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Khoshnur Jannat
Department of Biotechnology & Genetic Engineering, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh

Rownak Jahan
Department of Biotechnology & Genetic Engineering, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh

Mohammed Rahmatullah
Department of Biotechnology & Genetic Engineering, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh

Corresponding Author:
Mohammed Rahmatullah
Department of Biotechnology & Genetic Engineering, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh

Oral glucose tolerance test with methanolic extract of *Zingiber montanum* Link ex A. Dietr. (Zingiberaceae) flowers

Khoshnur Jannat, Rownak Jahan and Mohammed Rahmatullah

Abstract
Zingiber montanum or cassumunar zinger is a common ginger species in Asia, which has ethnic uses against various ailments like headache, dysentery, constipation, skin diseases, inflammation, jaundice, dyspepsia, pain, ulcer, fever and common cold. The objective of the study was to evaluate the antihyperglycemic effect of methanol extract of flowers of the plant, which is used by the Marma tribe in the Bandarban area of Bangladesh to keep high blood glucose level under control. Antihyperglycemic activity was determined through oral glucose tolerance test (OGTT) with methanol extract of *Zingiber montanum* flower. The extract (MEZM) at doses of 50, 100, 200, and 400 mg per kg body weight each to glucose-loaded mice reduced blood glucose levels by 13.6, 21.8, 27.9, and 32.3%, respectively, compared to control (untreated) mice. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by40.8%. Our results validate the use of flowers by the Marma tribe to lower elevated blood glucose levels.

Keywords: *Zingiber montanum*, cassumnar, diabetes, OGTT, antihyperglycemic

Introduction
Diabetes is a disturbance of glucose homeostasis characterized by elevated levels of glucose in blood. This metabolic disorder can be caused through less secretion of insulin from pancreatic beta-islets or rising insulin resistance or less removal of glucose from the blood. The disease has been known from the ancient time; for instance in Ayurveda, the disease has been described almost five thousand years ago as modhu-meho (sweet test of urine), pro-meho (urinary problem), and bahumutro (frequent urination), all being characteristics of the disorder known in present time as diabetes, diabetes mellitus orDM.

The disease has already reached endemic proportion in the world and reaching epidemic proportion. The cause for the rapid increase in the number of diabetic patient throughout the world is not known but may have to do with a more sedentary life style and increased consumption of refined sugar. According to the World Health Organization (WHO), the global prevalence of diabetes among adults over 18 years age has risen from 4.7% in 1980 to 8.5% in 2014. It is estimated that the prevalence of diabetes is rising more rapidly in middle and low income countries than the developed countries [1].

In Bangladesh, several surveys were conducted to measure the percentage of pre-diabetic and diabetic patients in rural area. A survey conducted among people over 30 years of age from 96 villages showed combined prevalence of impaired fasting glucose, impaired glucose tolerance and diabetes as 26.1% among men and 34.9% among women, and which increased with age [2]. Studies showed that rural people have less knowledge about diabetes and they are not even aware of the consequences of the disease they are bearing [3].

Diabetes is mainly divided into two types. Type 1 diabetes is characterized by deficient insulin production because the pancreas can no longer produce insulin. As a result the body becomes unable to control blood sugar and requires daily administration of insulin. That is why this type is known as insulin dependent diabetes mellitus (IDDM). Symptoms appearing in this disease are excessive urination (polyuria), thirst (polydipsia), weight loss, constant hunger, changes in vision andfatigue.

Type 2 diabetes mainly happens because of ineffective use of insulin. It is a metabolic disorder which is the result of decreased pancreatic beta cell mass, so that the body becomes insensitive to the effect of insulin.

This type of diabetes is known as non-insulin dependent diabetes mellitus or NIDDM. Type 2 diabetes is now prevalent among the majority of people with diabetes around the world.

Another type of diabetes is called Gestational diabetes, which is blood glucose impairment during pregnancy. Women having this type of diabetes bear the risk of developing type 2 diabetes in the future.

Impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG) are intermediate conditions in the transition between normality and diabetes. People with IGT and IFG are at high risk of progressing to type 2 diabetes, although this is not inevitable [1, 4, 5].

Oral medications currently available for treating hyperglycemia in NIDDM include sulfonylureas, which increase the release of endogenous insulin as well as improve its peripheral utilization. This class of drug includes glimepiride, glyburide (glibenclamide), chlorpropamide, and glipizide (glydiazianide). Some possible side effects include low blood glucose (hypoglycemia), upset stomach, skin rash or itching, and/or weightgain.Another class of anti-diabetic drugs is biguanide, which includes metformin. This drug lowers the blood glucose by lowering the production of glucose by liver. Additionally this drug helps in improving blood fat and cholesterol level. Some possible side effects of metformin include nausea, diarrhea and other stomach symptoms, weakness or difficulty breathing, or a metallic taste in the mouth.

Alpha-glucosidase inhibitors are another class of drugs that block the enzyme that digest the starch that is taken. Drugs of this class are known under the generic names acarbose and miglitol. Possible side effects include stomach problems (gas, bloating, and diarrhea).Meglitinides are known under the generic name rapaglinide and rateglinide which are a class of anti-diabetic drugs that makes the pancreas produce more insulin after taking meal. Possible side effects include hypoglycemia and weight gain [6, 7].

