Amylase and Glucosidase Enzyme Inhibitory Activity of Ginger (*Zingiber officinale* Roscoe) an *in vitro* Study

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ABSTRACT.   *The prevalence of type 2 diabetes has reached to an epidemic proportion in Sri Lanka. The need for achieving better control of blood glucose level has been evident in diabetes management. However it is not easy to achieve this goal in a large proportion of patients. This is partly due to limitations of currently available pharmacological agents which stimulate research on novel anti-diabetic agents with different mechanisms. Digestive enzymes have been targeted as potential avenues for modulation of blood glucose concentration through inhibition of the enzymatic breakdown of complex carbohydrates to meal derived glucose absorption. Acarbose is a widely used oral anti-diabetic drug which inhibits the α-glucosidase, enzyme responsible for breaking down of disaccharides and polysaccharides into glucose. Many herbal extracts have been found to posses similar inhibitory effects. Ginger (<u>Zingiber</u> <u>officinale</u> Roscoe) has developed a reputation in treatment of several diseases. In vitro enzymic inhibitory effect of ginger was investigated in this study. Enzymes α -amylase and α -glucosidase treated with either Acarbose or ginger extract were allowed to react with cooked rice and percentages of glucose content were measured. The glucosidase and amylase activities on the rice were inhibited by addition of ginger cause significant reduction in glucose percentages (36.86± 1.05 to 26.87± 2.17, P<0.05 and 49.04±0.65 to 35.35±2.22, P<0.05) which showed comparable results with Acarbose on glucosidase activity (36.86± 1.05 to, 27.8±1.32 P<0.05). Results of the study indicates ginger as a potential plant based amylase and glucosidase inhibitor in carbohydrate digestion but usage in glycaemic control in human has to be investigated further.*

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) herbaceous aromatic plant belonging to family Zingiberaceae is grown commercially in most tropical regions of the world (Pieris, 1982). The rhizome which is valued for its flavour contains two classes of constituents such as essential oil and oleoresin (Balladin *et al*., 1998). The essential oil contributes to the characteristic flavour of ginger which is a good source of antioxidants (Balachandran, *et al*., 2006). The medicinal history of ginger is extensive. It has developed a reputation in the treatment of many gastrointestinal disorders and is often promoted as an effective herbal anti-emetic. It is been believed to possess anti-inflammatory, cholesterol lowering and anti-thrombotic properties. Many medicinal plants of herbal extracts have been found to inhibit

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the enzymatic activity. Polyphenols were capable of binding and precipitating protein, suggesting a potential ability to denature digestive enzymes. In addition, the inhibitory effects of tea polyphenols on α-amylase, pepsin, trypsin and lipase were studied. In the presence of 0.05 mg/mL tea polyphenols, the inhibition ratios of α-amylase, pepsin, trypsin and lipase were, respectively, 61%, 32%, 38% and 54%, suggesting that tea polyphenols might possess antinutritional properties. Several phenolic compounds are known to interact with proteins and inhibit enzymatic reactions (He *et al.,* 2006). Ginger is a medicinal plant rich in phenolic compounds. The major constituents of ginger are the pungent vanilloids, gingerol, paradol, shogaols and zingerone. Results of several studies showed that the gingerol-treated cells, insulin-sensitive glucose uptake were increased. Ginger enhance the insulin-sensitivity, and improve control of diabetes (Sekiya *et al.,* 2004). In a study with diabetic rats treatment with ginger produced significant increase in insulin sensitivity and a decrease in fasting glucose levels (Kar *et al.,* 2003).

The Glycaemic Index (GI) is a ranking system for carbohydrates based on the measurement of blood glucose after the ingestion of a carbohydrate (Powell *et al*., 2001). According to this system, individual foods are assigned values according to how fast they are digested and absorbed during the postprandial period. Dietary carbohydrates with different chemical compositions (*e.g*. sugars, oligosaccharides, starches, and non-starch polysaccharides) and physical structures are digested and absorbed at different rates in the human small intestine and therefore give rise to different blood glucose responses. The key enzymes involve in enzymatic break down of complex carbohydrates are pancreatic α - amylase and intestinal α - glucosidase. These two key enzymes are involved in hydrolysis of starch, thus producing glucose immediately (McCue *et al*., 2000). Carbohydrate foods that break down quickly during digestion have the highest GI values. Their blood sugar response is fast and high and they are associated with higher insulin levels. Carbohydrates which break down slowly, release glucose gradually into blood stream and low GI values.

