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Sapium ellipticum (Hochst) Pax ethanol leaf extract modulates glucokinase and glucose-6-phosphatase activities in streptozotocin induced diabetic rats

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ABSTRACT

Objective: To examine the effects of *Sapium ellipticum* (SE) leaf extract on the hepatic activities of glucokinase and glucose-6-phosphatase in streptozotocin-induced diabetic Wistar rats.

Methods: STZ-induced diabetic Wistar rats (four groups, $n = 8$) were used in this study. SE was assessed at two different doses, 400 and 800 mg/kg BW, in comparison with metformin (METF) (12 mg/kg BW) as a reference antidiabetic drug. All treatments were done orally (p.o), twice daily at 8 h interval for a period of 21 days. Glucokinase and glucose-6-phosphatase activities were respectively determined using standard protocols. Hepatic and muscle glycogen contents were estimated as well.

Results: STZ caused significant decrease in glucose-6-phosphatase activity and concomitant increase in glucokinase activity. SE extract especially at 400 mg dosage significantly reversed the alterations by increasing glucokinase activity by 40.31% and inhibiting glucose-6-phosphatase activity by 37.29% compared to diabetic control animals. However, the effects were significantly lower than that of METF which enhanced glucokinase activity by 94.76% and simultaneously inhibited glucose-6-phosphatase activity by 49.15%. The extract also improved hepatic glycogen level by 32.37 and 27.06% at 400 and 800 mg dosage respectively. HPLC-MS analysis of some SE fractions in dynamic MRM mode (using the optimized compound-specific parameters) revealed among other active compounds, the presence of amentoflavone, which has been associated with antidiabetic function.

Conclusions: The ability of SE extract to concurrently inhibit glucose-6-phosphatase and activate glucokinase in this study suggests that it may be a treatment option for type 2 diabetes patients, and the presence of amentoflavone in the plant extract may account for its anti-diabetic potential.

1. Introduction

Fasting hyperglycaemia in all forms of diabetes mellitus occurs primarily as a result of upsurge in hepatic glucose synthesis (HGS) [1,2]. Two specific enzymes, glucokinase (GK) and glucose-6 phosphatase (Glu-6-Pase) play crucial role in hepatic glucose production, utilization and homeostasis [3–7]. These enzymes catalyze very important enzymatic steps in the regulatory pathways of glucose in the liver. Glu-6-Pase which enables the liver to produce glucose catalyzes the final step of glycogenolysis and gluconeogenesis in which glucose-6-Phosphate is hydrolysed to yield glucose and phosphate [7]. The enzyme is like a double edged sword with the unusual capability to balance the concentrations of free glucose and stored glucose as glycogen. When body cells are energy starved due to unavailability of glucose, the activity of glucose-6-Phosphatase becomes a necessary counter-regulatory response which is often triggered by glucagon and other insulin antagonistic hormones. Hence, Glu-6-Pase activity is markedly increased in insulin-deficient diabetic rats or during short period of fasting [2].

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On the other hand, GK activity, which allows the liver to utilize glucose, is decreased during fasting. It catalyzes the rate limiting step of glycolysis by phosphorylating glucose to glucose -6-phosphate. GK also functions as a glucose sensor in ensuring appropriate secretion and release of insulin vis-à-vis plasma glucose concentration. The enzyme is activated by high plasma glucose concentration (>7.5 mM) and becomes deactivated when the glucose level drops to normal (<5.5 mM) [8]. These observations indicate that both GK and Glu-6-Pase are important regulators of HGS in diabetic conditions. Compounds that modulate the activity of these enzymes have been reported to enhance their regulatory function in drug-induced diabetes in animal model [4,9], and consequently contribute to the management of diabetes mellitus.

Sapium ellepticum (*S. ellipticum*) (Hochst) pax enjoy huge therapeutic application in the local treatment of a number of disease conditions [10,11], including diabetes (ethno-botanical survey). It belongs to the family Euphorbiaceae and is commonly referred to as jumping seed tree. *S. ellipticum* is widely distributed in eastern and tropical Africa. In southwest part of Nigeria, particularly among the Ilorin indigenes, the plant is popularly known as *aloko-ẹgbọ*.