The drugs that have been mentioned, besides causing adverse effects, are costly and not affordable for the low income people of Bangladesh. Illiteracy also makes the people mistake medication doses. Besides, the drugs cannot be found readily in rural and remote areas. Insulin injection is also so much expensive that rural people cannot afford it tocontinue. As a result, scientists including ourselves have been looking towards the plant kingdom in efforts to find newer, affordable and readily available drugs with better antihyperglycemic efficacies [8-29].

Towards finding other antidiabetic plants, which may contain compounds of greater interest, the objective of this study was to find out through oral glucose tolerant test (OGTT), the antihyperglycemic efficacy of methanolic extract of *Zingiber montanum* flower. The flowers of the plant is chewed by the Marma tribe of Bandarban district in the Chittagong Hill Tracts region of Bangladesh to lower high blood glucose levels in diabetic patient.

Methods

Plant material collection and extraction

Flowers of *Zingiber montanum* (Figure 1) were collected from Bandarban district, Bangladesh. Plant specimen was taxonomically identified by a trained botanist at the University of Development Alternative. Flowers were sliced into thin strips and dried in the shade for 72 hours. The sliced air-dried flowers were grounded into a fine powder and 34g of the powder was extracted with methanol (1:3, w/v) for 48 hours. The extract (MEZM) was evaporated to dryness at 50 ^{o}C and stored in small aliquots at -20 ^{o}C till use. The final weight of MEZM was 1.6g. The extract (MEZM) was suspended in DMSO prior to administration by gavaging to mice in oral glucose tolerance test.

Chemicals

All the chemicals were of analytical grade. Glucose and glibenclamide were obtained from Square Pharmaceuticals, Bangladesh.

Animals

Swiss albino mice, which weighed between 14-18g were used in the present study. The animals were purchased from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in Dhaka city, Bangladesh. The animals were acclimatized for three days prior toactual experiments. During this period, they were kept in a temperature controlled room (25 ^{o}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 and following European Union guidelines for experimenting with animals.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999) [30] with minor modifications. Briefly, mice fasted for sixteen hours were grouped into six 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). Groups 3-6 received MEZM at doses of 50, 100, 200, and 400 mg per kg body weight, respectively. All substances were administered by gavaging. 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 percentage lowering of blood glucose levels were calculated according to the formula describedbelow.

Percentage lowering of blood glucose level = (1 – We/Wc) X 100, where We and Wc represents the blood glucose concentration in glibenclamide or extract administered mice (Groups 2-6), and control mice (Group 1), respectively.

Statisticalanalysis

Experimental values are expressed as mean ± 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 allcases.

Results and Discussion

Administration of methanol extract of *Zingiber montanum* (MEZM) at doses of 50, 100, 200, and 400 mg per kg body weight each to glucose-loaded mice reduced blood glucose levels by 13.6, 21.8, 27.9, and 32.3%, respectively, compared to control (untreated) mice. In comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 40.8%. Thus at the highest dose tested, MEZM demonstrated good capacity to improve glucose tolerance in mice, although the percent lowering of blood glucose was not the same as demonstrated by glibenclamide. The results are shown in Table 1.

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

Treatment	Dose (mg/kg bodyweight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	5.88± 0.18	-
Glibenclamide	10 mg	3.48 ± 0.22	40.8*
(MEZM)	50 mg	5.08 ± 0.26	13.6*
(MEZM)	100 mg	4.6 ± 0.49	21.8*
(MEZM)	200 mg	4.24 ± 0.59	27.9*
(MEZM)	400 mg	3.98 ± 0.37	32.3*

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

The result revealed that administration of 400 mg/kg extract lowered blood glucose level by 32.3%, which was higher than the other doses. Thus the plant has a high probability of containing glucose-lowering constituent (s), which needs to be isolated and identified.

Any new drug, at the least, should possess two qualities. The first is displaying appropriate pharmacological property with appropriate pharmacodynamics. For example, for an anti-diabetic drug, the drug should be able to efficiently reduce elevated blood glucose level along with proper metabolism resulting in non-toxic products, which can be readily excreted through the kidneys and in the process avoiding both hepatic and renal damages. The second property should be that the drug should not demonstrate any cytotoxicity. It is expected that since various parts of *Zingiber montanum* are routinely taken orally by the Marma community, any component(s) of the plant will not be cytotoxic. Notably, pinene, phellandrene, zerumbone, veratric acid have been reported in rhizomes of the plant, which have anti-diabetic properties [31, 32]. These components may also be found in flowers.

Fig 1: Flowers of *Zingiber montanum*

Conclusion

A common antidiabetic drug metformin was isolated from the plant *Galega officinalis*. It means that plantscan be a good source for finding new drugs. As the people of Marma tribe chew the flower for anti-diabetic effect, it suggests that it is non-toxic. The plant is readily available in the Chittagong Hill Tracts and can be a readily available and affordable source of new anti-diabetic drug(s).

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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