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# IMPROVEMENT OF GLUCOSE TOLERANCE WITH A POLYHERBAL FORMULATION OF *BRASSICA RAPA* SUBSP. *RAPA* ROOTS AND *ZINGIBER OFFICINALE* RHIZOMES

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## ABSTRACT

**Background.** *Brassica rapa* subsp. *rapa* (Brassicaceae family) and *Zingiber officinale* (Zingiberaceae family) are two plants cultivated in Bangladesh, respectively, for consumption as vegetable and as spice. A number of Brassicaceae and Zingiberaceae family plants have previously been reported to have antihyperglycemic properties. It was therefore of interest to determine the antihyperglycemic potential of a polyherbal formulation containing the roots of the first and rhizomes of the second plant. **Methods.** Oral glucose tolerance test (OGTT) was done to evaluate antihyperglycemic potential. **Results.** In oral glucose tolerance tests, methanol extract of roots of *Brassica rapa* subsp. *rapa* (MEBRR), administered at a dose of 400 mg per kg body weight reduced blood glucose by 30.8%. Methanol extract of rhizomes of *Zingiber officinale* (MEZO), administered at the same dose reduced blood glucose by 33.6%. Administration of a combination of MEBRR and MEZO (1:1, w/w), tentatively termed MEBRZO, significantly and dose-dependently reduced blood glucose levels in glucose-loaded mice by 32.5, 36.3, 40.4, and 44.5%, respectively, at doses of 50, 100, 200 and 400 mg each per kg body weight in mice. By comparison, a standard antihyperglycemic drug, glibenclamide, reduced blood glucose levels by 46.9% at a dose of 10 mg per kg. **Conclusion.** MEBRZO can be used as a source for blood glucose lowering in diabetic patients in the absence of antihyperglycemic drugs.

**KEYWORDS**: Antihyperglycemic, *Brassica rapa* subsp. *rapa*, OGTT, Zingiber officinale.

## BACKGROUND

A number of Brassicaceae family species have been reported in the scientific literature for their antihyperglycemic properties. These include Brassicaceae mustards[1,2], and cabbage, cauliflower, broccoli and kohlrabi.[3,4] *Zingiber officinale* rhizomes in combination with *Allium sativum* cloves have been reported to improve oral glucose tolerance.[5] Other Zingiberaceae family plants like *Kaempferia rotunda* also reportedly possess antihyperglycemic potential.[6]

Diabetes mellitus, primarily characterized by elevated blood glucose levels, is one of the leading causes of death in the world. Its global prevalence was 8% in 2011 but was predicted to rise to 10% by 2030. In Bangladesh, the number of diabetic patients has already reached 10%.[7] The treatment costs for diabetes are high; in USA, it ranges from $55,000 to $130,000 with an average of $82,500. It may be mentioned in this context that around a third of the population of Bangladesh lives below the poverty level income, defined as less than $2 per day. As a result, such people cannot afford diabetes treatment. On top of it, modern doctors and hospitals are not available to most people of Bangladesh because of non-availability or non-affordability, and diabetic medications like insulin injections are cumbersome to take for many diabetic patients.

To circumvent these problems, one approach can be to find out readily available and affordable glucose lowering drugs. Plants can be a good source, more so if simple consumption of the plants, plant parts, plant decoctions or plant juice can result in decrease of blood glucose levels. Towards that, we had been experimenting with various local plants and formulations for their blood glucose lowering effects.[8-20]. The objective of the present study was to evaluate the oral glucose tolerance efficacy in glucose-loaded mice with methanol extract of a combination of *Brassica rapa* subsp. *rapa* roots and *Zingiber officinale* rhizomes. The two plants belonged to the Brassicaceae or Cruciferae and the Zingiberaceae family, respectively.

## METHODS

***Plant material collection:*** Roots of *Brassica rapa* subsp. *rapa* (English: turnip; Bengali: shalgom) and rhizomes of *Zingiber officinale* (English: ginger; Bengali: ada) were collected from a vegetable market in Dhaka city, Bangladesh in January 2017.

***Preparation of methanolic extract of Brassica rapa subsp. rapa roots and Zingiber officinale rhizomes:*** For preparation of methanol extract of roots of *Brassica rapa* subsp. *rapa* (MEBRR), roots were at first thoroughly dried and pulverized into a fine powder. 100g of the powder was extracted with 500 ml methanol over 48 hours. Methanol was evaporated at 50$^{o}$C and the extract was dissolved in Tween 20 prior to administration to mice by gavaging. The final weight of the extract was 7.586g. For rhizomes of *Zingiber officinale*, the rhizomes were sliced and thoroughly dried prior to pulverizing into a fine powder. 100g of the powder was extracted with 500 ml methanol over 48 hours. Methanol was evaporated at 50$^{o}$C and the extract was dissolved in Tween 20 prior to administration to mice by gavaging. The final weight of the extract (MEZO) was 7g.

