acid, quercetin and rutin are the major compounds. Antidiabetic properties of these compounds have been shown by several reports²⁶⁻²⁸. Hence, it can be predicted that these molecules have prominent roles in controlling diabetes and its related complications. But, other molecules present may also get involved, especially in the protection of pancreatic damage and in controlling associated biochemical parameters.

Diabetic animals tend to lose their weight due to altered metabolism³. During the course of experiment, the body weight of normal control animals significantly increased after 28 days of treatment, whereas a significant decrease was observed in diabetic control rats. STZ is known to increase glucose levels in experimental animals by decreasing the production of insulin causing damage to pancreatic cells. The observed values are comparable to metformin treated group rats²⁹.

Decreases in the concentration of HDL and high levels of cholesterol, lipoprotein and triglycerides along with the altered cholesterol/HDL ratio

are the predictors of coronary heart disease including atherosclerosis³⁰. Diabetes is also associated with profound alterations in plasma lipid, triglycerides and lipoprotein profile. Consequently these may lead to heart ailments, which are the major causes of morbidity and deaths in diabetic subjects³¹. Therefore, to evaluate the effect of MTLMAF on these lipids, we estimated their concentrations in all our experimental groups. *Salacia L.* (Celastraceae) species have been widely used as antidiabetic medicine in ayurveda from several years. Chronic oral administration of the *S. oblonga* Wall. root extract reduced the cardiac TG and other lipid contents as demonstrated by³². The results which obtained with MTLMAF were compared with those of *salacia* species^{33,34}. The liver function is assessed by SGOT, SGPT and ALP. Any alteration in the activity of these enzymes is the indication for liver malfunctioning²³. Ohaeri²⁴ found that liver was necrotized and the activities of these enzymes were increased in STZ-induced diabetic rat serum. This may be mainly due to leakage of these enzymes from liver cytosol into blood stream. To further validate the change in the activities of these enzymes, we performed the histopathological studies of liver. These results indicated the liver protective effect of MTLMAF in diabetic animals. Increased levels of urea and creatinine in the serum are considered as significant markers of renal dysfunction, which are generally evident in chronic diabetes³⁰. In this study no significant changes in these were observed and hence studies on kidney damage was not considered.

Several *in vitro* and *in vivo* studies have demonstrated that superoxide generation is increased in diabetes which is associated with β -cell dysfunction along with the decrease in the activities of endogenous antioxidants³⁵. The main players of endogenous antioxidant defense of the islets are SOD, catalase, glutathione and GPx enzymes and glutathione molecule³⁶. Therefore, induction of these endogenous antioxidants may strengthen islets against detrimental effect of ROS. Several compounds such as flavonoids isolated from plants have antioxidant properties. The plant extracts which are used in treatment of diabetic complications have shown to ameliorate the activities of these antioxidants¹. In the present study we found that treatment of diabetic animals with MTLMAF increased the activities of catalase, SOD and glutathione when compared to untreated diabetic rats. Hence, MTLMAF effectively controlled the oxidative stress under disease conditions by increasing the activities of these antioxidants.

Histopathological and immunostaining studies of the pancreatic and liver tissues indicated the protective effect of MTLMAF in diabetic animals, which is in accordance with previous reports³⁰. This supports our other performed experiments. Since diabetes is a chronic metabolic disease which requires long term treatment, determining the safety of the drug is crucial. Hence, we evaluated the toxicity of MTLMAF up to the dose level of 1000 mg/kg. No significant changes either in the body weight or behavior of treated animals were observed. Several biochemical parameters were investigated and histopathological observations were made. But no noticeable change in any of the tested parameters during the entire treatment phase was observed. Thus it can be concluded that MTLMAF does not produce any adverse toxic effect on treated animals.

CONCLUSION

MTLMAF from *Memecylon talbotianum* plant helps to control the STZ induced diabetes. Improves lipid profile, reduce the risk of oxidative stress and, ameliorate liver and pancreatic damage. However, detailed mechanistic studies are needed for validation of the active compound(s), before developing a therapeutically potential antidiabetic molecule.

