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Research Article

ANTIDIABETIC POTENTIAL OF *MEMECYLON TERMINALE DALZ* EXTRACTS IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Memecylon terminale Dalz is an endemic plant that is exclusively present in the Western Ghat region of Southern India. The traditional healers in this region have been using this plant extract to cure disorders such as dysentery, diabetes, diarrhea, piles, haemoptysis, menorrhagia and shown to possess carminative stomachic astringent property. The present study was carried out to evaluate the hypoglycemic activity of different extracts of *M. terminale Dalz* in alloxan induced diabetic rats. Chloroform and methanolic extracts of *M. terminale Dalz* leaves were screened for hypoglycemic activity in alloxan induced diabetes in Swiss albino rats. The lipid profile was also examined in diabetic rats administered with these extracts. The efficacy of these extracts was also analyzed for their ability to inhibit α-glucosidase and α-amylase. Of the two extracts, chloroform extract (500 mg/kg body weight) showed dose dependent decrease in blood glucose level which was comparable to that of control after 24 h of extract administration. On the other hand, chloroform extract also showed good lipid profile that was comparable with standard drug. As compared to chloroform extract, the methanolic extract showed good inhibition of α-amylase and α-glucosidase enzyme activity. The results of our study indicate that *M. terminale Dalz* plant possesses significant protective effects against alloxan induced diabetes. However, detailed structure function analysis of active ingredients of the extract is needed to be validated.

Key Words: *M. terminale Dalz*, hypoglycemic activity, alloxan, α-amylase, α-glucosidase

INTRODUCTION

Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment [1]. Although herbs have been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. It is a well-established fact that plant-derived compounds offer potential sources of new antibiotics [2]. World Health Organization (WHO) recognizes diabetes mellitus (DM) as an epidemic and which is the only non-infectious disease in the world that is recognized as epidemic disease. In the United States, 8.3% of the total population in 2010 suffered from DM. Due to this reason DM ranked among the top ten causes of mortality around the world. According to WHO, more than 180 million people are suffering from DM worldwide, which may double by 2030. DM is characterized by hyperglycemia and it is the most common serious metabolic disorder that considered to being one of the live leading causes of death in the world [3]. There are a number of reasons for the increase in number of diabetic patients that include unhealthy diet, sedentary lifestyle, urbanization, aging populations, soaring incidences of obesity and population growth [4]. DM can be divided into two

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major types: type I and type II, type I is insulin-dependent diabetes mellitus (IDDM), whereas type II is non-insulin-dependent diabetes mellitus (NIDDM), the latter one is more common and more than 90% of diabetic patients are suffering from type II diabetes only. Diabetes also leads to cardiovascular risks, renal failure, stroke, cerebrovascular disease, blindness, neurological complication and limb amputation and thus it is now considered as a major public health challenge [5-11]. Totally ~65% of the mortality was seen in the diabetic patients due to cardiac problems or stroke. The nitric oxide (NO) and prostacyclin (PGL2) are the two important vasodilator substances for normal functioning of heart, but in the case of diabetes, biosynthesis of these two substances is reduced and leads to endothelial dysfunction which intern leads to critical cardiovascular complication such as cardiac inflammation, thrombosis, hypertension, remodeling and atherosclerosis [12]. Since the synthetic drugs have undesirable side effects or contraindication, the WHO has recommended the evaluation of traditional plant treatment for diabetes [13]. *Memecylon terminale Dalz,* belonging to the family Melastomataceae, is a small erect shrub found exclusively in Western Ghats of Karnataka, India. Nearly three hundred *Memecylon species* are distributed in different habitats like semi evergreen, evergreen, deciduous and mountain shoals with a wide range of altitude from the sea level [14]. All parts of this plant are being used by traditional healers in this region for curing various ailments such as dysentery, fever, diabetes, diarrhea, piles and haemoptysis [14]. However, no information is available regarding the antidiabetic activity of *M. terminale Dalz.* In this regard, the present study was carried out to find out the novelty of different extract of *M. terminale Dalz* against the alloxan induced diabetes and its related enzyme inhibition studies.

