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# Oral glucose tolerance test with methanol extract of leaves of *Ocimum minimum* L. (Lamiaceae) in Swiss albino mice

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**Abstract**
**Background:** Diabetes is a metabolic disorder characterized by high blood sugar caused due to either insufficient production of insulin from the pancreas or development of insulin resistance, which causes polyuria, polydipsia, and polyphagia. In recent years, the disorder is increasing at a rapid rate in virtually all countries of the world. Existing conventional drugs suffer from problems of non-affordability, adverse effects, and non-availability in particularly rural and remote areas of developing countries like Bangladesh. As a result scientists are continually striving for discovery of new anti-diabetic drugs, which can fulfill the needs of all diabetic patients.
**Methods and Findings:** The *Ocimum* genera within the Lamiaceae family contains a number of plants reported to possess anti-diabetic properties. However, very little is known about the pharmacological including anti-diabetic properties of *Ocimum minimum* L., the leaves of which are chewed by the indigenous people in the Chittagong Hill Tracts region in the south-eastern part of Bangladesh to control diabetes. Oral glucose tolerance test (OGTT) was conducted with Swiss albino mice and methanol extract of *Ocimum minimum*, using glibenclamide (a standard anti-diabetic drug) as the comparison drug. The methanolic extract of *Ocimum minimum* (MEOM) showed only significant anti-hyperglycemic activity at a dose of 400 mg/kg body weight; at that dose blood glucose was lowered by nearly 19%. By comparison, glibenclamide at a dose of 10 mg/kg lowered blood glucose by 37%.
**Conclusions:** Methanol extract of *Ocimum minimum* leaves did not show any remarkable lowering of blood glucose in OGTT except at high doses. However, the plant has an advantage of growing profusely in the Chittagong Hill Tracts region, which is mountainous, forested, and inhabited by a number of indigenous communities with little contact with modern medical facilities. As such, the inhabitants of this region can chew, if necessary, as much as possible of the leaves of *Ocimum minimum* and bring their blood glucose under control.

**Keywords:** Phytotherapy, OGTT, *Ocimum minimum*, Bangladesh

## Introduction
Diabetes mellitus (DM) is a metabolic disorder characterized by high blood sugar caused due to either insufficient production of insulin from the pancreas or development of insulin resistance, which causes polyuria, polydipsia, and polyphagia [1]. Two types of diabetes usually occur in people; type 1 DM is where there is auto-destruction of insulin producing cells causing little or no production of insulin, while type 2 DM is where there is impairment of glucose utilization in the body leading to high glucose levels in the blood. Both types of DM end up with high blood glucose levels (hyperglycemia). In turn, hyperglycemia induces oxidative stress, which activates a number of pro-inflammatory pathways further leading to diabetic nephropathy, diabetic retinopathy, and cardiac myopathy [2]. Neither DM nor DM-induced disorders as mentioned above are curable; they can only be controlled and slowed down.
In recent years, diabetes has been spreading quite widely. The American Diabetes Association (ADA) statistics show that in 2019, 37.3 million Americans constituting 11.3% of the population had diabetes. Moreover, 1.4 million new diabetic persons are diagnosed every year with 29.2% patients at or above 65 years of age [3]. The Centers for Disease Control and Prevention (CDC), USA report for diabetes in 2022 is more serious. CDC estimates that

around 130 million people with DM and pre-DM are living currently in the United States [4], that is around 39% of the total population has diabetes or are inclined to develop diabetes.A recent report from Bangladesh concludes that among the adult population of 18-95 years age, 9.2 and 13.3% had diabetes and pre-diabetes, respectively. The alarming feature of this report was that only 30.4% of the diabetic people had their disease under control [5]. Needless to say that this places a heavy health burden on the society, but in a country like Bangladesh, this is unavoidable because of the remoteness of rural areas, lack of proper medical facilities in rural and remote areas, and the high cost of medicines to the people who need them most but can ill-afford.

On the other hand, Bangladesh is blessed with nearly 6,000 plant species of which around 1200 are recognized as medicinal plants. All plants produce secondary metabolites (phytochemicals), which are gaining recognition as therapeutics for their various pharmacological activities [6]. From that realistic view point every plant should be a medicinal plant, since all plants produce secondary metabolites. Since 2010, we had been surveying various areas of Bangladesh and interviewing traditional, folk and tribal medicinal practitioners about the plant parts/formulations that they use in the cure for a wide variety of diseases. We have built up a database, and uses of plants (as well as animals and other items) in some areas have been published [7-27]. In our laboratory we have utilized this database to conduct OGTT (anti-hyperglycemic), antinociceptive, brine shrimp lethality, and other pharmacological activity studies on some of these plant extracts [28-35].

The *Ocimum* genera (Lamiaceae family) comprises of a number of medicinally important plants. Among the plants that are more familiar from the medicinal point of view are *Ocimum americanum* L., *Ocimum basilicum* L., *Ocimum gratissimum* L., and *Ocimum tenuiflorum* L. [36]. The plants are useful in a number of diseases, the common diseases being gastrointestinal, hepatic and lung disorders. On the other hand, despite the high antioxidant capacity of *Ocimum minimum* [37], very little has been reported on this plant. The essential oil from the plant has been reported to contain nine monoterpene hydrocarbons, six oxygenated monoterpenes, fourteen sesquiterpene hydrocarbons, six oxygenated sesquiterpenes, as well as six other compounds [38]; the nature of a number of compounds can be correlated with anti-oxidative properties. In alloxan-induced diabetic rats, administration of extract of aerial parts of *Ocimum basilicum* led to improved oral glucose tolerance and inhibition of the enzymes α–amylase and α–glucosidase [39]. It was therefore of interest to see whether extract of *Ocimum minimum* leaves can improve oral glucose tolerance in glucose-loaded mice.

