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Inhibitory effect of aqueous extract of different parts of *Gossypium herbaceum* on key enzymes linked with type 2 diabetes and oxidative stress in rat pancreas *in vitro*

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ABSTRACT

This study sought to determine the inhibitory effect of aqueous extract of different parts (bark, leaf, and flower) of cotton plant (*Gossypium herbaceum*) on key enzymes linked with type 2 diabetes and oxidative stress in rat pancreas *in vitro*. The aqueous extract (1:10 w/v) of *Gossypium herbaceum* was prepared and the ability of the extract to inhibit the activity of α-amylase and α-glucosidase as well as activities of pro-oxidant Fe2+-induced lipid peroxidation was determined spectrophotometrically. The results revealed that the three varieties were able to inhibit the activity of α-amylase and α-glucosidase in rat's pancreas in a dose dependent manner (0–88.8 mg/ml). Also, the incubation of pancreas tissue homogenate in the presence of Fe^{2+} caused a significant increase (233.3%) in the malondialdehyde (MDA) content of pancreas homogenate, nevertheless, the introduction of the aqueous extract inhibited MDA production dose dependently (0–33.33 mg/ml) and also exhibited further antioxidant properties as represented by their high radical scavenging and Fe^{2+} chelating abilities. Inhibition of α-amylase and α-glucosidase activities has been the primary treatment for the management/prevention of type 2 diabetes. Therefore, the α-amylase and α-glucosidase inhibitory activities of aqueous extracts of different parts of *Gossypium herbaceum* in rat pancreas and prevention of lipid peroxidation in the tissue may be attributed to the presence of polyphenol content of the plant.

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1. Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism (Kwon et al., 2007). Diabetes mellitus often referred to as diabetes is a condition in which the body either does not produce enough, or does not properly respond to insulin, a hormone produced in the pancreas. However, of the several types of diabetes mellitus, non-insulin dependent diabetes mellitus (NIDDM) is the most common form of diabetes, accounting for 90% of all cases. The inhibition of enzymes involved in the breakdown of starch (α-amylase) and uptake of glucose (α-glucosidase) have been suggested to be a useful approach to the management and prevention of Type-2 diabetes (Kwon et al., 2007; Oboh et al., 2013). Diabetes is the most common endocrine disorder, and by the year 2010, it was estimated that more than 200 million people worldwide will have DM and 300 million will subsequently have the disease by 2025 (American Diabetes Association, 2005). Diabetes is best controlled by either diet alone or exercise (non-pharmacological), or diet with herbal or oral hypoglycemic agents or insulin (pharmacological). Majority of people with type 2 diabetes are overweight and usually have other metabolic disorders of the insulin resistance syndrome. The major aims of dietary and lifestyle changes are to reduce weight, improve glycemic control and reduce the risk of coronary heart disease (CHD), which accounts for 70% to 80% of deaths among those with diabetes. α-Amylase is the major form of amylase found in humans and other mammals, it hydrolyzes α (1–4) glycosidic bond in starch (Fried et al., 1987). For most humans, starch is the major source of carbohydrates in the diet; its digestion begins in the mouth, where salivary α-amylase hydrolyzes the internal glycosidic linkages of starch, producing short polysaccharide fragments or oligosaccharides. In the stomach, salivary α-amylase is inactivated by the low pH, but a second form of α-amylase, secreted by the pancreas into the small intestine, continues the breakdown process (Perry et al., 2004) before the action of α-glucosidases which breaks down disaccharides into monosaccharide (glucose) which is readily absorbed through the epithelia cells of the small intestine into blood stream (Fried et al., 1987). An effective strategy for type-2 diabetes management has been through the inhibition of α-glucosidase and pancreatic α-amylase (Krentz and Bailey, 2005; Oboh et al., 2013). Many commercially available α-glucosidase inhibitors (acarbose and miglitol) used in the management of the disease has employed this mechanism. Intestinal α-glucosidase inhibition, which delays the absorption of glucose following starch and sucrose conversion moderates the postprandial blood glucose elevation, and thus mimics the effects of dieting on hyperglycemia (Kwon et al., 2006). Pancreatic α-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of carbohydrates, and less glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short-term effect of drug therapies is to decrease current blood glucose levels: the long-term effect is a small reduction in hemoglobin level (Samantha and Aschenbrenner, 2008).

Natural remedies of medicinal plants are considered effective and safe alternative treatment for hyperglycemia (Ripsin et al., 2009). There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience, and relatively low cost (Gupta et al., 2005). Cotton is usually used as a textile while making clothing and can be made into yarns and sheets of fabric. It has been cultivated for women's menstrual cycle pains and irregular bleeding, expulsion of placenta after birth and increase of lactation. Cotton has been used for gastrointestinal issues also, such as hemorrhages, nausea, diarrhea, as well as fevers and headaches especially in the southern United States (Grieve, 2014). Also, orally administered ethyl ether and ethanol extracts of *Gossypium herbaceum* has been seen to significantly decreased the blood glucose level (Velmurugan and Bhargava, 2014), but there is dearth of information on the effect of the aqueous extract which is commonly used in Nigeria in the prevention/management of type 2 diabetes in folklore medicine. It is therefore expedient to assess the inhibitory effect of aqueous extract of different parts of cotton plant (flower, bark, and leaf) on the key enzymes (α-amylase and α-glucosidase) linked to type 2 diabetes in other to provide some possible mechanism of action by which they exert their anti-diabetic properties.

2. Materials and methods

2.1. *Materials*

2.1.1. *Sample collection*

The cotton flower, back and leaf was collected from Iworoko farm in Ado-Ekiti metropolis, and the authentication was carried out in the Department of Plant Science, Ekiti state University, Ado-Ekiti. Ekiti State, Nigeria.

2.1.2. *Aqueous extract preparation*

The cotton flower, bark, and leaf were washed with distilled water to remove contaminants, air dried and blended. 10 g of the sample was weighed into 100 ml of distilled water, centrifuged and filtered to obtain a clear supernatant which was then stored in the refrigerator for subsequent *in vitro* analysis (Oboh et al., 2007).

2.1.3. *Chemicals and reagents*

Chemicals and reagents used, such as thiobarbituric acid (TBA), 1,10-phenanthroline, deoxyribose, garlic acid, Folin–Ciocalteu's reagent were procured from Sigma-Aldrich, Inc., (St Louis, MO). Trichloroacetic acid (TCA) was sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany), dinitrophenyl hydrazine (DNPH) from ACROS Organics (New Jersey, USA), hydrogen peroxide, methanol, acetic acid, thiourea, $CuSO_4.5H_2O$, H_2SO_4, sodium carbonate, $AlCl_3$, potassium acetate, Tris-HCl buffer, sodium dodecyl sulphate, $FeSO_4$, potassium ferricyanide and ferric chloride were sourced from BDH Chemicals Ltd., (Poole, England), while the water was glass distilled.

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