MATERIALS AND METHODS

Chemicals and Reagents: Alloxan, p-nitrophenyl-α-D-glucopyranoside, α-glucosidase, α-amylase and acarbose were obtained from the Sigma Chemical Co. (St. Louis, USA). All other chemicals and reagents were of analytical grade procured from Himedia labs, Mumbai. The solvents used were distilled prior to use.

Preparation of *M. terminale Dalz* extracts: Fresh *M. terminale Dalz* plant leaves were collected at the flowering stage from Hulikal region of Hosanagara Taluk, Shimoga district, Karnataka state, which belongs to Western Ghats (latitude of 13˚ 43' 47" to North and 75˚ 00' 38" to East, temperature 24°C, altitude 850 meters). The identification and authentication of the plant were made by the Department of Botany, Kuvempu University, Shankaraghatta, Shimoga, Karnataka, India. The dried and powdered plant leaves were subjected to Soxhlet's extraction using three different solvents with increase in polarity (petroleum ether, chloroform, and methanol). After the extraction, the solvent was filtered out and evaporated using a rotavapor. The crude extract obtained was stored at -40° C until further use.

Animals: Wistar rats weighing 140±20 g of either sex was procured from the S.S.I medical college, Davangere, Karnataka, India. The animals were housed at controlled conventional condition (22±2ºC temperature, 50±10% relative humidity, 12 h light-dark cycle) and fed with the standard pellet and drinking water *ad libitum* throughout the experiment. The animals were kept under starvation for 24 h before starting the experiment. All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg.no.No-628/02/c/CPCSEA).

Hypoglycemic activity: The condition of hyperglycemia was induced by intraperitoneal administration of alloxan hydrate at a dose of 150 mg/kg body weight (bw) of rats in saline [15]. The animals were kept under observation for 48 hours and then tested for blood glucose levels using the Accu-Check Active Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. The rats having a plasma glucose level above 400 mg/dl were selected for further experiments and divided into seven groups of six animals each and treated orally as follows. Group 1 was the normal control, which was given only the vehicle (1 ml of 1 % CMC/kg/day, orally). The groups 2 and 3 were diabetes induced control that was untreated and treated with standard drug (100 mg/kg, orally), respectively. The groups 4 – 7 were diabetes induced that received chloroform extract (250 and 500 mg/kg bw) and methanolic extract (250 and 500 mg/kg bw), respectively. Blood samples were drawn from the tail vein of the rats at 0, 2, 6, 24 and 72 hours after administration of standard drug or plant extracts. The animals were treated for 14 days and during this period the animals was given free access to food and water *ad libitum*. On 15th day, the animals were sacrificed by decapitation and blood was collected from the arterial jugular and serum was separated. The serum was used for the estimation of lipid profile.

***In vitro* α- glucosidase inhibition assay:** The α-glucosidase inhibition assay was carried out

according to the procedure described earlier with suitable modifications Ohta *et al* [16]. Briefly, 0.1 ml of different extracts of *M. terminale Dalz* at different concentrations (10, 50, 100, 250 and 500 µg/ml equivalent to GAE), 1 ml 0.1 M phosphate buffer (pH 6.8), 0.2 ml of p-nitrophenyl-α-D-glucopyranoside (NPG: 50 mM in 0.1 M phosphate buffer) and 0.2 ml of α-glucosidase solution (0.15 U/ml in phosphate buffer) were mixed and incubated for 60 min at 37°C. The reaction was terminated by adding 1.5 ml of 0.25 M sodium carbonate solution. The supernatants were transferred to a 96 well plate and the absorbance was read at 405 nm. The α-glucosidase inhibitory activity was expressed as IC_{50} values.

% inhibition= $(\Delta A_{Control} - \Delta A_{Sample})/ \Delta A_{Control}$ X100

Where, A_{sample}= Absorbance of the test sample and $A_{control}$= Absorbance of the control

***In vitro* α-amylase activity:** The α-amylase activity of plant extracts was carried out using the procedure described earlier with little modifications [17]. The experiment was performed by using 1 ml of enzyme solution (1 unit/ml in 20 mM PBS, pH 6.9), 1ml of different concentration of plant extracts (10, 50, 100, 250 and 500 µg/ml equivalent GAE) in dimethyl sulfoxide (DMSO). The reaction mixture was diluted with 9 ml of distilled water and the absorbance of the resulting solution was measured at 540 nm to determine the inhibition of enzyme activity. The inhibitory effect of plant extracts was compared with standard salivary α-amylase inhibitor acarbose at the same concentration. The percent inhibition of α-amylase ($I_{\alpha\text{-amylase}}$) was plotted against the sample concentration and a logarithmic regression curve established in order to calculate the IC_{50} value (inhibitory concentration). This represents the concentration of sample (µg/ml) required for the inhibition of α-amylase activity by 50%.

