

Oral Glucose Tolerance Test with Methanolic Extract of *Homalomena aromatica* (Araceae) Whole Plant

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Abstract

Homalomena aromatica (Araceae) is an aromatic medicinal herb found in tropical Asia. Since scientific studies are absent on the anti-hyperglycemic efficacy of this herb so far, the objective of the present study was carried out to evaluate the possible antihyperglycemic potential of methanolic extract of whole plant, which is traditionally used by the Marma tribe of Chittagong Hill Tracts, Bangladesh to keep elevated blood glucose level under control. Oral glucose tolerance test (OGTT) was used for evaluation of antihyperglycemic activity of methanolic extract of whole plant of *Homalomena aromatica* (MEHA). The results showed blood glucose levels were significantly reduced in glucose-challenged mice at the two evaluated doses of 200 and 400 mg MEHA per kg body weight by 62.0 and 68.3%, respectively, compared to untreated mice (control). Glibenclamide (an antihyperglycemic drug used as standard), when given to mice at a dose of 10 mg per kg body weight, resulted in a decrease of blood glucose level by 61.0%, demonstrating that in this regard both doses of MEHA were better than glibenclamide. The results suggest that the Marmas are valid in using the whole plant to decrease glucose levels in blood.

Keywords: *Homalomena aromatica*; Antihyperglycemic Efficacy; OGTT; Type 2 Diabetes Mellitus

Introduction

Traditional herbal medicine, commonly known as phytomedicine, have re-emerged as an integral part of primary health care system in most of the developing and industrialized countries due to its lesser or no side effects and less expense compared to synthetic drugs [1,2]. Herbal medicinal plants had been traditionally used for the treatment of diabetes [3], an endocrinological disorder that is rapidly reaching almost endemic proportions throughout the world. According to the World Health Organization, the world diabetic population increased from 108 million in 1980 to 422 million in 2014. Among adults over 18 years of age, the percent of diabetic persons rose from 4.7% in 1980 to 8.5% in 2014, and this percent has been rising more rapidly in the less rich countries [4]. Diabetes is characterized by high blood glucose levels, passing of glucose in urine and frequent urination, but the disease may quickly progress to more complicated disorders like diabetic retinopathy, neuropathy and nephropathy [5].

In Bangladesh, the prevalence of diabetes is gradually rising in both urban and rural areas. Because of lack to modern clinical facilities and doctors, many tribal and below poverty level income diabetic patients resort to medicinal plants administered by folk and tribal medicinal practitioners for treatment of diabetes. We had been surveying and screening medicinal plants used by folk and tribal medicinal practitioners against diabetes for a number of years through oral glucose tolerance tests in mice [6-8].

Homalomena aromatica (Spreng.) Schott (Araceae) also known as 'Sugandhmantri' is a very popular aromatic medicinal herb mostly prevalent in the deep shady forests in north eastern regions of India and in the hilly areas of Chittagong and Moulvibazar districts of Bangladesh. The antidiabetic efficacy of this herb has not been investigated before, although in visits to the Marma inhabited area (Bandarban district, Bangladesh) the authors found them to chew the whole plant for blood glucose lowering purpose.

Objective of the Study

The objective of the present study was to determine whether methanolic extract of *Homalomena aromatica* whole plant possess anti-hyperglycemic activity.

Materials and Methods

Plant material collection

The whole plant of *Homalomena aromatica* (tubers and aerial parts) was collected from the Marma tribal community in Ramu Upazila (sub-district) of Bandarban district in Chittagong Hill Tracts region of Bangladesh. Members of the community took the authors within the forested hill tract some distance from their habitat and showed us some areas from where the plants could be collected in the wild. Plants were collected in November 2018, when the dry season has started leaving only a few leaves of the plant above the ground. It was not possible to prepare any voucher specimens from the plant samples collected at Ramu. However, a few tubers of the plant, brought at the same time were planted in containers at the University of Development Alternative, where they remained dormant till April 2019. Around May 2019, when the rainy season started, the tubers sprouted and identification was made of the plants. A picture of the plant in a container is shown in figure 1. Plant specimen was then taxonomically identified at the University of Development Alternative, Dhaka, Bangladesh by a plant taxonomist on the basis of the actual plant itself and its tuber, the plant being deposited with the Medicinal Plant Collection Wing of the University of Development Alternative, and cultivation presently being done in both container and soil. The plant specimen was given an accession number of MPCW-UODA 143/2019.



Figure 1: *Homalomena aromatica* plant growing in container at the University of Development Alternative.

Preparation of methanolic extract of *Homalomena aromatica* whole plant (MEHA)

For preparation of methanol extract of whole plant of *Homalomena aromatica*, whole plants were cut into small to medium sized pieces and shade-dried for 72 hours. 100g of powdered dried plants were extracted with 5 volumes of methanol for 48 hours. The extract (MEHA) was dried and freed completely from methanol at 50°C and preserved in small amounts at 4°C till use to avoid repeated freeze-thawing. The final dry weight of MEHA was 3.66g. The extract (MEHA) was suspended in dimethyl sulfoxide (DMSO) prior to administration directly to mice by gavaging in oral glucose tolerance test (OGTT).

