

## Oral Glucose Tolerance Test with a Combination of Methanol Extract of *Stemona tuberosa* roots and *Bulbophyllum neilgherrense* Fruits

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### Abstract

Diabetes (both types 1 and 2) are quite prevalent in Bangladesh. Because of price of glucose-lowering medications and absence of modern hospitals and doctors, considerable number of diabetic rural folks depend on local folk medicinal practitioners and herbal formulations for keeping their blood glucose levels under control. For these reasons, discovery of available and affordable glucose-lowering drugs with lesser side effects is of utmost importance for the low income people of developing countries like Bangladesh. The objective of the present study was to conduct oral glucose tolerance test (OGTT) in Swiss albino mice with a combination of methanolic extracts of roots of *Stemona tuberosa* and fruits of *Bulbophyllum neilgherrense*, plant parts of which have previously been shown individually to reduce blood glucose (BG) levels in glucose-challenged mice. The methanolic extracts of roots of *Stemona tuberosa* (MEST) and fruits of *Bulbophyllum neilgherrense* (MEBN) reduced BG levels by 40.3 and 41.0%, respectively, at a dose of 400 mg/kg body weight. When given in combinations of 50, 100, 200 and 400 mg/kg each, (MEST + MEBN) reduced BG levels, respectively, by 34.1, 37.5, 43.3 and 46.1%. A standard BG lowering drug, glibenclamide, administered at 10 mg/kg, reduced BG by 43.3%. The results indicate that a polyherbal formulation containing MEST and MEBN may be more effective in control of elevated BG levels in diabetic patients than glibenclamide.

**Keywords:** Diabetes; *Stemona tuberosa*; *Bulbophyllum neilgherrense*; OGTT; Glibenclamide

### Introduction

*Stemona tuberosa* Lour. (Stemonaceae) is a vinous flowering plant that can be found in Bangladesh. It is known in English as 'wild asparagus' and locally as 'lal guraniya alu'. The plant and its various plant parts have diverse ethnic uses in many countries of the world; these uses include coughs, chest complaints, tuberculosis, respiratory disorders, helminthiasis, and jaundice (Reviewed by Bharali., *et al.* [1]). *Bulbophyllum neilgherrense* Wight (Orchidaceae) is known locally as 'ek pata ek fol' and fruits of the plant are used for treatment of gastric disorders and lessening anger [2]. The plant has been reported from Rema-Kalenga Wildlife Sanctuary in Habiganj District, Bangladesh. Methanolic extract of aerial parts of the plant (excluding fruits) have been reported to lower blood glucose levels in oral glucose tolerance tests (OGTTs) in glucose-challenged mice [3]. Lowering of blood glucose in OGTTs in Swiss albino mice has also been reported for methanolic extract of roots of *Stemona tuberosa* [4].

Diabetes, although non-contagious, is rapidly acquiring a magnitude in Bangladesh, which approximates a contagious disease. Diabetes (Types 1 and 2) is essentially a disorder in which the body does not produce enough insulin or becomes resistant to insulin, leading to

alterations in normal glucose homeostasis, and which is manifested in the form of elevated blood glucose and passing of glucose in urine. Left unchecked, diabetes can rapidly lead to disorders of the retina, kidney and nervous system and may even necessitate amputation of limbs [5]. Diabetes is more prevalent in Bangladesh among the rural poor; a cross-sectional survey in 2016 found prevalence of diabetes to be 8.9 and 11.4% in men and women of 30 years age or more. Furthermore 17% men and 23% women had intermediate hyperglycemia or impaired glucose tolerance [6].

A large number of drug groups (both oral drugs and insulin injections) are available for controlling diabetes (Type 2), but they are not without side-effects, and as a result complementary therapies have been proposed [7]. Complementary therapies for diabetes most often involve plants (referred to as antidiabetic plants), their various parts, and phytochemicals. In India alone, about 800 plants are regarded to have antidiabetic potential from their ethnic use reports [8]. Diabetes and antidiabetic plants have been mentioned in ancient medical treatises of Ayurveda from about 5,000 years ago [9]. Since Bangladesh is rich in flora, and at the same time drugs to control diabetes are not readily available and affordable to the rural poor, herbal medicines can serve a realistic means for diabetes control. For that reason, we had been systematically conducting a survey of local plants and building up a data base of the antidiabetic plants as determined from OGTT tests on methanolic extracts of various plants and plant parts [10-15]. Previously we have reported OGTTs done on methanolic extracts of roots of *Stemona tuberosa* [4] and aerial parts (minus fruits) of *Bulbophyllum neilgherrense* [3]. From the results obtained in those two studies, we thought it would be of interest to evaluate in OGTTs a combination of methanolic extract of roots of *Stemona tuberosa* (MEST) and methanolic extracts of fruits of *Bulbophyllum neilgherrense* fruits (MEBN) for any synergistic glucose lowering effects.