Several observational studies have shown that the chronic consumption of a diet with a high GI (HGI) is independently associated with an increased risk of developing type 2 diabetes, cardiovascular disease, and certain cancers. Feeding HGI diets for longer periods showed increasing risk of developing insulin resistance and cardio vascular disease in rats (Chandrasekara *et al.,* 2005). In diabetic patients, studies suggest that replacing HGI carbohydrates with low glycaemic index (LGI) forms will improve glycaemic control (Willett *et al*., 2002). Hyperglycemia is widely recognized as one of the earliest disease makers in the prediction of subsequent micro-vascular and macro-vascular complications that can progress to full symptomatic type 2 diabetes (Ratner, 2001). Diabetic mellitus ranks the seventh among the leading causes of death. This disease leads to variety of other major diseases including cardio vascular disease (Whitney and Rolfes, 1999).

Type 2 diabetes constitutes more than 95% of diabetic patients in Sri Lanka. Its prevalence is constantly increasing and has already reached epidemic proportions, particularly in urban Sri Lanka. The complications of diabetes can be prevented or postponed by achieving persistent and tight metabolic control. The need for achieving better control has been evident. However, it is not easy to achieve this goal of persistent and tight metabolic control in a large proportion of patients. This is partly due to limitations of currently available modalities of treatment, and partly due to patient's noncompliance with prescribed antidiabetic medications as well as with diet and exercise prescriptions.

Traditional Indian and Chinese medicinal plant extracts have long been used as anti diabetic agents (Chen *et al*., 2001). Herbal extracts of many medicinal plants have been found to inhibit the enzymatic activity of α -glucosidase and α -amylase (Kim *et al*., 2000). Acarbose is a widely used anti-diabetes medication which lowers the digestion of carbohydrates, and lengthens the time it takes for carbohydrates to convert to glucose, thereby facilitating better blood glucose control. Acarbose, originally developed in Germany, is being widely used for post-prandial glucose regulation. It is usually used in type 2 diabetes either as a monotherapy or in conjunction with insulin secretagogue or insulin sensitizer medications. It belongs to a class of drugs called α -glucosidase inhibitors. Acarbose was approved by the Food and Drug Administration of USA for use in 1995. Inhibition of α - glucosidases by acarbose retards the absorption of ingested carbohydrates and attenuates postprandial hyperglycemia (Rosenbaum *et al.,* 2002).

In a multinational study including treated and placebo group suggests that treating impaired glucose tolerance patients with acarbose is associated with a significant reduction in the risk of cardiovascular disease and hypertension. Decreasing postprandial hyperglycemia with acarbose was associated with a 49% relative risk reduction in the development of cardiovascular events (Chiasson *et al.,* 2003).

In this study, we investigated the potential of aqueous extracts of ginger for anti amylase and anti glucosidase activities and the significance of plant based amylase inhibitors for modulation of carbohydrate breakdown in the context of preventing hyperglycemia and diabetes mellitus.

MATERIALS AND METHODS

Materials

Ginger (variety *Sidda*), rice (variety *Suduru*), D – glucose, pancreatic α -amylase and α -glucosidase, phenol (5%), H_2SO_4 (72% and 95.5%), spectrophotometer, glassware, laboratory blender, centrifuge, refrigerator and filter papers (Whatman No. 1).

Preparation of ginger extract

Thoroughly cleaned, peeled 10 g of ginger sample was homogenized in 100 mL of distilled water for one minute using a laboratory blender set on "high". The homogenate was centrifuged at 4000 rpm for 20 min. The supernatant was vacuum-filtered through Whatman No. 1 filter paper and used as the crude extract for each rice sample.

Preparation of enzymes (McCue *et al.,* 2005*)*

1. Preparation of α -glucosidase
    Fifty milligrams of powdered α - glucosidase was added to 27 mL of distilled water and pH was adjusted to 6.9. It was diluted to 30 mL total volume.
2. Preparation of pancreatic amylase
    Fifty milligrams of powdered pancreatic amylase was added to 27 mL of distilled water and pH was adjusted to 8. It was diluted to 30 mL total volume.

