

An herbal formulation containing *Zingiber officinale* rhizomes and *Allium sativum* cloves can increase oral glucose tolerance in mice

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Abstract

Zingiber officinale rhizomes and *Allium sativum* cloves are two plant parts widely consumed in Bangladesh as spices, and which are readily available and affordable. The two plants also have ethnomedicinal uses, one of which uses being to treat diabetes. Since the plant parts are consumed in culinary dishes (which involves boiling or steaming), the objective of this study was to determine whether methanol extract of steamed *Z. officinale* rhizomes (MEZO) and steamed *A. sativum* cloves (MEAS) can both individually as well as more so in combination improve glucose tolerance in oral glucose tolerance tests (OGTT) in mice. Administration of either MEZO or MEAS to mice at a dose of 400 mg per kg body weight led to reductions in blood glucose levels in glucose-challenged mice by 37.0 and 34.9%, respectively. Administration of a combination of (MEZO + MEAS) at individual doses of 100, 200 and 400 mg each led to dose-dependent reductions in blood glucose levels, respectively, by 22.5, 30.4, and 42.2%. Thus, the two extracts demonstrated a synergistic effect when administered in combination. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10mg per kg lowered blood glucose level by 43.9%. Thus, at the highest dose tested, the combination of the two extracts lowered blood glucose levels in a comparable manner to that of glibenclamide. Since the extracts were prepared from steamed plant parts, the results suggest that partaking of the two spices in culinary dishes can be effective in controlling blood glucose in diabetic patients.

Introduction

Diabetes mellitus is a disorder primarily characterized by high glucose levels in blood and glucose intolerance. This disorder, which is rapidly increasing throughout the world, can lead to complications of various organs of the body including kidneys, eyes, and heart [1], which in turn can lead to untimely death. Allopathic medicine cannot cure this disorder; however, orally taken drugs are present to increase insulin production from the pancreas and to lower elevated blood glucose levels. Blood glucose level can also be controlled through regular insulin injections. The various oral drugs used to lower blood glucose include sulfonylureas, meglitinides, biguanides, thiazolidinediones, and alpha-glucosidase inhibitors. However, these drugs can have adverse effects ranging from hypoglycemia to gastrointestinal disorders and even increased risks of bladder cancer and non-fatal heart attacks. Also for countries like Bangladesh, the rural and the poorer sections of the population does not have affordability and availability to these medications. As a result, more affordable and readily available substitutes are necessary.

Zingiber officinale Rosc. (Zingiberaceae) and *Allium sativum* L. (Liliaceae) are two commonly cultivated and widely used spice plants of Bangladesh. Rhizomes of the first, known in English as ginger and locally as ada, and cloves of the second, known in English as garlic and locally as roshun, are used in a large number of dishes of Bangladesh, which includes vegetable, fish and meat dishes. The anti-diabetic efficacy of *Z. officinale* rhizomes have been reviewed [2,3]. The efficacy of *A. sativum* cloves against diabetes and diabetes-induced complications have also been variously reported [4-6]. However, the available reports deal with raw rhizomes or cloves or extract there from.

Since the rhizomes or cloves are consumed following cooking (which involves boiling or steaming), it was of interest to determine if steamed plant parts still maintain their antihyperglycemic efficacies, for then cooked rhizomes or cloves as present in various culinary dishes can form an easy means to reduce elevated blood glucose levels. For the last few years we had been screening various medicinal plants including spices for their antihyperglycemic potential as determined through oral glucose tolerance tests or OGTT [7-12]. The objective of the present study was to evaluate whether methanolic extract of steamed rhizomes of *Z. officinale* and steamed cloves of *A. sativum* individually or in combination can improve glucose tolerance in glucose-loaded mice and as such demonstrate antihyperglycemic activity.

Materials and methods

Plant material collection

Rhizomes of *Z. officinale* and cloves of *A. sativum* were collected from a local market in Dhaka city, Bangladesh in January and April 2016, respectively. The plant parts were steamed for 20 min and thoroughly dried separately and finely powdered prior to extraction with methanol.