***Chemicals and Drugs***

Glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade. Glucometer and strips were purchased from Lazz Pharma, Bangladesh.

***Animals***

Swiss albino mice, which weighed between 12-15g 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 time, the animals were fed with mice chow (supplied by ICDDR,B) 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 for evaluation of antihyperglycemic activity***

Oral glucose tolerance tests (OGTT) were carried out as per the procedure previously described by Joy and Kuttan.[21] with minor modifications. Briefly, fasted mice were grouped into eight groups of five mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 20 in water, 10 ml/kg body weight) and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3 and 4 received MEBRR and MEZO, respectively, at a dose of 400 mg per kg body weight. Groups 5-8 received, respectively, (MEBRR + MEZO, otherwise denoted as MEBRZO) at doses of 50, 100, 200 and 400 mg each extract per kg body weight. All substances were orally administered by gavaging. The amount of Tween 20 administered was same in both control and experimental mice. Following a period of one hour as described earlier[10, 16], all

mice were orally administered 2g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart following previously published procedures.[10,16] 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 = $(1 – W_e/W_c) X 100$,

where $W_e$ and $W_c$ represents the blood glucose concentration in glibenclamide or MEBRR, MEZO or (MEBRR + MEZO) administered mice (Groups 2-8), and control mice (Group 1), respectively. Gavaging was done carefully such that injuries do not happen, and no mice fatalities occurred during gavaging.

***Statistical analysis***

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 all cases.[16]

**RESULTS**

In oral glucose tolerance tests, methanol extract of roots of *Brassica rapa* subsp. *rapa* (MEBRR), administered at a dose of 400 mg per kg body weight reduced blood glucose by 30.8%. Methanol extract of rhizomes of *Zingiber officinale* (MEZO), administered at the same dose reduced blood glucose by 33.6%. Administration of a combination of MEBRR and MEZO (1:1, w/w), tentatively termed MEBRZO, significantly and dose-dependently reduced blood glucose levels in glucose-loaded mice by 32.5, 36.3, 40.4, and 44.5%, respectively, at doses of 50, 100, 200 and 400 mg each per kg body weight in mice. By comparison, a standard antihyperglycemic drug, glibenclamide, reduced blood glucose levels by 46.9% at a dose of 10 mg per kg. Thus the highest dose of MEBRZO gave almost equivalent results in lowering blood glucose compared to glibenclamide and as such can act as a substitute for the drug.

**Table. 1: Effect of MEBRR, MEZO and (MEBRR + MEZO) 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 | 5.84 ± 0.12 | - |
| Glibenclamide | 10 mg | 3.10 ± 0.15 | 46.9* |
| (MEBRR) | 400 mg | 4.04 ± 0.14 | 30.8* |
| (MEZO) | 400 mg | 3.88 ± 0.11 | 33.6* |
| (MEBRR + MEZO) | (50 + 50) mg | 3.94 ± 0.09 | 32.5* |
| (MEBRR + MEZO) | (100 + 100) mg | 3.72 ± 0.06 | 36.3* |
| (MEBRR + MEZO) | (200 + 200) mg | 3.48 ± 0.06 | 40.4* |
| (MEBRR + MEZO) | (400 + 400) mg | 3.24 ± 0.11 | 44.5* |

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

## DISCUSSION

We have previously reported improved glucose tolerance with a polyherbal formulation containing seeds of *Brassica alba*, *Coriandrum sativum*, and *Foeniculum vulgare*.[22] Improved oral glucose tolerance has also been observed with a polyherbal formulation containing *Momordica charantia* and *Brassica oleracea*.[23] Thus various members of the Brassica genus appear to be good candidates for improvement of glucose tolerance through synergistic action with other blood glucose lowering plants as well as also by themselves. In the present report, a synergistic antihyperglycemic action was noted between a *Brassica* species (*Brassica rapa* subsp. *rapa*) and *Zingiber officinale*. Since not all *Brassica* species plants or plant parts are found throughout the year, this opens up an opportunity for diabetic patients to use what *Brassica* species of plant is available at any given time and use that species by itself or in combination with other plants (or plant parts) to lower blood glucose. *Zingiber officinale* rhizome has also been previously reported by us to give synergistic antihyperglycemic action in combination with *Leucas aspera* aerial parts.[24] The rhizome of *Zingiber officinale* (ginger), by itself, has beneficial effects in diabetes.[25] As such, the polyherbal formulation as described in the present study may prove to be a good combination in the amelioration of diabetes and its side-effects.

## CONCLUSION

The results suggest that a combination of methanolic extract of *Brassica rapa* subsp. *rapa* roots and *Zingiber officinale* rhizomes can be used for lowering of blood glucose. Since the plants are cultivated, the plant parts are readily available and affordable. However, *Brassica*

*rapa* subsp. *rapa* is available in Bangladesh only during the winter season (October to February), which can be a point of limitation in the availability of this species year around.

## CONFLICTS OF INTEREST

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

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