The inhibition percentage of α-amylase was assessed by the following formula:

$I_{\alpha\text{-amylase}} = (\Delta A_{control} - \Delta A_{sample})/ \Delta A_{control}$X100

Where, A_{sample}= Absorbance of the test sample and $A_{control}$= Absorbance of the control

Statistical analysis: All the experimental results were expressed as mean ±SEM and ANOVA was performed to determine the significant differences between groups using IBM SPSS (version 20). All statement of significance were based on a probability of $p<0.05$.

RESULTS

Hypoglycemic activity of *M. terminale Dalz*: The plasma glucose level of diabetic control rats (472.1±3.4) was higher as compared with those of the normal rats (104.7±5.2) (Table 1). When compared with the diabetic control group, the extracts of *M. terminale Dalz* treated rats showed significant reduction in the blood glucose levels. Among these, the rats receiving chloroform extracts (103.8±4.1) showed a significantly pronounced antidiabetic activity as compared with rats receiving methanolic extracts (213.1± 7.1) at 500 mg/kg bw after 24 hours. On the other hand, the blood glucose levels were 228.1±4.7 and 326.6±4.8 at 250 mg/kg bw for the chloroform and methanolic extract, respectively, indicating the difference in the efficacy levels of these two extracts (Table 1). It was observed that the standard drug at 100 mg/kg bw maintained the glucose level at 101.7±5.9 whereas chloroform extract (500 mg/kg bw) showed a value of 105.1±5.2 after 72 hours (Table 2).

Modulation of serum lipid profile by *M. terminale Dalz*: The effect of *M. terminale Dalz* extracts in reducing the diabetes induced hyperlipidemia was also investigated and the results are shown in Table 3. It was observed that due to increase in blood glucose level there was a concomitant increase in the total cholesterol level (186.2±2.0 mg/dl) as well as triglyceride level (109.3±4.6) in the diabetic control group. In this group, the HDL level was reduced (30.1±2.5) and the LDL level was elevated (130.2±1.3), but there were no significant variation in the VLDL level (23.3±2.2) when compared to untreated rats (Table 3). The cholesterol and triglyceride level of chloroform extract treated rats was 155.7±2.7 and 98.5±5.0 (mg%) which was comparable to standard drug treated (151.3±4.2 and 95.7±3.6), and untreated rats (140.3±1.6 and 90.3±1.4), respectively (Table 3). Similarly, the HDL, LDL and VLDL levels of chloroform extract treated rats were 40.7±4.3, 94.1±4.3 and 21.5±1.2, which were comparable to standard drug treated rats having a value of 40.1±2.4, 81.8±5.6 and 20.4±1.1, and untreated control rats (43.1±1.3, 78.2±2.4 and 19.6±0.7), respectively. On the other hand, the methanolic extract failed to show lipid reducing activity in the diabetic induced rats (Table 3).

Inhibition of α-glucosidase and α-amylase by *M. terminale Dalz*: The percentage inhibition of α-glucosidase by different extracts of *M. terminale Dalz* was determined using NPG as substrate. The extent of enzymatic inhibition by different extracts was determined by calculating the IC_{50}. The IC_{50} of α-glucosidase inhibition was 721µg/ml, 821µg/ml, and 1440 µg/ml for the methanolic, chloroform, and petroleum ether extracts, respectively (Table 4). However, the standard (acarbose) was found to possess an IC_{50} value of 578 µg/ml, indicating that

only methanolic extract has moderate activity (Table 4).

The α-amylase inhibition activity of different extracts of *M. terminale Dalz* was determined using starch as substrate. The sensitivity of glycosidases to various inhibitors depends on the concentration of flavonoids and phenolic contents [18]. As shown in Table 4, the methanolic extract showed potent inhibitory activities with an IC_{50} value of 629 µg/ml. The chloroform extract, petroleum ether extract, and the standard acarbose showed a value of 738 µg/ml, 1127 µg/ml, and 533 µg/ml, respectively.