ACKNOWLEDGEMENT

The authors acknowledge the support form Ministry of Human Resource Development and University Grant Commission under Institution of

Excellence scheme awarded to University of Mysore (F. No. 8-3/2008-U.I) and UGC fellowship scheme (Or. No. DV9/192/NON-NETFS/2013-14 dated: 11-11-2013).

CONFLICT OF INTEREST

None declared.

ABBREVIATION USED

STZ - 2-deoxy-2- (3- (methyl-3-nitrosoureido) - D-glucopyranose, MTLMAF - *Memecylon talbotianum* leaf methanol active fraction, OECD - Organisation for Economic Co-operation and Development, ALP - alkaline phosphatase, AST - aspartate transaminase, ALT - alanine transaminase, CAT - catalase, GSH - glutathione, SOD - superoxide dismutase, HE- hematoxylin-eosin, PBS- phosphate buffered saline, HRP - Horseradish peroxidase, PI - Propidium iodide.

Source(s) of support: UGC fellowship scheme

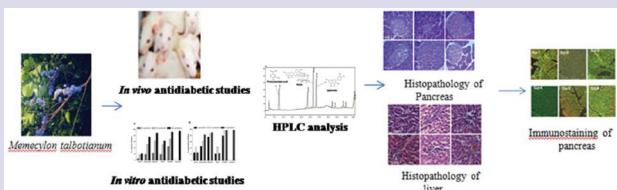
Acknowledgement: The authors acknowledge the support from UGC fellowship scheme (Or. No. DV9/192/NON-NETFS/2013-14 dated: 11-11-2013) and Ministry of Human Resource Development and University Grant Commission, Government of India, under the Institution of Excellence scheme awarded to the University of Mysore, Mysore, India (F. No. 8-3/2008-U. I).

REFERENCES

- Mukherjee PKK, Maiti K, Mukherjee, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. *J Ethnopharmacol.* 2006; 106: 1-28.
- Mohideen S, Hari Babu L, Anbuselvam C, Balasubramanian MP. Antimicrobial activity of *Memecylon edule* ROXB, and *Memecylon umbellatum* BURM.F. *Int. J. Pharm. Sci. Rev. Res.* 2012; 15(1): 79-82.
- Amalraj T, Ignacimuthu S. Evaluation of the hypoglycaemic effect of *Memecylon umbellatum* in normal and alloxan diabetic mice. *J Ethnopharmacol.* 1998; 62: 247-250.
- Bharathi TR, Nadafi R, Prakash HS. *In vitro* antioxidant and anti-inflammatory properties of *Memecylon talbotianum* Brandis. *Int. J. Phytochem.* 2014; 4: 148-152.
- Bharathi TR, Madhusudan MC, Chandranayaka S, Prakash HS. Antimicrobial potential of *Memecylon L.* species from Western Ghats against clinical isolates of pathogenic bacteria. *Res. J. Pharm. Biol. Sci.* 2015; 6: 1280-1287.
- Bharathi TR, Sampath Kumara KK, Prakash HS. *Memecylon* Species: A Review of traditional information and taxonomic description. *Int J Phar & Pharm Sci.* 2016a; 8: 26-34.
- Bharathi TR, Shaiasree S, Sampath Kumara KK, Madhusudan MC, Prakash HS. Metabolite profiling by UPLC-PDA-ESI/HDMS and antibacterial activity of *Memecylon talbotianum* Brandis. *Pharmacogn. Commn.* 2016b; 6: 225-231.
- Ruangchakpet A, Sajaanantakul T. Effect of browning on total phenolic, flavonoid content and antioxidant activity in Indian gooseberry (*Phyllanthus emblica* Linn.). *Kasetsart. J. Nat. Sci. Biol. & Med.* 2007; 41: 331-337.
- Subramanian R, Asmawi MZ Sadikun A. *In vitro* alpha-glucosidase and alpha-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. *Acta. Biochim. Polonica.* 2008; 55: 391-398.
- Akshatha VJ, Nalini MS, D'Souza C, Prakash HS. Streptomyces endophytes from antidiabetic medicinal plants of the Western Ghats inhibit alpha-amylase and promote glucose uptake. *Lett. Appl. Microbiol.* 2013; 58: 433-439.
- Kumar KA, Umamaheswari M, Sivashanmugam AT, Subhadraadevi V, Somanathan SS, Ravi TK. Protective effect of *Asystasia gangetica* reduced oxidative damage in the small intestine of streptozotocin-induced diabetic rats. *Oriental Phar & Exp. Med.* 2009; 9: 307-314.
- Matteucci E, Giampietro O. Proposal open for discussion: defining agreed diagnostic procedures in experimental diabetes research. *J Ethnopharmacol.* 2008; 115: 163-172.
- Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* 1974; 47: 469-474.
- Aebi H. Catalase. In: Bergmeyer HU, ed. *Methods in Enzymatic Analysis*. Vol. 3. New York: Academic Press Inc, 1974. 673-86.
- Ellman GL. Tissue sulphhydryl groups. *Archives Biochemistry & Biophysics.* 1959; 82: 70-77.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 1951; 193: 265- 275.
- Ghaffari H, Venkataramana M, ChandraNayaka S, Ghassam BJ, Angaswamy NC, Shekar S, SampathKumara KK Prakash HS. Hepatoprotective action of *Orthosiphon*

