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Antioxidant and antihyperglycemic potential of methanolic extract of bark of *mimusops elengi* l. In mice.

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Abstract

Ayurveda refers *Mimusops elengi* L. for the treatment of the diabetes. Considering the traditional claim of M. *elengi* in management of diabetes and the possible involvement of oxidative stress in pathogenesis of diabetes, the present study was aimed to evaluate the in vitro antioxidant and in vivo antihyperglycemic property of methanolic extract of bark of M. *elengi* (MEMeOH). In vitro antioxidant activity of MEMeOH was evaluated using reducing power assay, DPPH and hydroxyl radical scavenging assay. MEMeOH offered significant in vitro reducing power capacity and radical scavenging activity. In acute study in alloxan induced diabetes, MEMeOH exhibited significant ($p< 0.001$) antihyperglycemic effect. The onset of antihyperglycemic effect was observed at 2nd hr; peak activity was demonstrated at 6th hr. The antihyperglycemic effect of MEMeOH 400mg/kg, p.o. was persistent up to 24th hr after drug administration. MEMeOH produced significant ($p < 0.01$) reduction in elevated glucose levels in glucose loaded non diabetic animals. The onset of action in non diabetic oral glucose tolerance test was found to be at 60th min and peak activity was observed at 120th min after oral glucose load. MEMeOH demonstrated significant ($p < 0.01$) reduction in elevated glucose levels 2hr before glucose administration and 6 hr after glucose load in oral glucose tolerance test in diabetic animals. MEMeOH has demonstrated antihyperglycemic activity in diabetic as well as non diabetic glucose loaded mice. MEMeOH should be further explored against diabetes and related complications.

Keywords: *Mimusops elengi*; antihyperglycemic, antioxidant, DPPH, diabetic OGTT

Introduction

Diabetes mellitus is a chronic incurable condition due to insulin deficiency that affects 10% of the population worldwide. The number of diabetic people is expected to rise from present estimate of 150 million to 230 million in 2025 [1]. Impaired glucose tolerance and the metabolic syndrome often lead to development of type 2 diabetes. A number of important epidemiological studies have highlighted the relationship between hyperglycemia and an increased risk of cardiovascular disease. The risk of cardiovascular disease is increased threefold in patients with established diabetes. Micro- and macro vascular disease can be traced back to hyperglycemia and the metabolic syndrome [2].

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The detrimental effects of diabetic complications are mainly mediated and complicated through oxidative stress [3]. Diabetes is usually associated by increased production of the molecules of reactive oxygen species (ROS) and/or impaired antioxidant defense systems, which result oxidative damage leading to ROS mediated diabetic pathogenesis [4]. Although numerous synthetic drugs were developed to combat against diabetes but the situation has only marginally improved. Many natural products have been used successfully since ancient era to counteract diabetes and its associated complications [5]. Natural products are being prescribed widely considering their safety, effectiveness, and availability [6]. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agent from medicinal plants has been more important from researcher's viewpoint [7]. Natural antioxidant compounds have been found fruitful against diabetes and associated complication [8]. So, it is worthy to develop a natural lead with a stirring prospect for the expansion of an alternative way of treatment of diabetes.

Mimusops elengi (ME) Linn (Sapotaceae) commonly known as Bakul, is a small to large evergreen tree found all over the different parts of India. It is cultivated in gardens as an ornamental tree. Earlier report revealed that the fruits are used in chronic dysentery, constipations; flowers are used as snuff to relive headache, lotion for wounds and ulcers. Barks are used to increase fertility in women and known to have antiulcer activity [9]. Bark is used as a tonic [10, 11], febrifuge, as a gargle for odontopathy, inflammation and bleeding of gums [9]. *Mimusops elengi* is a rich source of tannin, saponin, alkaloids and glycosides [12].

Sushrut Samhita [13] in the *Chikitsa* chapter explains the treatment strategies for the diabetes. *Mimusops elengi* is endowed with the antidiabetic potential.

The present study was aimed to evaluate the in vitro antioxidant and antihyperglycemic property of Methanolic extract of bark of ME (MEMeOH).