Preparation of rice samples

Five grams of raw rice was taken to each boiling tube and 25 mL of distilled water was added and boiled for 30 min in a water bath.

Determination of total glucose content in raw rice, cooked rice and raw ginger

1.   Preparation of standards

Sugar solutions (D - glucose) concentrations 1.5, 3, 4.5, 6.0, 7.5 µg/mL were prepared. Two milliliters of each sugar solution was added to colorimetric tubes and 1 mL of 5% phenol and 5 mL of concentrated H_2SO_4 were also added. The tubes were placed in a water bath for 15 min and the absorbance readings were measured at 490 nm and the standard curve was constructed.

2.   Determination of percentage glucose

Thoroughly cleaned, peeled 5 g of ginger was well crushed using motor and pestle and a dilution series was prepared. The total glucose content was determined using standard spectrophotometer method using glucose standards prepared as above. Five grams of rice was well crushed using motor and pestle and a dilution series was prepared. The total glucose content and available carbohydrate was determined using standard spectrophotometer method. Five grams of rice was added to boiling tubes and 25 mL of distilled water was added to each tube. Samples were cooked using a water bath for 30 min. Samples were well crushed using a motor and pestle and a dilution series was prepared and glucose content determined.

Treatment of enzymes with ginger extract

Two milliliters of ginger extract was added to prepared two enzyme solutions separately and incubated for 12 hrs at 4°C with stirring. For the control 2 mL of distilled water was added in place of ginger extract.

**Determination of anti glucosidase and anti amylase activity of ginger**

Previously prepared enzymes treated with the ginger extract were added to cooked rice samples separately. It was digested for 1 hr in a water bath at 50 - 60°C and for the control instead of 2 mL of ginger 2 mL of distilled water was added and digested. Total glucose content of each sample was analyzed in duplicate and anti glucosidase and anti amylase activity potential of ginger was determined.

Statistical analysis

All values are expressed as the mean±SE. Student's "t''" test was performed, and significance was assumed P<0.05.

RESULTS AND DISCUSSION

In this study, the potential inhibition of ginger on amylase and glucosidase enzymic activity for modulation of blood glucose concentration through reduction of carbohydrate digestion investigated. Table 1 shows the potential anti-glucosidase activity of ginger.
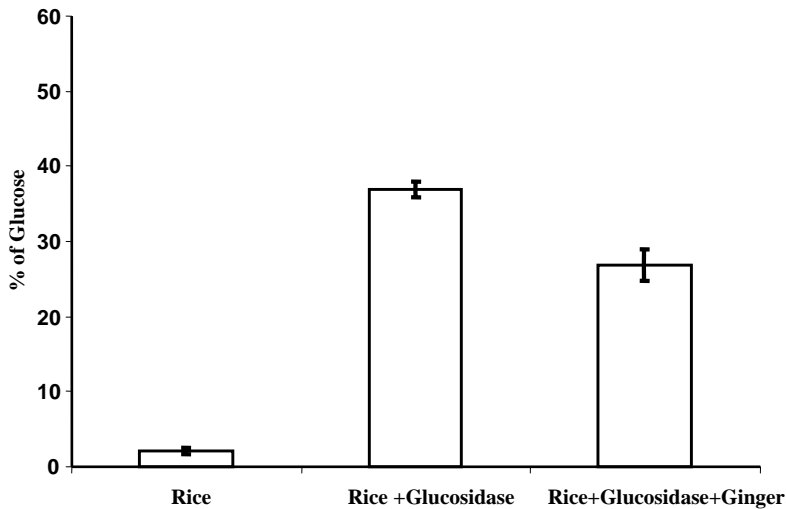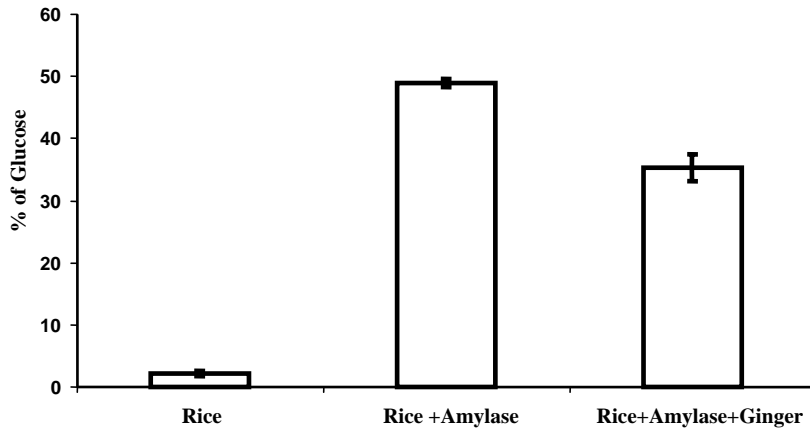

Figure 1.   Effect of ginger on anti-glucosidase activity on *in vitro* digestion of rice.

