



**ORAL GLUCOSE TOLERANCE TESTS WITH COMBINATION OF
METHANOLIC EXTRACT OF AERIAL PARTS OF *Bulbophyllum
neilgherrense* AND GLIBENCLAMIDE**

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ABSTRACT

Background: *Bulbophyllum neilgherrense* Wight is an epiphytic monocot belonging to the Orchidaceae family and found in Bangladesh and India. Since orchids have always been used in indigenous medicines, it was therefore of interest to evaluate the antihyperglycemic activity of aerial parts of this orchid and synergistic effect, if any, with an antihyperglycemic drug, glibenclamide. **Methods:** Antihyperglycemic activity was determined through oral glucose tolerance tests (OGTT) in mice. **Results:** Administration of methanol extract of *Bulbophyllum neilgherrense* aerial parts (MEBN) at a dose of 400 mg per kg to glucose-loaded mice reduced blood glucose level by 25.7%. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg

body weight, reduced blood glucose level by 38.3%. MEBN, when administered at doses of 200 and 400 mg per kg along with glibenclamide at 10 mg per kg, respectively reduced blood glucose levels by 43.3, and 44.0%. **Conclusion:** Methanolic extract of aerial parts of *Bulbophyllum neilgherrense* (MEBN) can act synergistically with antihyperglycemic drugs like glibenclamide, and so can reduce glibenclamide dependence.

KEYWORDS: Antihyperglycemic, *Bulbophyllum neilgherrense*, Glibenclamide, OGTT, Mice.

BACKGROUND

Bulbophyllum neilgherrense Wight (local name: purusharantha) belongs to the Orchidaceae family and is considered a medicinal species by various tribes of India.^[1] The Gowli tribals of Uttara Kanada of Karnataka use the plant to treat various ailments like heart diseases, rheumatism, and leucoderma.^[2] The pseudobulbs are used as a tonic by some tribals in Bangladesh.^[3] The Irulars of Vellingiri Hills, Coimbatore, India, use whole plants to cure pimples and skin allergy.^[4] The local population residing around the Rema-Kalenga Wildlife Sanctuary in Habiganj district, Bangladesh, uses fruits of the plant to increase sexual strength, gastric disorders, and to lessen anger.^[5]

According to the World Health Organization (WHO), overall 8% of the people in Bangladesh suffer from diabetes (8.6% males, 7.4% females).^[6] Another study found age-standardized prevalence of diabetes was 9.2%. Moreover, there was a huge inequality in the awareness, treatment and control of diabetes between the poor and the wealthy; for instance, 56.6% of the wealthy people could afford treatment versus 15.8% of the poor.^[7] The prevalence of diabetes is increasing in people who are in a transitional state of urbanization.^[8]

Diabetes is characterized by elevated blood glucose levels, which if not controlled can lead to occurrences of cardiovascular disorders, kidney failures, and blindness of eyes, among other disorders. Although drugs are available to control blood glucose levels, cost of drugs, lack of accessibility to trained doctors and diagnostic centers, and adverse effects of antidiabetic drugs can be factors in lack of treatment. As such, we had been screening medicinal plants of Bangladesh for their blood glucose lowering properties towards finding an easily available and affordable solution to the incidental medicinal costs of the people suffering from diabetes.^[9-34] Also, it would be beneficial to the diabetic population if a safer substitute can be found to reduce dependency on existing antidiabetic drugs. The objective of the present study was to evaluate the antihyperglycemic potential of methanolic extract of *Bulbophyllum neilgherrense* aerial parts (MEBN) along with an antidiabetic drug, glibenclamide through oral glucose tolerance tests (OGTT) in mice.

METHODS

Plant material collection and extraction

Aerial parts of *Bulbophyllum neilgherrense* were collected from Rema-Kalenga Wildlife Sanctuary in Habiganj district, Bangladesh during December, 2016. Plant specimen was taxonomically identified by a trained botanist at the University of Development Alternative.

The sliced air-dried aerial parts of *Bulbophyllum neilgherrense* were grounded into a fine powder and 76g of the powder was extracted with methanol (1:5, w/v) for 48 hours. The extract was evaporated to dryness. The final weight of the extract (MEBN) was 1.852g. The extract was stored at -20°C till use.

Chemicals and Drugs

Glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals

Swiss albino mice, which weighed between 15-20g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The animals were acclimatized for three days prior to actual experiments. During this period, they were kept in a temperature controlled room (25°C) and given standard mice chow and water *ad libitum*. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Oral glucose tolerance tests (OGTT) for evaluation of antihyperglycemic activity

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999) ^[35] with minor modifications. Briefly, fasted mice were grouped into five groups of five mice each. The various groups received different treatments like Group 1 received vehicle and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Group 3 received MEBN at a dose of 400 mg per kg body weight. Groups 4 and 5 received, respectively, 10 mg/kg glibenclamide plus 200 and 400 mg per kg MEBN. All substances were orally administered. Following a period of one hour, all mice were orally administered 2g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured with a glucometer. 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 various extracts administered mice (Groups 2-5), and control mice (Group 1), respectively.

Statistical analysis

Experimental values are expressed as mean \pm SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.^[22]

RESULTS**Oral glucose tolerance test (OGTT) results**

Administration of MEBN at a dose of 400 mg per kg to glucose-loaded mice reduced blood glucose level significantly by 25.7%. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 38.3%. A combination of 10 mg/kg glibenclamide plus 200 and 400 mg MEBN lowered blood glucose levels, respectively, by 43.3, and 44.0%. The results are shown in Table 1 and suggest that MEBN can give a synergistic antihyperglycemic effect (that is improve oral glucose tolerance) when administered with glibenclamide. Thus the results indicate that administration of MEBN may lower dependency on glibenclamide for lowering blood glucose.

Table 1: Effect of MEBN and glibenclamide 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	6.00 \pm 0.07	-
Glibenclamide	10 mg	3.70 \pm 0.08	38.3*
(MEBN)	400 mg	4.46 \pm 0.11	25.7*
(MEBN + glibenclamide)	(200 + 10) mg	3.40 \pm 0.04	43.3*
(MEBN + glibenclamide)	(400 + 10) mg	3.36 \pm 0.05	44.0*

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

DISCUSSION

Glibenclamide is a drug used in Bangladesh and other countries of the world to lower elevated blood glucose levels in diabetic or glucose-impaired patients. However, the drug has some adverse effects like nausea, vomiting, constipation, and diarrhea. It has also been suggested that glibenclamide may cause fatal hypoglycemic coma in the elderly patients.^[36] As such, concomitant MEBN administration with glibenclamide can necessitate lesser intake of glibenclamide and may reduce the adverse side-effects noted with the compound. The other advantage is that since *Bulbophyllum neilgherrense* can be grown locally, it may prove

to be more affordable, at least in its extract form. Furthermore, the possibility of discovery of new and better antidiabetic drugs from *Bulbophyllum neilgherrense* cannot be ruled out.

CONCLUSION

The results suggest that methanolic extract of aerial parts of *Bulbophyllum neilgherrense* can act synergistically with glibenclamide for lowering blood glucose in glucose-loaded mice.

CONFLICTS OF INTEREST

The author(s) declare that they have no competing interests.

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