Materials and methods

Collection and authentication of plant material

Mimusops elengi bark was collected during May and June from Rajgurunagar, Pune District, Maharashtra State, India. The plant was identified and authenticated by Botanical Survey of India and a voucher specimen was deposited at Botanical Survey of India (voucher specimen sample no. GG 01).

Drugs and chemicals

Glyburide (Ranbaxy Pharma Ltd., India), alloxan monohydrate (Spectrochem, India), glucose estimation kit (Accurex Biomedical Pvt.Ltd.), India, were purchased from respective vendors.

Preparation of methanolic extract of Mimusops elengi (MEMeOH)

The stem bark of *Mimusops elengi* was shade-dried and powdered in a grinder. The powdered material (100 g) was extracted with methanol using soxhlet extraction at 40^{o}C. The extract was dried on a tray dryer at 40°C (yield – 10.2 % w/w). The extract was suspended in 1% w/v CMC and used for pharmacological studies.

In vitro antioxidant activity Sample preparation

100 µg/ml stock solutions were prepared by dissolving 5 mg of test compound and ascorbic acid to 50 ml with distilled water. Different concentrations of test and ascorbic acid like 10, 25, 50 and 100 µg/ml were prepared from stock solution.

Ferric ion reducing antioxidant power (FRAP) [14]

1ml of different concentrations of test and ascorbic acid as a standard compounds were mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50^{0}C for 20 min. 2.5 ml of tri chloro acetic acid (TCA) was added to the mixture, which was then centrifuged at 3000 *g* for 10 min. 2.5 ml of upper layer solution was taken and mixed with 2.5 ml distilled water and 0.5 ml of $FeCl_3$ solution and the absorbance was measured

at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Free radical scavenging activity by DPPH method [15]

1ml of different concentration of test and standard were employed in different test tubes. To this 5 ml of methanolic solution of DPPH was added, shaken well and incubated at 37^0C for 20 min. The absorbance was measured against methanol as blank at 517 nm.

Hydroxyl radical scavenging activity assay [14]

The scavenging activity for hydroxyl radicals was measured with Fenton reaction. Reaction mixture contained 60 µl of 1.0mM FeCl3, 90 µl of 1mM 1,10- phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H_2O_2, and 1.5 ml of test compounds and standard at various concentrations. Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560nm was measured with a spectrophotometer.

Experimental animals

Swiss albino mice (25–30 g) of either sex were obtained from Serum Institute of India, Pune, India. Animals were maintained at a temperature of 25±1 °C and relative humidity of 45–55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India) and water was available *ad libitum*.

Acute oral toxicity studies

Adult albino mice of either sex were subjected to acute toxicity studies as per guideline (425) suggested by Organization for Economic Co-operation and Development (OECD). The mice were observed for 2 h for behavioral, neurological and autonomic profiles and for any lethality during next 48 h.

Induction of experimental diabetes and determination of serum glucose level

Diabetes was induced in Swiss albino mice by a single intravenous injection of aqueous alloxan monohydrate (80 mg/ kg, i.v.) solution in normal saline. After 48 h, the animals showing serum glucose level above 300mg/dl were selected for the study.

Effect of acute administration of MEMeOH in alloxan induced diabetes in mice [16]

Diabetic Swiss albino mice of either sex were divided into different groups (*n*=6) *viz*; Group I, vehicle (1% w/v CMC; 10ml/kg, p.o.); Group II, glyburide (10mg/kg, p.o.); Group III, MEMeOH (100 mg/kg, p.o.), Group IV, MEMeOH (200 mg/kg, p.o.) and Group V, MEMeOH (400 mg/kg, p.o.). Acute study involved determination of serum glucose level at 0, 2, 4, 6 and 24 hr after glyburide and MEMeOH administration.

Antihyperglycemic activity

Effect of MEMeOH on oral glucose tolerance test (OGTT) in non- diabetic mice.

The non diabetic mice were fasted overnight before the experiment. The animals were divided into different groups (n = 6): group I - vehicle (CMC 1%, 10 ml/kg, p. o.), group II - glyburide (10 mg/kg, p.o.), group III, IV and V – MEMeOH (100, 200 and 400 mg/kg, p.o.) respectively. D-Glucose (2.5 g/kg, p.o.) was administered 0.5 hr of pretreatment with glyburide or MEMeOH. Serum glucose levels were estimated at 30, 60, and 120 min after glucose loading through retro orbital plexus. Serum glucose level is estimated using GOD-POD method.