- diffusus* (Benth.) methanol active fraction through antioxidant mechanisms: An *in vivo* and *in vitro* evaluation. *J Ethnopharmacol.* 2013; 149: 737-44.
18. Zhang Z, Jiang J, Yu P, Zeng X, Lerrick JW, Wang Y. Hypoglycemic and beta cell protective effects of andrographolide analogue for diabetes treatment. *J. Trans. Med* 2009; 7: 1-13.
 19. Ohly P, Gleichmann H. Metallothioneine: *in vitro* induction with zinc and streptozotocin in pancreatic islets of mice. *Exp. Clin. Endocrinol.* 1995; 78-82.
 20. World Health Organization. 1999. Definition, diagnosis and classification of diabetes mellitus and its complications: Report of a WHO Consultation. Part 1. Diagnosis and classification of diabetes mellitus. Geneva, World Health Organization.
 21. World Health Organization. 2014. Global Health Estimates: Deaths by Cause, Age, Sex and Country, 2000-2012. Geneva, WHO.
 22. World Health Organization. 1980. The WHO expert committee on diabetes mellitus. Technical report series.
 23. Kasetti RB, Rajasekhar MD, Kondeti VK, Fatima SS, Kumar EGT, Swapna S, Rao CA. Antihyperglycemic and antihyperlipidemic activities of methanol- water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. *Food. Chem. Toxicol.* 2010; 48: 1078-1084.
 24. Ohaeri OC. Effect of garlic oil on the levels of various enzyme in the serum and tissue of streptozotocin diabetic rats. *Bioscience. Reproduction.* 2001; 21:19-24.
 25. Honda M, Hara Y. Inhibition of rat small intestinal sucrase and alpha-glucosidase activities by tea polyphenols. *Biosci. Biotechnol. Biochem.* 1993; 57: 123-124.
 26. Montpied P, de Bock F, Rondouin G, Niel G, Briant L, Courseau AS, Lerner-Natoli M, Bockaert J. Caffeic acid phenethyl ester (CAPE) prevents inflammatory stress in organotypic hippocampal slice cultures. *Mol. Brain. Res.* 2003; 115: 111-120.
 27. Rifai RA, El-Tahawy NF, Ali Saber E, Ahmed R. Effect of quercetin on the endocrine pancreas of the experimentally induced diabetes in male albino rats: A histological and immuno-histochemical study. *Sci. Rep.* 2012; 1: 330.
 28. Kakkar S, Bais S. A review on protocatechuic acid and its pharmacological potential. *Pharmacology.* 2014; 1-9.
 29. Nitre NT, Ansari AA, Naik SR. Anti-hyperglycemic activity of rutin in streptozotocin-induced diabetic rats: an effect mediated through cytokines, antioxidants and lipid biomarkers. *Indian. J. Exp. Biol.* 2014; 52: 720-7.
 30. Kumar S, Kumar V, Prakash O. Antidiabetic, hypolipidemic and histopathological analysis of *Dillenia indica* (L.) leaves extract on alloxan induced diabetic rats. *Asian Pac J. Trop. Med.* 2011; 347-352.
 31. Aguilera AFJ, Ramos RR, Gutierrez PS, Contreras AA, Weber CCC, Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. *J Ethnopharmacol.* 1998; 61: 101-110.
 32. Ronald A, Codario MD. Diabetic dyslipidemia. Type 2 diabetes, pre-diabetes, and the metabolic syndrome. *Curr. Clin. Practice.* 2011; 213-238.
 33. Huang TH, Yang Q, Harada M, Uberai J, Radford J, Li GQ. *Salacia oblonga* root improves cardiac lipid metabolism in zucker diabetic fatty rats: modulation of cardiac PPAR-alpha-mediated transcription of fatty acid metabolic genes. *Toxicol. Appl. Pharm.* 2006; 210: 78-85.
 34. Yoshikawa M, Shimoda H, Nishida N, Takada M., Matsuda H. *Salacia reticulata* and its polyphenolic constituents with lipase inhibitory and lipolytic activities have mild antiobesity effects in rats. *J. Nutr.* 2002; 132: 1819-24.
 35. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and β cell dysfunction. *Diabetes.* 2003; 52(1): 1-8.
 36. Gul M, Kutay FZ, Temocin S, Hanninen O. Cellular and clinical implications of glutathione. *Indian. J. Exp. Biol.* 2000; 38, 625-634.