Chemicals and drugs

Square Pharmaceuticals Limited, Bangladesh was the source of glibenclamide and glucose. All chemicals were of analytical grade. Glucometer and strips were obtained from a drug store known as Lazz Pharma, Bangladesh.

Animals

The present study used Swiss albino mice of both sexes, which weighed between 14 - 18g. Mice were purchased from the Animal House of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). All mice were of the same age group of close to seven weeks and obtained from the same batch. As such, it was expected that there will be no metabolic differences within the groups. Moreover, we have done oral glucose tolerance tests with other plant extracts dozens of times previously and published our results, so we followed the same procedure in this experiment also. The animals were kept in the laboratory to accustom them 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 feed and water without any restrictions in the daily feed and water intake. 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. Precautions were taken to ensure the comfort of animals throughout the whole period of study. Every means was taken to minimize any pain to the experimental animals.

Oral glucose tolerance tests for evaluation of antihyperglycemic activity

Oral glucose tolerance tests were done as described previously by Joy and Kuttan [9] with minor modifications to their procedure. Mice fasted for 16 hours were randomly distributed into four groups of 4 mice each. Treatments varied according to the Group number like 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 MEHA at doses of 200 and 400 mg per kg body weight, respectively. Gavaging was the method used for administration of glucose, glibenclamide or MEHA. After giving the mice a period of one hour following oral administration of MEHA or glibenclamide, as described earlier, all mice were given by gavaging 4g 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 administered mice (Groups 2 - 4) and control mice (Group 1) [7]. It may be mentioned in this regard that in a number of previous experiments with similar age and weight mice, we have tried to obtain blood by tail vein puncture but have failed because of the low quantity of blood drawn and that drawing of blood only possible once. It could be that the mice were young and small in size. So we resorted to blood collection by puncturing heart.

Statistical analysis

Experimental values are expressed as mean \pm SD (standard deviation). For statistical comparison, independent Samples t-test was carried out. A p value < 0.05 in all cases indicated statistical significance [7].

Results

Methanol extract of *Homalomena aromatica* (MEHA) at doses of 200 and 400 mg per kg body weight each administered to glucose-loaded mice resulted in reduction of blood glucose levels by 62.0 and 68.3%, respectively. Glibenclamide, which is known as an antihyperglycemic or glucose level normalizing drug, when given to mice at a dose of 10 mg per kg body weight, reduced blood glucose level by 61.0%. Thus, at both the doses tested, MEHA demonstrated better oral glucose tolerance in mice. The results are shown in table 1.

| Treatment | Dose (mg/kg body weight) | Blood glucose level (mmol/l) | % lowering of blood glucose level |
|---------------|--------------------------|------------------------------|-----------------------------------|
| Control | 10 ml | 6.85 ± 0.19 | - |
| Glibenclamide | 10 mg | 2.68 ± 0.30 | 61.0* |
| (MEHA) | 200 mg | 2.60 ± 0.71 | 62.0* |
| (MEHA) | 400 mg | 2.18 ± 0.53 | 68.3* |

Table 1: Effect of MEHA on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading. All administrations were made orally. Values represent mean ± SD (standard deviation), (n = 4); *p < 0.05; significant compared to hyperglycemic control animals.

Discussion

The two doses of MEHA did not result in any visible toxic effect in mice, which was not surprising considering that the Marma tribal people chew whole plants without showing any toxic symptoms. Preliminary phytochemical analysis of methanolic rhizome, leaf and petiole extracts of *Homalomena aromatica* have showed the presence of alkaloids, tannins, saponins, cardiac glycosides, phenolic compounds, terpenoids, and coumarins [10]. In a previous experiment on oral glucose tolerance in mice, phytochemical screening of the methanolic extract of the aerial parts of *Alternanthera sessilis* plants [7] showed the presence of tannins, alkaloids, and flavonoids, thus showing some similarities with phytochemical screening of *Homalomena aromatica*. Although not investigated in the present study, these groups of compounds may be responsible for the observed glucose-lowering effects. To our knowledge this is the first report on the anti-diabetic potential of this plant and further investigation to authenticate the use of *H. aromatica* as a natural plant anti-diabetic is recommended. Even as of present, the plant can serve as a useful and affordable substitute to costly blood glucose lowering drugs.

Conclusion

The results obtained in the present study merit potential for further investigations on isolation and characterization of active principle(s) from the extract that is responsible for the hypoglycemic effect. Besides, the study reveals the importance of *H. aromatica* in the primary healthcare of the tribal community, as well as in the whole society. Hence, it is necessary to conserve the indigenous knowledge of this valuable herb and their therapeutic uses before it is lost forever.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

RRN, KJ, and RAM did the actual OGTT experiment. MR and RJ supervised the experiment and wrote the first draft of the manuscript. PD analyzed the results and also supervised the experiment. All authors contributed to edition of the first draft and agreed to the final draft. Actual plant samples were collected by MR, KJ and RAM from Bandarban district.

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