DISCUSSION

DM is one of leading and most common chronic diseases associated with hyperlipidemia and co-morbidities such as obesity and hypertension [19]. Insulin not only maintains the proper blood glucose level in the body but also plays very important role in the regulation of metabolism of lipids and it is an important inhibitor of lipolysis. Insulin prevents the release of free fatty acids from the adipose tissue by suppressing the hormone sensitive lipases [20]. In diabetic condition, decreased insulin concentration leads to increased lipolysis by enhancing the activity of enzymes and releases more free fatty acids into the blood which results in an increase in the concentration of acetyl CoA and cholesterol in the blood leading to hypercholesterolemia [21]. The increase in blood cholesterol level results in a relative molecular ordering of the residual phospholipids which leads to decrease in membrane fluidity of the cells [22].

Phospholipids are important part of cell membrane, rich in PUFA and these lipids are susceptible substrates for O_2^- and $OH^\bullet$ free radicals [23]. This increased triglyceride is hydrolyzed in to triglycerides by the presence of lipoprotein lipase enzymes in the body and it is activated in the presence of insulin [24]. The coenzyme A oxidase and fatty acyl coenzymes activity increases drastically in diabetic condition resulting in β-oxidation of fatty acids and leads to lipid peroxidation [25, 26]. Lipid peroxidation strongly destabilizes the membrane functions by decreasing membrane fluidity and it alters the enzyme activities which are bound to cell membrane [26].

By the administration of chloroform extract of *M. terminale Dalz* plant, the cholesterol and triglyceride levels in the test animals reduced significantly. There are several antidiabetic drugs in the market and their potency depends upon multiple factors such as inhibition of carbohydrate-digesting enzymes, impairment of glucose uptake from small intestine, stimulation of insulin secretion from beta cells of the pancreas, insulinomimetic or insulin sensitizing activity at insulin target sites and antagonism activity of glucagon [27]. Glibenclamide is one of such sulphonylureas drug which increases the pancreatic insulin secretion from the existing β-cells in STZ-induced diabetic rats by membrane depolarization and stimulation of Ca^{+2} influx, an initial key step in insulin secretion [28]. Several medicinal plants are reported to possess hypoglycemic effect due to the presence of terpenoids, iridoid glycosides, flavonoids and other phenolic compounds [29]. These isolated bioactive compounds were reported to modulate pancreatic beta-cells and stimulate insulin secretion through exertion distal to K^+-ATP channels and L-type Ca^{2+} channels and activation of the cAMP/PKA signaling pathways [30, 31]. A number of other plants have also been reported to have antihyperglycemic activity, among which *Phyllanthus niruri* (contains tolbutamide) extracts show very good activity in streptozotocin diabetic rats [32]. The phenolic compounds in plants have long been recognized to inhibit the activities of digestive enzyme because of their ability to bind to proteins [18]. Different *in vitro* assays have shown that many plant phenols possess carbohydrate hydrolyzing enzyme inhibitory activities. The methanolic extract of *M. terminale Dalz* contains good percentage of phenols and flavonoids which could be responsible for its potent enzyme inhibition activity that is comparable with that of standard acarbose. It has been reported that the polyphenols of sweet potato, green tea and berry have the ability to bind carbohydrate metabolizing enzymes and inhibits their activity [33-35]. Nowadays, α-glucosidase enzyme inhibitors are the most commonly used oral drugs for improving postprandial hyperglycemia. Normally these inhibitors bind to different sites of enzyme and delay carbohydrate digestion and glucose absorption with diminution of postprandial hyperglycemic excursions. Delay in the carbohydrate digestion do not cause any net loss of nutrition, instead it slows the carbohydrate absorption by the body. The inhibitor such as acarbose is a pseudotetrasaccharide containing a nonhydrolyzable nitrogen linked bond which play very important role in reducing the activity of enzyme by competitive reversible inhibition [36]. To determine the true efficiency of plant extracts as α-glucosidase inhibitors, studies in human subjects are necessary.