PICTORIAL ABSTRACT



SUMMARY

- The *Memecylon talbotianum* is an indigenous medicinal plant used in ethno medicine including ayurveda and siddha mainly used in the treatment of skin diseases.
- Diabetes was induced in male Wistar rats by using STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose).
- *in vitro* and *in vivo* antidiabetic properties of MTLMAF including protection against tissue damage was evaluated.
- Our study shows that MTLMAF of *Memecylon talbotianum* helps in controlling glucose level by its protective effect on pancreatic damage in STZ induced diabetic rats along with modulating other physiological parameters.

ABOUT AUTHORS



Dr. Bharathi, T. R. is a Scientist (EU-alba), Department of studies in Biotechnology, University of Mysore, Mysuru. Her research is focused on DNA barcoding, metabolite profiling, characterization and assaying bioactive potential of Medicinal plants including seed technology.



Dr. Prakash, H. S. Professor, Department of studies in Biotechnology, University of Mysore, Mysuru has research interests in Plant and microbial biotechnology, molecular diagnostics, molecular markers and metabolite diversity, metagenomics, bioprospecting of medicinal plants and endophytes. He has several publications and patents to his credit. At present he is handling a three crore European Union coordinated project on "Novel strategies to increase the yield of sorghum and millets", along with partners from Denmark, Burkina Faso and Tanzania. He has visited USA, Japan, Italy, Denmark, Germany, China, Tanzania, Bangladesh, Nepal and Vietnam on academic assignments. He has demonstrated the role of peroxidise, lipoxygenase, proton-ATPase, Superoxide dismutase, their isoforms and transcriptomes in biotic and abiotic stress. He has shown the diversity of bark endophytes in medicinal trees, their bioactive potential and characterized novel metabolites. He has identified promising disease-tolerant lines for various diseases. The role of PGPR and RNA interference in virus disease management was demonstrated. He has developed serological and molecular diagnostic tools for the identification of plant.