A few scientific investigations have been carried out on it. Adesegun *et al.*, [12] in their *in vitro* study credited substantial antioxidant properties to the stem bark extract of the plant. Cytotoxicity screening of selected Nigerian plants used in traditional cancer treatment on HT29 (colon cancer) and MCF-7 (breast cancer) cell lines (HeLa cervix adenocarcinoma cells) indicated that *S. ellipticum* leaf extract expressed the highest cytotoxic activity among other plants with anticancer potential which was comparable to the reference drug, ciplastin [13]. The phythochemical constituents, *in vitro* antioxidant capacities and antiplasmodial activities of *S. ellipticum* stem bark extracts were documented by Nana *et al.*, [10]. Edimealem and colleagues [14] in their study demonstrated the presence of Lupeol, lupeol acetate and stigmasterol in the stem bark extract of *S. ellipticum*. This present study sought to investigate the effects of the plant leaf extract on glucose metabolizing enzymes such as glucokinase and glucose-6-phosphatse.

2. Materials and methods

2.1. Collection of Sapium ellipticum leaves

Fresh *S. ellipticum* (SE) leaves were harvested in the month of December, 2012 from a forest in a suburb of Ibadan, south-west of Nigeria. The harvested leaves were taxonomically authenticated by a botanist (Mr. T.K. Odewo) at the Lagos University Herbarium (LUH), Nigeria, where a specimen was deposited and assigned a voucher number, LUH 5423.

2.2. Preparation of Sapium ellipticum leaf extracts

The plant material was freed of extraneous materials; air dried at room temperature and milled to a fine powder, using a Waring blender. 300 g of the powdered sample was macerated in 2.5 L of the extracting solvent (ethanol). The mixture was allowed to stand for 72 h and stirred intermittently with a glass rod to facilitate extraction. Sieving of the mixture was achieved with a muslin cloth (maximum pore size 2 mm). The resulting filtrate on sieving was further filtered through Whatman filter paper (No 42) and subsequently reduced in volume with a rotary evaporator at 40 °C. Final elimination of solvent and drying was done using a regulated water bath at 40 °C.

2.3. Induction of diabetes mellitus with streptozotocin in experimental rats

Single intraperitoneal (i.p) dose (55 mg/kg BW) of freshly prepared streptozotocin (STZ) was administered to a batch of normoglycaemic rats starved for 16 h.

2.4. Experimental design and management of animals

Eight normoglycemic animals constituted a control group (group 1). Thirty-two STZ-treated Wistar rats were randomly assigned to four groups (groups 2, 3, 4 and 5) containing eight animals each. Group 1 animals were administered olive oil (0.5 ml) and served as normal control. Group 2 animals were left untreated and served as diabetic control. Groups 3 and 4 were respectively treated with 400 and 800 mg/kg of *S. ellepticum* and the last group was treated with metformin (12 mg/kg BW), a reference anti-diabetic drug. All treatments were done orally (p.o), twice daily at 8 h interval for a period of 21 days. All procedures for maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health [15]. This was approved by the Ethical Committee of the College of Bioscience, Federal University of Agriculture, Abeokuta.

2.5. Preparation of tissue homogenate and post-mitochondrial fraction (PMF)

At the end of 21 days of treatments, the rats were fasted over night and sacrificed by cervical dislocation. The liver of each rat was harvested, rinsed with ice-cold 1.15% KCl solution, blotted and immediately suspended in an appropriate homogenizing buffer depending on the assay.

2.5.1. Estimation of hepatic glucokinase activity in rats

Glucokinase activity was measured as described by Zhang *et al.*, [16] with slight modification. Briefly, 100 mg of liver tissue was homogenized in 1 mL of ice-cold homogenization buffer containing 100 mM KCl, 25 mM HEPES (N′-2-Hydroxyethylpiperazine-N′-2 ethanesulphonic acid), 7.5 mM $MgCl_2$, 4 mM dithiothreitol (pH 7.4) using Potter Elvehjem type homogenizer. The resultant homogenate was then lysed overnight at 4 °C.

2.5.2. Estimation of hepatic glucose 6-phosphatase activity in rats

Glucose 6-phosphatase (G6Pase) activity was assayed according to the method of Baginsky *et al.* [17] by estimation of the inorganic phosphate (Pi) liberated from glucose 6-phosphate (G6P).

One gram of frozen liver tissue was homogenized in ice-cold sucrose solution using Potter Elvehjem type homogenizer. The homogenate was centrifuged sequentially at 11 000 g for 30 min, then at 105 000 × g for 1 h using an ultracentrifuge (Beckman Inc., CA, USA). The solid pellet was re-suspended in ice cold sucrose/EDTA solution and used as the source of the enzyme.

2.5.3. Estimation of hepatic glycogen content in rats

Glycogen contents in hepatocytes were determined by the method of Seifter with slight modifications as reported in Methods in Enzymology Vol. 111 [18].

Hundred milligram of the tissue sample was digested in 1 mL of 30% KOH. The digested tissue was dissolved and in 1.25 mL

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