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INVESTIGATION OF THE MECHANISM OF ACTION OF GARCINIA KOLA (BITTER KOLA) AS AN ANTIDIABETIC

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ABSTRACT

Evaluation of the mechanism of action of Garcinia kola (Bitter kola) as an ant diabetic. Diabetes mellitus is the seventh leading cause of death in the United States and it is now a worldwide epidemic. Currently available drugs are expensive and have side effects that can compromise compliance in patients. There is an urgent need to introduce drugs that are effective and that have less troublesome side effects. The objective of this study was to examine the antidiabetic activity and mechanism of action of Garcinia kola seed used traditionally to treat diabetes. Garcinia kola was evaluated for its ability to inhibit alpha-glucosidase as a possible mechanism of action. In vitro method was used and alpha-glucosidase from two sources, Bacillus stearothermophilus, and Saccharomyces cerevisiae were used. The alpha-glucosidase was exposed to different concentrations of aqueous extracts of Garcinia kola (bitter kola). Our results indicate that Garcinia kola inhibited alpha-glucosidase from the two sources, an indication that it acts like acarbose (that is already marketed for the treatment of diabetes) and may be useful in treating hyperglycemia.

KEYWORDS: *Diabetes, Alpha-Glucosidase, Medicinal Plants, Mechanism of Action*

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INTRODUCTION

Diabetes mellitus is a life-long disease characterized by high levels of sugar in the blood. It is defined as an elevated blood glucose associated with absent or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action. The disease states underlying the diagnosis of diabetes mellitus are now classified into four categories: Type 1, Type 2, other, and gestational diabetes mellitus [1]. It is noted that about 5% to 10% of all diabetic patients have Type 1 Diabetes; the vast majority of the diabetic patients have Type 2 [1, 3]. Type 1diabetes which accounts for 5%–10% of diabetes and is due to the autoimmune-mediated destruction of the β cells of the islet, leading to total or near-total insulin deficiency [3]. Type 2 diabetes is associated with impaired insulin secretion and action. A common factor in Type 2 diabetes is obesity or overweight and occurs in about 80% of affected individuals. Type 2 diabetes results when there is insufficient insulin action to maintain plasma glucose levels in the normal range and it has a strong genetic factor. The "other" designation refers to multiple *other* specific causes of elevated blood glucose for example pancreatectomy, pancreatitis, non-pancreatic diseases, drug therapy, etc. Gestational diabetes (GDM) is defined as any abnormality in glucose levels noted for the first time during pregnancy [1]. The long-term complications can be

serious, costly, and deadly. They include heart disease, stroke, kidney damage (chronic kidney disease and kidney failure), blindness, and amputations of the legs and feet. Diabetes-related complications are more likely and more severe among people whose diabetes is not well managed and those who have had diabetes longer [4,5]. Diabetes-related complications are more severe among people whose diabetes is not well managed and those who have had diabetes longer [3]. According to the World Health Organization (WHO), it is estimated that about 300 million people have diabetes [5]. Worldwide, there has been a dramatic increase in the number of diabetic cases because of changes in lifestyle and diet. In the United States of America, it is the 7th leading cause of death and the major cause of End Stage Renal Disease [6]. Effective control of hyperglycemia in diabetic patients is critical for reducing the risk of micro-and macrovascular disease. Currently, insulin, oral hypoglycemic and other parenteral drugs e.g. amylin and incretin mimetic agents are the major drugs used for treating diabetes [7]. Despite the progress made with synthetic drugs available, the search continues for newer drugs because the existing ones have limitations. The search for new drugs is pointing towards phytochemicals with the belief that drugs from plant origin may cause fewer side effects.

Acarbose, miglitol, and viglibose have been introduced as alpha-amylase and alpha-glucosidase inhibitors. Some of these agents are not only expensive but have side effects. Acarbose and miglitol delay the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes in the digestive tract. However, they cause some side effects like flatulence and abdominal bloating. They need to be replaced by new alternatives that produce fewer side effects. Therefore, the objective of this study was to investigate the ability of *Garcinia kola* to inhibit α- glucosidase from different sources, *Bacillus stearothermophilus,* and *Saccharomyces cerevisiae*.

Garcinia kola is also known as bitter kola largely grows in sub-Saharan Africa (West and Central Africa) and has been referred to as "wonder plant" because almost every part of the plant has used medicinally [8]. Antidiabetic and aldose reductase activities of biflavanones of *Garcinia kola* have been demonstrated [9]. Extract of *Garcinia kola* is used in treating laryngitis, cough, ulcer, it is also anti-inflammatory, and antidiabetic [10,11,12]. It is different from *Garciniacambogia* believed to cause weight loss. Other workers found that the scavenging of hydrogen peroxide (H_2O_2) by *Garcinia kola* was similar to that with vitamin E particularly at high doses [13,14,15]. Some researchers have also investigated the cardioprotective effect of kolaviron (*Garcinia kola* seed extract) in cholesterol-fed rat [16]. Our objective was to examine the mechanism of action of *Garcinia kola* in vitro to see if it has some antidiabetic potential similar to acarbose that is already in the market for the treatment of diabetes. Current evidence supports the observation that the known α-glucosidase inhibitors such as acarbose and viglibose potentially reduce the progression of diabetes as well as micro-and macrovascular complications including retinopathy, nephropathy, and neuropathy [17] The objective of this study was to demonstrate that *Garcinia kola* used traditionally to treat hyperglycemia produces its antidiabetic action by inhibiting alpha-glucosidase from *Bacillus stearothermophilus,* and *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Alpha-Glucosidase Inhibitory Assay