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Key words: *Zingiber officinale*, *Allium sativum*, ginger, garlic, OGTT

Received: January 03, 2017; **Accepted:** January 18, 2017; **Published:** January 20, 2017

Preparation of methanolic extracts

The plant parts were dried thoroughly following steaming for 20 min and finely powdered, and 100g of dried and powdered individual plant parts (rhizomes or cloves) were extracted with methanol (w:v ratio of 1:5). The final weight of the methanol extract of *Z. officinale* rhizome (MEZO) was 5.8g, and the final weight of *A. sativum* clove (MEAS) was 4.2g. The extracts were 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

Thirty-Five Swiss albino mice, which weighed between 15-18g 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 (72 hours) prior to actual experiments. The study was carried out over a period of 4 days (3 day-night period of acclimatization and one further day to conduct the actual experiment). No mice died during this period. 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) [13] with minor modifications. Briefly, fasted mice were grouped into seven groups of five mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 20 in water, 10ml/kg body weight) and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Group 3 received methanol extract of *Z. officinale* rhizome (MEZO) at a dose of 400 mg per kg body weight. Group 4 received methanol extract of *A. sativum* clove (MEAS) at a dose of 400mg per kg body weight. Groups 5-7 received (MEZO + MEAS) at doses of 100, 200, and 400mg each (that is 100mg of MEZO + 100 mg of MEAS combined) per kg body weight, respectively. 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 by glucose oxidase method [14]. The percent lowering of blood glucose levels were calculated according to the formula described below.

$$\text{Percent lowering of blood glucose level} = (1 - W_e/W_c) \times 100,$$

where W_e and W_c represents the blood glucose concentration in glibenclamide or various extracts administered mice (Groups 2-7), 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 indicated by a p value < 0.05 in all cases [15].

Results

Administration of either MEZO or MEAS to mice at a dose of 400mg per kg body weight led to reductions in blood glucose levels in glucose-

challenged mice by 37.0 and 34.9%, respectively. Administration of a combination of (MEZO + MEAS) at individual doses of 100 (that is 100 mg each of MEZO + MEAS), 200 and 400 mg each led to dose-dependent reductions in blood glucose levels, respectively, by 22.5, 30.4, and 42.2%. Thus, the two extracts demonstrated a synergistic effect when administered in combination. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10mg per kg lowered blood glucose level by 43.9%. Thus, at the highest dose tested, the combination of the two extracts lowered blood glucose levels in a comparable manner to that of glibenclamide. The results are shown in Table 1 and strongly indicate that steaming does not reduce the oral glucose tolerance effect of rhizomes of *Z. officinale* or cloves of *A. sativum*. In fact, both extracts possessed substantial antihyperglycemic effects and the combination was more or less effective as glibenclamide at the highest dose (400 mg each) of the two extracts.

Discussion

Ginger extract and gingerols present in rhizomes has been shown to increase glucose uptake in skeletal muscle cells, which can account for the antihyperglycemic activity of *Z. officinale* [16]. It is interesting that methanolic extract of steamed ginger also demonstrated improved oral glucose tolerance, that is, antihyperglycemic effects. As such, cooked ginger (added to cuisines) can also form the basis of dietary regimen to control blood glucose. Interestingly, the same conclusion has also been reached by an earlier study [17]. The hypoglycemic effect of garlic (*A. sativum*) has been attributed to its sulfur-containing compounds [18]. It is quite possible that such compounds like allicin may either be resistant to breakdown during heating or may form other more active antihyperglycemic compounds during the steaming (heating) process. The identifications of relevant bio-active components and their mechanism of action needs further research and are currently being undertaken in our laboratory. The fact, however, remains that the two spices are readily available, affordable and consumed in Bangladesh by all sections of the people. As such, they can form a suitable substitute for costlier and difficult to obtain allopathic drugs for blood glucose control in diabetic and glucose metabolism impaired persons.

Authorship and contributorship

MS, SR, NR, IJT, and SH collected the plant materials, prepared the extracts and did the experiments. MR guided the work and wrote the manuscript. All authors read the manuscript and made necessary changes to it where applicable. The final version has been approved by all the authors connected with the research work.

Acknowledgement

The authors thank the University of Development Alternative for providing necessary support.

Table 1. Effect of MEZO, MEAS, and (MEZO + MEAS) 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	5.78 \pm 0.12	-
Glibenclamide	10 mg	3.24 \pm 0.05	43.9*
(MEZO)	400 mg	3.64 \pm 0.09	37.0*
(MEAS)	400 mg	3.76 \pm 0.07	34.9*
(MEZO + MEAS)	100 mg each	4.48 \pm 0.09	22.5*
(MEZO + MEAS)	200 mg each	4.02 \pm 0.07	30.4*
(MEZO + MEAS)	400 mg each	3.34 \pm 0.12	42.2*

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

Funding information

This research was performed with internal funding from the University of Development Alternative.

Competing interests

The authors declare that they have no competing interests.

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