Effect of MEMeOH on oral glucose tolerance test (OGTT) in diabetic mice.

The diabetic mice were fasted overnight before the experiment. The animals were divided into different groups (n = 6): group I - vehicle (CMC 1%, 10 ml/kg, p. o.), group II - glyburide (10 mg/kg, p.o.), group III, IV and V – MEMeOH (100, 200 and 400 mg/kg, p.o.) respectively. D-Glucose (2.5 g/kg, p.o.) was administered in diabetic mice at the 4th hr of pretreatment with glyburide or MEMeOH. Serum glucose levels were estimated before and 2 hr after glucose loading in diabetic mice using GOD-POD method.

Statistical analysis

Data was expressed as mean ± SEM and statistical analysis was carried out by one-way

ANOVA with *post hoc* Dunnett's test performed using Graph Pad InStat. P value was considered significant when $P < 0.05$.

Results

The reducing power of MEMeOH displayed a concentration-dependent antioxidant activity there was increase in the absorbance with increase in concentration (Fig. 1).

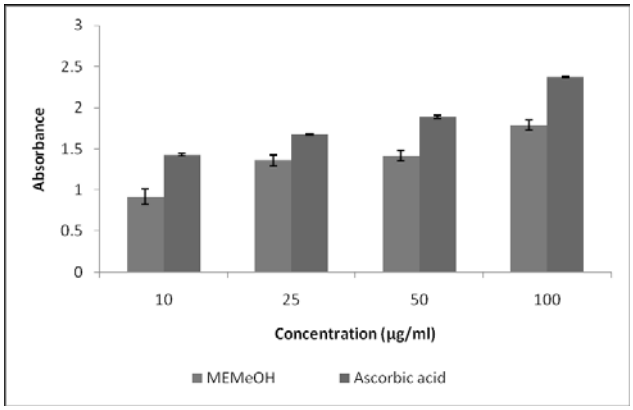

Fig. 1: Effect of MEMeOH on in vitro Reducing Power Assay

It was observed that MEMeOH scavenged the DPPH radical in concentration dependent manner. The percent scavenging of DPPH radical by MEMeOH and ascorbic acid at 100µg/ml concentration were found to be 71.55 and 77.23 respectively (Table 1).

Hydroxyl radical scavenging activity assay exhibited the potential of MEMeOH in scavenging hydroxyl free radical in concentration dependent manner. The percent scavenging of hydroxyl radical by MEMeOH and ascorbic acid were found to be 82.11 and 93.91 respectively (Table 1).

Table 1: In vitro DPPH and hydroxyl radical cavenging activity of MEMeOH.

Conc. (µg/ ml)	% DPPH scavenging		% hydroxyl scavenging	
	MEMeOH	Ascorbic acid	MEMeOH	Ascorbic acid
10	42.55±3.12	62.50±3.04	31.44± 1.12	41.23± 2.34
25	53.06±2.77	69.12±5.12	49.99± 2.11	62.34± 2.77
50	64.12±5.17	72.87±4.24	68.88± 2.86	83.11±6.01
100	71.55±3.22	77.23±3.32	82.11± 4.20	93.91± 6.11

Acute oral toxicity studies

Acute toxicity studies revealed that MEMeOH was found safe up to a dose level of 5000 mg/kg, p.o. in mice. No lethality or any toxic reactions were observed up to the end of the study period. LD50 of MEMeOH is found to be > 5000mg/ kg, p.o.

Effect of MEMeOH on serum glucose in alloxan induced diabetic mice

Single dose administration of MEMeOH 100 mg/kg, p.o. significantly reduced the serum glucose level at 2nd hr. of administration. MEMeOH produced highest activity at 6th and the onset of activity was 2nd hr. The onset of antihyperglycemic effect of MEMeOH 100, 200, 400mg/kg and glyburide (10mg/kg, p.o.) was observed at 2nd hr; peak antihyperglycemic effect was demonstrated at 6th hr. The antihyperglycemic effect of MEMeOH 400mg/kg was persistent up to 24th hr. after drug administration (Fig. 2).