## Materials and Methods

### Plant material collection

Both plant materials were collected from Rema-Kalenga Wildlife Sanctuary in Habiganj district, Sylhet Division as described previously [3,4]. The Sanctuary occupies an area of nearly 1796 hectares and is home to a number of endangered floral and faunal species. Whole plants (at least one each of *Stemona tuberosa* and *Bulbophyllum neilgherrense*) were collected, dried and pasted onto herbarium sheets for identification purposes. Roots of *Stemona tuberosa* and fruits of *Bulbophyllum neilgherrense* were collected separately. Forest officials and local villagers who reside by the edges of the forest area assisted in searching for and collecting the plants. Plant specimens were taxonomically identified at the University of Development Alternative, Dhaka, Bangladesh by a plant taxonomist on the basis of the whole plant itself. The herbarium sheets were deposited with the Medicinal Plant Collection Wing of the University of Development Alternative. Plant collections were done during February 2017. The plant specimens were given an accession number of MPCW-UODA 78/2017 and MPCW-UODA 79/2017 for *Stemona tuberosa* and *Bulbophyllum neilgherrense*, respectively.

### Preparation of methanolic extract of *Stemona tuberosa* roots (MEST) and *Bulbophyllum tuberosa* fruits (MEBN)

For preparation of methanol extract of roots of *Stemona tuberosa*, the roots were first washed thoroughly with water to clean any attached soil and were then dabbed with tissue paper to soak up the water. Roots were then cut into small pieces and air-dried in the shade for 72 hours till they were dry enough to be powdered. 100g of powdered roots were extracted with 5 volumes of methanol for 48 hours. Methanol was evaporated at 50°C and the extract (MEST) was preserved in small amounts at 4°C. The final dry weight of MEST was 1.98g.

For preparation of methanol extract of *Bulbophyllum neilgherrense* fruits, fruits were cut into small pieces following washing and draining off the water and then air-dried for 72 hours in the shade till they were dry enough to be powdered. 100g of powdered fruits were extracted with 5 volumes of methanol for 48 hours. Methanol was evaporated at 50°C and the extract (MEBN) was preserved in small amounts at 4°C. The final dry weight of MEBN was 2.04g.

The extracts (MEST and MEBN) were suspended in 10% dimethyl sulfoxide (DMSO) prior to administration to mice by gavaging in oral glucose tolerance tests.

### **Chemicals and drugs**

Glibenclamide and glucose were purchased from Square Pharmaceuticals Limited, Bangladesh. All chemicals were of analytical grade. Glucometer and strips were obtained from a drug store known as Lazz Pharma, located in Dhaka, Bangladesh [16].

### **Animals**

Swiss albino mice of both sexes and weighing around 14-16g (seven weeks old and same batch) were used in the present study. Mice were purchased from the Animal House of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The animals were kept in the laboratory so that they can get accustomed with the laboratory conditions for three days prior to actual experiments. During their 3 days at the laboratory, they were kept at 25°C, and fed with standard mice chow (obtained from ICDDR,B) and water *ad libitum*. Approval from the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh was obtained prior to commencement of experiment. During the experiment, the European Union (EU) Directive 2010/63/EU for animal experiments was adhered to. Every means was taken to minimize any pain to the experimental animals [16].

### **Oral glucose tolerance tests for evaluation of antihyperglycemic activity**

Oral glucose tolerance tests were done as described previously by Joy and Kuttan [17] with a few minor modifications to their procedure. Mice were fasted for 16 hours before being randomly distributed into eight groups of 5 mice each. Group 1 mice received only vehicle (10% DMSO in water; 10 ml/kg body weight) and served as control, Group 2 mice were administered a standard drug (glibenclamide, 10 mg/kg body weight). Groups 3 and 4 mice received MEST and MEBN, respectively, at a dose of 400 mg per kg body weight. Groups 5-8 mice received, respectively, 50, 100, 200, and 400 mg each of (MEST + MEBN) per kg body weight. All administrations were done orally by gavaging. After giving the mice a period of one hour following oral administration of MEST, MEBN, (MEST + MEBN) or glibenclamide, as described earlier; all mice were given by gavaging 2g glucose per kg of body weight. After 120 minutes of glucose administration, heart was punctured to collect blood for glucose measurement. A glucometer was used to measure blood glucose. Lowering of blood glucose levels (%) was calculated according to the formula (below):

Percentage lowering of blood glucose level =  $(1 - W_e/W_c) \times 100$ ,

Where  $W_e$  and  $W_c$ , respectively, represents the concentration of blood glucose in glibenclamide or extract(s) administered mice (Groups 2-8), and control mice (Group 1) [14]. It may be mentioned that we preferred puncturing heart to obtain enough blood for glucose measurements. Mice are small animals, and while tail vein puncturing to obtain enough blood works for rats, it does not work satisfactorily for mice in our experiences.