Effect of ginger on glucosidase enzyme activity

Figure 1 shows glucose percentages of cooked rice which has very low amount (2.14%) without any treatment. One hour of digestion at 50-60°C in a water bath with α-glucosidase the glucose percentage increases to the level of 36.86% (P<0.05). But the addition of ginger extract during the digestion caused about 10% reduction in percentage of glucose (26.87%, P<0.05). These results show a strong anti glucosidase activity of ginger.

Effect of ginger on amylase enzyme activity

All values are expressed as the mean±SE. Student's "*t″* test was performed, and significance was assumed P<0.05.

**Figure 2.** **Effect of ginger on anti amylase activity on *in vitro* digestion of rice.**

After an hour of digestion with α- amylase, percentage glucose in the rice sample increased significantly. When the rice samples treated with ginger, it showed an inhibition of digestion of the enzyme resulting about 15% reduction in glucose (amylase treated vs. amylase and ginger extract treated; P< 0.05). A strong anti amylase activity was can be observed in this study.

Effect of Acarbose on glucosidase enzyme activity

Figure 3 shows the effect of acarbose drug on percentage glucose reduction on *in vitro* digested rice samples.
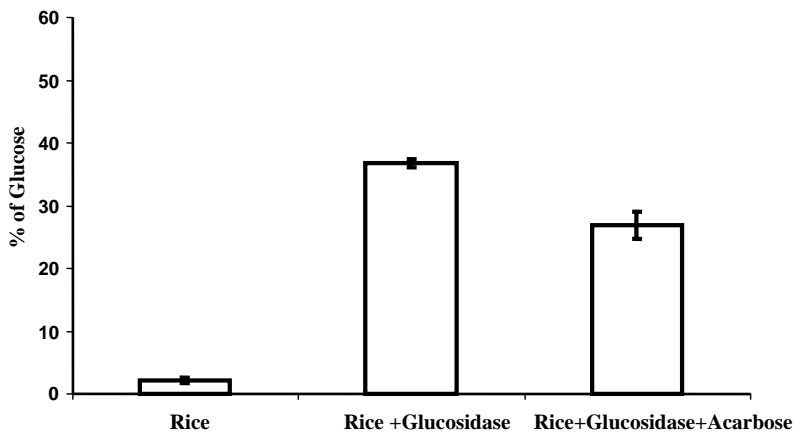
Figure 3. **Effect of Acarbose on glucosidase activity on *in vitro* digested rice samples.**

There was a significant reduction of percentage glucose on *in vitro* digested rice samples with the α- glucosidase enzyme and the Acarbose drug (P< 0.05). Acarbose drug

showed a significant anti glucosidase activity. There was no significant difference between the percentage glucose reduction with the acrbose treated and the ginger extract treated *invitro* glucosidase digested rice samples (27.8±1.32 vs. 26.87± 2.17, P> 0.05).

Majority of available synthetic anti diabetic drugs target the dual metabolic effects that characterized impaired insulin secretion and insulin resistance and some of these drugs can have negative effects at high doses (Ohmura *et al*., 1998). According to the results of the present study ginger has a potential to be a natural and safe herbal based glucosidase and amylase inhibitor to modulate the carbohydrate digestion and control blood glucose elevation. Investigating further of anti diabetic activity and the Glycaemic Index lowering ability of ginger in a mixed food are avenues for further research in this area.

CONCLUSIONS

Ginger has strong anti amylase and anti glucosidase activity. This *in vitro* study showed potential in use of ginger as a natural plant based amylase and glucosidase inhibitor for glycaemic modulation. The therapeutic usages of ginger in human subjects on controlling blood glucose response have to be investigated further.

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