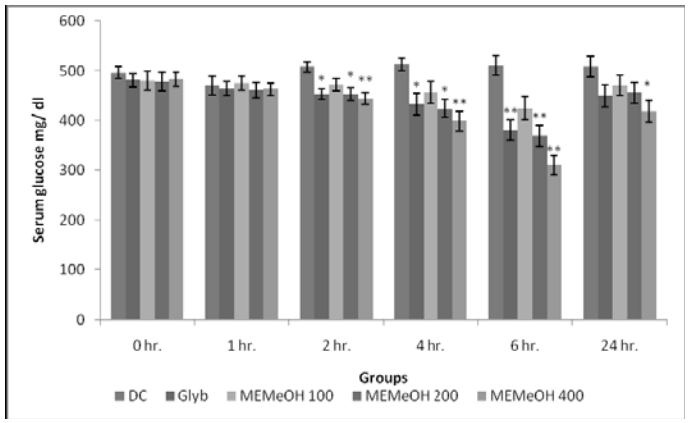

Fig. 2: Effect of MEMeOH on serum glucose in alloxan induced diabetic mice

Effect of MEMeOH on oral glucose tolerance test (OGTT) in diabetic mice

At 6th hr. of administration; glyburide (10mg/kg, p.o.) and MEMeOH 100, 200 and 400 mg/kg, p.o. produced significant ($p < 0.01$) reduction in elevated glucose level. Glyburide (10mg/kg, p.o.) and MEMeOH 100, 200 and 400 mg/kg, p.o. produced significant ($p < 0.01$) reduction in elevated glucose level 2 hr. before administration of glucose load (Fig. 3).

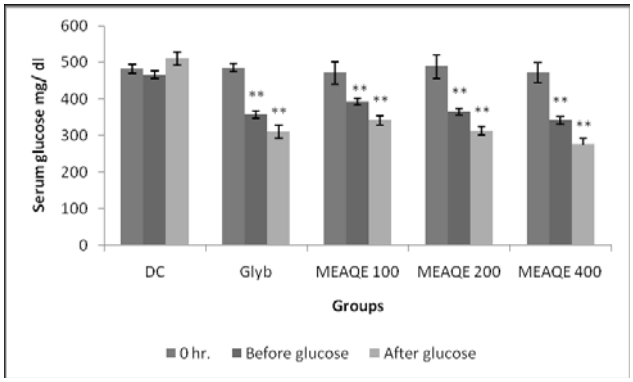

Fig. 3: Effect of MEMeOH on oral glucose tolerance test (OGTT) in diabetic mice

Effect of MEMeOH on oral glucose tolerance test (OGTT) in non diabetic mice

Glyburide (10mg/kg, p.o.) and MEMeOH 200, 400 mg/kg, p.o. produced significant ($p < 0.01$) reduction in elevated glucose levels at 120 min after oral glucose load. MEMeOH 200 mg/kg, p.o. demonstrated significant ($p < 0.05$) reduction in serum glucose level at 60 min. MEMeOH 400 mg/kg, p.o. demonstrated significant ($p < 0.001$) reduction in serum glucose level at 60 min. MEMeOH 100 mg/kg, p.o. demonstrated significant ($p < 0.05$) reduction in serum glucose level at 120 min (Fig. 4).

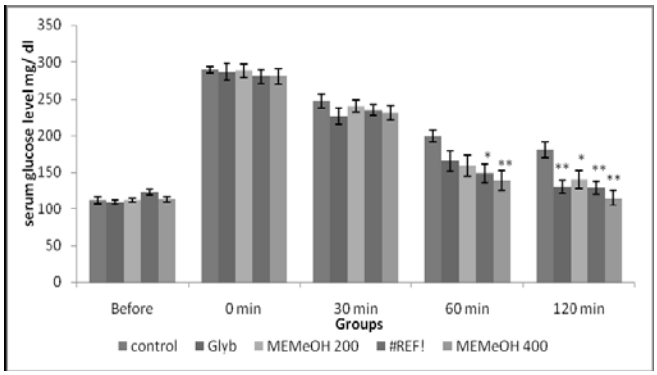

Fig. 4: Effect of MEMeOH on oral glucose tolerance test (OGTT) in non diabetic mice

Discussion

Oxidative stress induced due to enhanced generation of reactive oxygen species (ROS) has been implicated in the etiology of diabetes. Antioxidants that can scavenge these reactive oxygen species or neutralize these radicals are beneficial in reducing this oxidative stress. In this context natural components with antioxidant activities are very important [17]. Reactive oxygen species play an important role in the development of diabetes and mainly in further complications of diabetes [18]. Oxidative stress is believed to be a common pathway linking diverse mechanisms for the pathogenesis of complications of diabetes. There is strong evidence for increased levels of indicators of oxidative stress in diabetic individuals suffering from complications [19].