Analysis of alpha-glycosidase inhibition was performed according to the method of Remirez et al.[18] with some modifications and as previously reported [19]. A total of 2.7 mg of *Bacillus stearothermophilus*, (Sigma-Aldrich USA) was dissolved in 2.7 mL of 0.2 phosphate buffer. 1 mL of this solution was pipetted into a mixture containing 200 mg of bovine serum albumin (BSA) and 99 mL of 0.2M phosphate buffer. Each test well consisted of 2.5 µL of various dilutions of the extract, 122.5µl of 0.2M phosphate buffer and 62.5 µL of 5mM para-nitrophenyl-α-D-glucopyranoside (PNPG) the USA.

This was pre-incubated at 37^0C for 5 min followed by addition of 62.5 µL of the α-glucosidase solution. The mixture was incubated for 10 min. Alpha-glucosidase activity was determined on a Thermo Scientific Multiscan Spectrophotometer at an absorbance 400nm. A similar assay was run using alpha-glucosidase from another source, *Saccharomyces cerevisiae*. Acarbose was used as a positive control of α-glucosidase inhibition and was measured similarly.

Plant Extract (Sample) Preparation

Bitter Kola dry powder, 100 g was mixed with 900 mL of de-ionized water. The resulting mixture was stored for 72 hours at 4^0C with frequent shakes followed by vacuum filtration at the end of 72 hours.. The solution was lyophilized. To prepare the bitter kola extract for the assay, 200 mg of the extract was dissolved in 800 µL 0.2 M Phosphate buffer and 200 µL DMSO to form a concentration of 200 mg/mL. The extract was vortexed to ensure that the solution was fully homogenized.

$$\% \text{ Inhibition} = \frac{A410 \text{ control} - A410 \text{ test}}{A410 \text{ control}} * 100$$

The IC50 values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined by performing the assay as above with varying concentration of the plant extracts ranging 20 to 100 µg. The IC50 values were determined from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

RESULTS

The results of this study are shown in figures 1-3. *Garcinia kola*, bitter kola aqueous extracts inhibited the alpha-glucosidase in a dose-dependent fashion from *Saccharomyces cerevisiae* and *Bacillus stearothermophilus*. The IC50 values were as follows: Acarbose: 0.53 E-5±3.59 mg/ml (*Bacillus stearothermophilus*) *Garcinia kola*: 1.37±1.32 mg/ml (*Bacillus stearothermophilus*) and *Garcinia kola*: 0.26 ±45 mg/ml. The result shows that Garcinia kola inhibited alpha-glucosidase from both sources in a dose-dependent fashion just like acarbose. The inhibitory potency of acarbose was significantly greater than that by *Garcinia kola*. Acarbose was administered in a pure form, it is already marketed. *Garcinia kola,* on the other hand, was prepared and used in this experiment in its crude form.

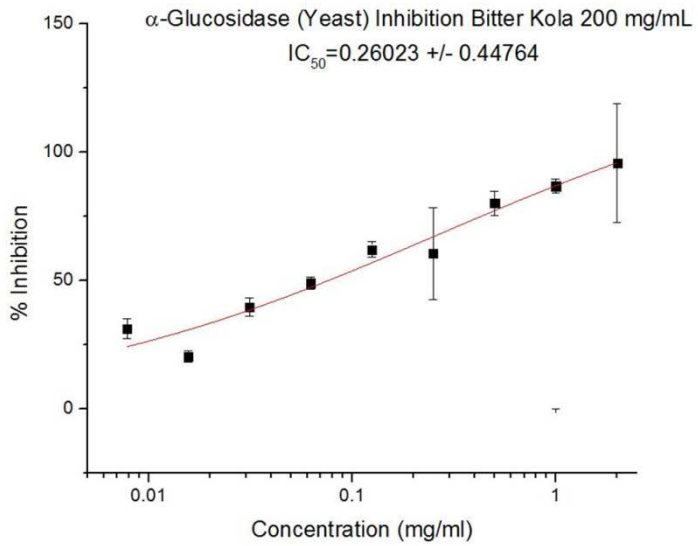

Figure 1: Dose-Response Curve of Inhibitory Activity of Aqueous extract of *Garcinia kola* on Alpha-Glucosidase from *Saccharomyces Cerevisiae* (from Yeast). Results are Expressed as Mean ±SEM