### **Statistical analysis**

Experimental values are expressed as mean  $\pm$  SEM (standard error of mean). For statistical comparison, independent Samples t-test was carried out. A p value < 0.05 in all cases indicated statistical significance [10-15].

### **Results and Discussion**

At a dose of 400 mg/kg body weight, MEST and MEBN, respectively, reduced blood glucose in glucose-challenged mice (OGTT) by 40.3 and 41.0% versus 43.3% obtained with the standard drug glibenclamide administered at 10 mg/kg body weight. All results are shown in

table 1. At the same time, the combinations of (MEST + MEBN) at 50, 100, 200 and 400 mg each/kg body weight, reduced blood glucose concentrations in glucose-challenged mice by 34.1, 37.5, 43.3, and 46.1%, respectively. The results suggest that the combination of (MEST + MEBN) at a dose of 400 mg each (Group 8) was better in lowering blood glucose levels than the standard antidiabetic drug glibenclamide (Group 2), even though the differences in percent reductions of blood glucose between the two groups were small.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	5.86 ± 0.14	-
Glibenclamide	10 mg	3.32 ± 0.12	43.3*
(MEST)	400 mg	3.50 ± 0.07	40.3*
(MEBN)	400 mg	3.46 ± 0.09	41.0*
(MEST + MEBN)	(50 + 50) mg	3.86 ± 0.14	34.1*
(MEST + MEBN)	(100 + 100) mg	3.66 ± 0.07	37.5*
(MEST + MEBN)	(200 + 200) mg	3.32 ± 0.11	43.3*
(MEST + MEBN)	(400 + 400) mg	3.16 ± 0.08	46.1*

**Table 1:** Effect of MEST, MEBN and (MEST + MEBN) on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

All administrations were made orally. Values represent mean ± SEM (standard error of mean), (n = 5); \*p < 0.05; significant compared to hyperglycemic control animals.

There are several directions to go in the future. One is to use the methanolic extract combinations of the two plant parts (MEST + MEBN) as an affordable and readily available antidiabetic agent. This approach has the advantage of providing to a potential diabetic patient through the crude extract many other antidiabetic compounds or phytochemicals that may be present within the crude extract, and which phytochemicals may offer protective action against diabetes-induced complications besides lowering elevated blood sugar. On the other hand, crude drugs or herbal formulations usually lack the necessary information on exact concentration(s) of the active ingredient(s) and may have toxic effects [18]. A second approach is to utilize bio-activity guided fractionation to identify possible compounds of interest, which is the modern or allopathic approach (also considered as the scientific approach), like discovery of artemisinin from *Artemisia annua* [18]. This approach has the advantage of discovery of possibly newer and more efficacious antidiabetic lead compounds or drugs. Another approach can be to examine the crude extract or bioactive isolated components on gene and protein level activation of Sirtuin 1 and other genes that are critical for insulin release and glucose lowering. It was not possible in our laboratory to extend the research further at the present time, but these studies are necessary and intended to be performed in the future.

In the present study, we have dealt with two plants with limited reports of phytochemicals and antidiabetic studies. That alone makes the two plants and their plant parts and their combination(s) providing scientific interests in the discovery of drugs against diabetes. Diabetes may be a more serious problem in the long term than the current COVID-19 pandemic because diabetes cannot be cured; diabetic symptoms like high blood glucose can only be alleviated with antidiabetic drugs. About 34.2 million persons of all ages had diabetes in USA in 2018, which was 10.5% of the US population, and about 1.5 million diabetes cases are being added every year [19]. In contrast, as of March 18, 2021 that was a little more than one year after the COVID-19 outbreak, the US had total COVID-19 infections of 30,301,478 persons [https://www.worldometers.info/coronavirus/], and the number of infections is expected to fall significantly now that vaccines against the COVID-19 virus, SARS-CoV-2 has been introduced [20].

## Conclusion

The results of the present study indicate that a combination of methanolic extract of roots of *Stemona tuberosa* and fruits of *Bulbophyllum neilgherrense* at doses of 400 mg/kg each can be more effective in lowering blood glucose levels than the standard antihyperglycemic

drug glibenclamide (10 mg/kg) in OGTT tests. As such, the plants can be considered for further studies towards discovery of new affordable antidiabetic drugs with possibly lower side-effects.

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### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

### **Author Contributions**

SA collected the plants and did the experiments. MR conceptualized the project, supervised the experiment, and wrote the first draft of the manuscript. Both authors edited and agreed to the final draft.

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