CONCLUSIONS

M. terminale Dalz is an endemic medicinal plant found in the Hulikal region of Western Ghats in Southern India. This plant is being used by the traditional healers in this region to treat a variety of diseases. However, in the literature, there is no reported systematic study of this plant to correlate

the structure-activity relationship. In this study, we have analyzed the plant extracts for the *in vitro* and *in vivo* antidiabetic activity including lipid reduction ability. Among the two extracts, chloroform extract showed good hypoglycemic activity whereas methanolic extract showed highly promising enzyme inhibition activities. These results indicate that *M. terminale Dalz* has a rich source of novel biologically active compounds that are antidiabetic. Further detailed studies on the isolation, purification, and characterization of these active compounds are in progress.

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Table 1: Effect of *M. terminale Dalz* extracts on the blood glucose levels[a]

	0th hour	2nd hour	6th hour	24th hour	72nd hour
Normal control	104.3±5.2	92.1±2.5	96.7±4.6	95.2±3.1	89.7±6.2
Diabetic control	472.1±8.4	479.3±5.3	472.1±8.4	465.2±5.5	395.3±3.7
Standard (100 mg/kg)	485.2±4.3	403.5±5.1**	338.7±8.0***	128.2±5.3**	101.7±5.9**
Chloroform Extract	467.3±1.2	444.8±4.2*	371.1±4.8**	228.1±4.7**	168.0±4.3***
Methanolic extract	473.3±3.6	454.8±5.2*	400.0±3.8*	326.6±4.8***	231.0±3.8**

Values are mean ±S.E.M, ***P<0.001 - Highly significant when compared with diabetic control, **P<0.005 - Significant when compared with diabetic control. *P<0.05 - Not significant when compared with diabetic control.

[a] Given by oral route at a dose of 250 mg/kg bw.

Table 2: Effect of *M. terminale Dalz* extracts on the blood glucose levels[a].

	0th hour	2nd hour	6th hour	24th hour	72nd hour
Normal control	104.3±5.2	92.1±2.5	96.7±4.6	95.2±3.1	89.7±6.2
Diabetic control	472.1±8.4	479.3±5.3	472.1±8.4	465.2±5.5	395.3±3.7
Chloroform Extract	472.3±3.2	301.3±13.7***	180.6±4.4**	103.8±4.1***	105.1±5.2***
Methanolic extract	496.5±5.8	441.1±5.1**	352.1±7.4**	213.1±7.1***	166.5±4.3**

Values are mean ±S.E.M, ***P<0.001 - Highly significant when compared with diabetic control, **P<0.005 - Significant when compared with diabetic control. *P<0.05 - Not significant when compared with diabetic control.

[a] Given by oral route at a dose of 500 mg/kg bw.

Table 3: Effect of *M. terminale Dalz* plant extracts on total cholesterol (TC), triglyceride (TG), HDL, LDL and VLDL levels in rats [a]

Test	Normal control	Diabetic control	Standard Drug (100 mg/kg bw)	Chloroform Extract	Methanol extract
TC (mg%)	140.3±1.6	186.2±2.0	151.3±4.0**	155.7±2.7**	170.3±2.3*
TG (mg%)	90.3±1.4	109.3±4.6	95.7±3.6***	98.5±5.0*	104.6±6.4**
HDL (mg%)	43.1±1.3	30.1±2.5	40.1±2.4**	40.7±4.3**	35.8±0.6***
LDL (mg%)	78.2±2.4	130.2±1.3	81.8±5.6**	94.1±4.3*	110.4±2.8*
VLDL (mg%)	19.6±0.7	23.3±2.2	20.4±1.1***	21.5±1.2***	22.6±0.8*

Values are mean ±S.E.M, ***P<0.001 - Highly significant when compared with diabetic control, **P<0.005 - Significant when compared with diabetic control. *P<0.05 - Not significant when compared with diabetic control.

[a] Given by oral route at a dose of 500 mg/kg of bw.

Tables 4: Inhibition of α-glucosidase and α-amylase by *M. terminale Dalz*[a]

Compounds	IC_{50} value (ug/ml)	
	α-glucosidase	α-amylase
Acarbose	578.3±1.4	533.1±0.3
Petroleum ether extract	1440.2±2.3	1127.9±2.1
Chloroform extract	821.8±0.8	738.6±1.5
Methanolic extract	721.1±0.5	629.4±1.8

[a] Values represents mean ± S.D of three parallel measurements

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