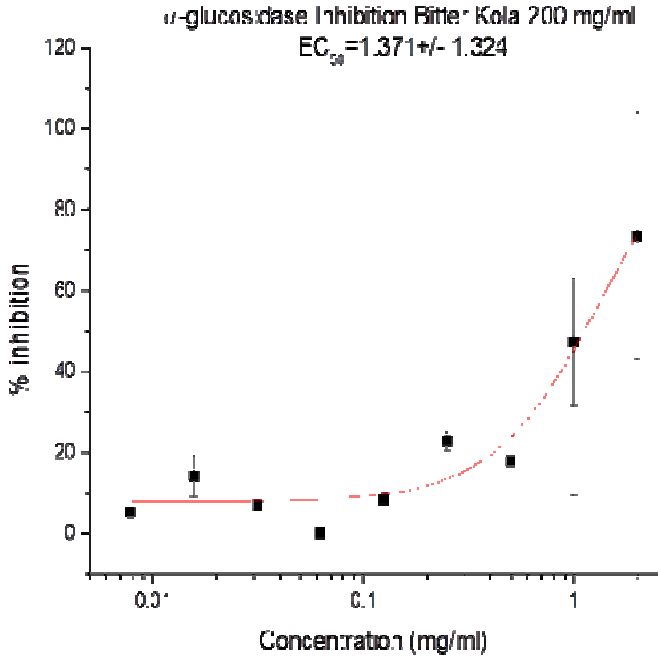

Figure 2: Dose-Response Curve of Inhibitory Activity of Aqueous Extract of *Garcinia Kola* on Alpha-Glucosidase from *Bacillus Starothermosphilus*. Results are Expressed as Mean ±SEM

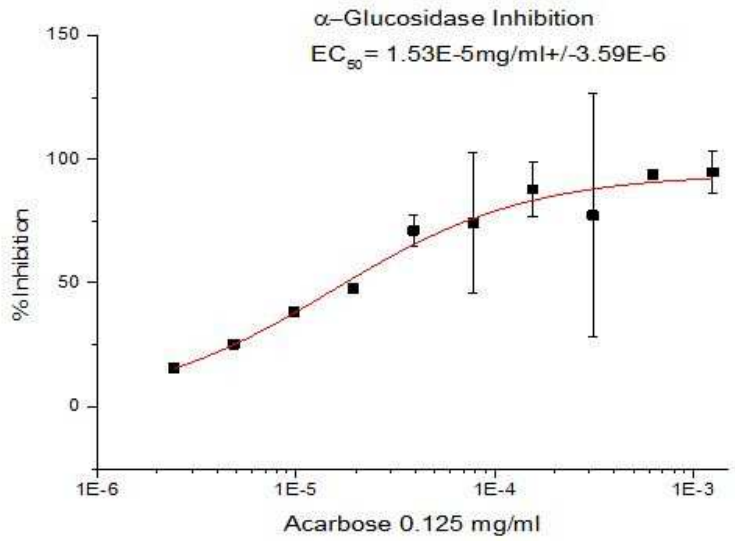

Figure 3: Dose-Response Curve of Inhibition of Alpha-Glucosidase from *Bacillus Starothermosphilus* by Acarbose. the Results are Expressed in Mean± Standard Deviation

DISCUSSIONS

The results of this study showed that the extracts of *Garcinia kola* inhibited alpha-glucosidase like acarbose, the positive control. The result indicates that *Garcinia kola* shows the same mechanism of action with acarbose and therefore shows a promise as an antidiabetic. Acarbose is an α-glucosidase inhibitor, it competitively inhibits the intestinal α-glucosidase enzymes and reduces post-meal glucose excursions by delaying the digestion and absorption of starch and disaccharides. Alpha-glucosidase inhibitors also reduce triglycerides and postprandial glucose [20]. Because of these benefits, there has been extensive research on α-glucosidase inhibitors. The inhibitory activities of α-glucosidase from natural sources have been studied. Sources from plants, foodstuff, and bacteria have been studied [21, 22, 23, and 24]. Many of the existing antidiabetics including acarbose have a lot of side effects and need replacement. Their costs and side effects adversely affect patient compliance and livelihood. In 2012 alone, diabetes-associated medical expenditures cost patients over 245 million dollars [4]. Consequently, there is a great need for alternative therapies with less adverse drug events and reduced medical costs. Recent studies point to phytotherapy as a viable alternative in the treatment of hyperglycemia. Diabetes is a serious disease and traditional methods using medicinal herbs to control diabetes is gaining ground [24, 25, 26, and 27]. *Garcinia kola* was used in this study because of supporting evidence in the literature and its widespread use in traditional medicine including diabetes.

CONCLUSIONS

In this study, *Garcinia kola* has demonstrated the ability to inhibit alpha-glucosidase from two sources, *Bacillus Starothermosphilus* and *Saccharomyces cerevisiae* (yeast). This may be an indication that it can inhibit the intestinal α-glucosidase enzymes and reduces post-meal glucose excursions by delaying the digestion and absorption of starch and disaccharides. Further work will include fractionation and purification of *Garcinia kola* and testing its antidiabetic activities *in vitro* and in animals.

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