The potential of the antioxidant constituents of plant materials for the maintenance of health and protection from coronary heart disease and diabetes is raising interest among scientists [20, 21]. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [22]. The antioxidant activity of natural compounds is mainly due to their redox properties [23], which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [24]. Therefore, use of antioxidants is reasonable in cure of diabetes and an antidiabetic drug with antioxidant potential will have a dual advantage. We have evaluated in vitro antioxidant potential of MEMeOH in the present investigation.

A reducing power is an indicative of a reducing agent having the availability of atoms which can donate electrons and react with free radicals, and then convert them into more stable metabolites and terminate the radical chain reaction. From the present investigation it is very clear that MEMeOH has concentration dependent reducing ability which could possibly be one of the modes of its antioxidant activity.

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The method of scavenging the stable DPPH radical is widely used for relatively rapid evaluation of antioxidant activity compared to other methods. The reduction of the DPPH radical is determined by its decrease in absorbance at 517 nm. The extent of reduction of

DPPH free radical is visualized as a discoloration from purple to yellow [25]. The present investigation indicated that MEMeOH has radical scavenging capacity.

The highly reactive oxygenated radical is assumed to attack cellular molecules including hepatic tissue, because it is located less than a few nanometers from the site of their generation [26]. For experimental purposes, ·OH is being produced by the reaction of transition metal ions including iron and copper with H_2O_2, ultraviolet (UV) photolysis, and the electro catalysis of H_2O_2, namely photo-Fenton and electro-Fenton reactions, respectively [27]. In the present work, we focused on the scavenging activity of ·OH to evaluate the antioxidant activity of MEMeOH because ·OH is biologically available radical and has the role to play in the pathogenesis of different diseases. Scavenging ability of MEMeOH reflected potential use of antioxidant mechanism of MEMeOH in diabetes and related complications.

Alloxan, induces "chemical diabetes" in a wide variety of animal species by damaging the insulin secreting cells of the pancreas. This damages a large number of β cells, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased use of glucose by the tissues [28]. Our study showed that alloxan-induced diabetic mice presented obvious hyperglycemic symptoms and significant injury in islets. The administration of MEMeOH significantly decreased serum glucose which could be treating the early clinical symptom of diabetes i.e. hyperglycemia. These results suggested that MEMeOH has a protective action in the hyperglycemic conditions of diabetes mellitus.

In the present study, alloxan injection produced significant hyperglycemia in all the test animals. The MEMeOH showed peak antihyperglycemic effect at 6 hr. The onset of antihyperglycemic activity was at 2nd after alloxan administration. The effect was persistent up to 24 hr.

Diabetic animals have impaired glucose tolerance. Additional load of glucose was found to impair the tolerance further. As acute study with alloxan revealed that the peak antihyperglycemic action is at 6th; the OGTT experiment was designed in such a way that glucose load was administered at 4th hr after pretreatment with MEMeOH and serum glucose was determined 2nd hr after glucose load. MEMeOH produced significant reduction in elevated blood glucose levels before glucose load and after glucose load in diabetic animals. It can be concluded that MEMeOH may act by increasing peripheral utilization of glucose. Glyburide (10mg/kg) was more effective in showing antihyperglycemic effect as well as glucose tolerance.

Non diabetic OGTT revealed that MEMeOH demonstrated significant lowering of elevated glucose level in glucose loaded animals. The onset of action was found to be 60 min and duration of action was longer than 2hr.

Conclusion

Taking into consideration the traditional claim of ME as having antioxidant and antihyperglycemic potential, MEMeOH has demonstrated antihyperglycemic activity in diabetic as well as non diabetic glucose loaded mice. MEMeOH should be further explored against diabetes and related complications.

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