## Methods

### Plant material collection

Leaves of *Ocimum minimum* were collected from Bandarban district, Bangladesh. Plant specimen was taxonomically identified by a trained botanist at the University of Development Alternative. Leaves were air-dried in the shade for 72 hours.

### Preparation of methanolic extract of Ocimum minimum leaves (MEOM)

The air-dried leaves were grinded into a fine powder and 50g of the powder was extracted with methanol (1:3, w/v) for 48 hours. The extract (MEOM) was evaporated to dryness at 40ºC and stored in small aliquots at -20ºC till use. The final weight of MEOM was 1.9g. The extract was suspended in DMSO prior to administration by gavaging to mice in oral glucose tolerance test.

### Chemicals and drugs

Source of glibenclamide and glucose was Square Pharmaceuticals Ltd., Bangladesh. Glucometer along with strips were purchased from Lazz Pharma, Bangladesh.

### Animals

Swiss albino mice of both sexes, (12-15g weight) were used in the present study. Mice were purchased from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Acclimatization period prior to actual experiment was 3 days. The animals were fed with mice chow (supplied by ICDDR, B) and water *ad libitum* during these three days. The study was approved by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

### Oral glucose tolerance test (OGTT)

The procedure of Joy and Kuttan [40] was followed for OGTT with minor modifications. Briefly, the mice were divided into six groups of five mice each. Group 1 received vehicle (1% Tween 20 in water, 10 ml/kg body weight) and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received, respectively, MEOM at doses of 50, 100, 200 and 400 mg per kg body weight. All substances (extract, vehicle, and drug) were orally administered by gavaging. The amount of Tween 20 administered was same in both control and experimental mice. Following a period of one hour as described earlier [41, 42], all mice were administered orally 2g glucose per kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart following previous procedures [41, 42]. A glucometer was used to measure blood glucose levels. The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = $(1 – W_e/W_c) \times 100$, where $W_e$ and $W_c$ represents the blood glucose concentration in glibenclamide or MEOM administered mice (Groups 2-6), and control mice (Group 1), respectively.

### Statistical analysis

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance (marked by *) was considered to be indicated by a p value < 0.05 in all cases [43].

## Results and Discussion

MEOM did not cause any lowering of blood glucose in OGTT at the two lowest doses of 50 and 100 mg/kg body weight. At a dose of 200 mg/kg, MEOM lowered blood glucose in glucose-loaded mice by 7%, which was not significantly different. At a dose of 400 mg/kg, MEOM lowered blood glucose significantly by nearly 19%. The standard blood glucose lowering drug glibenclamide, when administered at a dose of 10 mg/kg lowered blood glucose by 37%. The results are shown in Table 1.

**Table 1:** Effect of MEOM on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

| Treatment | Dose (mg/kg body weight) | Blood glucose level (mmol/l) | % Lowering of blood glucose level |
|---|---|---|---|
| Control | 10 ml | 12.82 ± 0.67 | - |
| Glibenclamide | 10 mg | 8.08 ± 0.53 | 36.97* |
| (MEOM) | 50 mg | 14.98 ± 1.25 | - |
| (MEOM) | 100 mg | 13.42 ± 1.45 | - |
| (MEOM) | 200 mg | 11.92 ± 1.14 | 7.02 |
| (MEOM) | 400 mg | 10.42 ± 0.98 | 18.72* |

All administrations were made orally. Values represented as mean ± SEM, (n=5); $^{*}P < 0.05$; significant compared to hyperglycemic control animals.

Interestingly, MEOM did not prove to be highly antihyperglycemic. Perhaps this is the reason why we observed the indigenous people to chew large number of leaves of *Ocimum minimum* for a large amount of time in one stretch (at least an hour or more) and going through several stretches every day. This begs the question as to why? There can be several reasons like absence or scarcity of other non-toxic anti-diabetic plants in the area, or chewing of *Ocimum minimum* leaves is producing other benefits besides lowering blood glucose. More studies and interviewing of local inhabitants needs to be carried out in the future to satisfactorily answer the questions raised.

Diabetes increases oxidative stress, which in turn is caused by hyperglycemia [2]. Eleven phenolic compounds (rosmarinic acid, lithospermic acid, vanillic acid, *p*-coumaric acid, hydroxybenzoic acid, syringic acid, caffeic acid, ferulic acid, cinnamic acid, hydroxyl phenyllactic acid, and sinapic acid) have been reported for *Ocimum minimum* [37]. Together, a number of these compounds, if not all, because of their antioxidant properties, can alleviate oxidative stress. For instance and to mention only three of these compounds, the antioxidant properties of rosmarinic acid have been reported [44]. Ferulic acid is considered to be a superior antioxidant because of its low toxicity and high free radical scavenging properties [45]. In type 2 streptozotocin-induced diabetes as well as high fat diet-induced insulin resistance in male Wistar rats, administration of sinapic acid at a concentration of 25 mg/kg bw/rat/day for 30 days resulted in decline in levels of plasma lipid peroxides, hydroperoxides, and protein carbonyls, demonstrating the antioxidant efficacy of sinapic acid [46].

Reduction of oxidative stress can play a major protective action against development and progression of various diabetes-induced disorders. As such, *Ocimum minimum* can play a protective role against development and progression of diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, and cardiac myopathy. The OGTT results also suggest that phytochemical(s) present in MEOM may play a role in reducing blood glucose (antihyperglycemic activity) by a yet to be determined mechanism, which deserves further research. To our knowledge, this is the first report of the antihyperglycemic property of *Ocimum minimum*.

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