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The leaf and seed aqueous extract of *Phyllanthus amarus* improves insulin resistance diabetes in experimental animal studies

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ABSTRACT

Ethnopharmacological relevance: Amongst the Yoruba tribe (Southwest Nigeria), water decoction of the leaf and seed of *Phyllanthus amarus* Schum. and Thonn. is reputably used for the local management of diabetes mellitus, obesity and hyperlipidemia.

Aim of the study: The present study seeks to evaluate the effectiveness and elucidate mechanism(s) of action of the aqueous leaf and seed extract of *Phyllanthus amarus* (PAE) in normal and 10% sucrose-induced hyperglycemia and dyslipidemia as an experimental model of insulin resistance diabetes mellitus.

Materials and methods: In this study, the repeated oral antihyperglycemic action of 150–600 mg/kg/day of PAE was evaluated in normal and 10% sucrose-induced insulin resistance rats using indicators such as fasting blood glucose (FBG), insulin and insulin resistance indices. The extract's weight losing, antihyperlipidemic and anti-atherogenic effects were also evaluated by measuring the effect of the extract on the body weight, plasma levels of triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and atherogenic indices.

Results: In normal rats, PAE caused significant ($p<0.05$, $p<0.01$ and $p<0.001$) and dose related decreases in body weight, FBG, TG, TC, LDL-c, and atherogenic indices. Repeated oral treatment with 10% sucrose drink for 30 days was associated with significant ($p<0.001$) weight gain, hyperglycemia, insulin resistance indices, hyperlipidemia and atherogenic indices. However, pre-treatment with PAE significantly ($p<0.05$, $p<0.01$ and $p<0.001$) and dose-dependently attenuated increase in any of these measured parameters.

Conclusions: Overall, results of this study showed PAE to effectively control insulin resistance DM which was mediated via improvement in insulin resistance, thus, validating its ethnomedical use in the local management of DM.

1. Introduction

Diabetes mellitus (DM) remains the most common non-communicable and metabolic disorder in the world. In the year 2011, the International Diabetes Foundation (2011) estimated the number of adults living with DM to be about 366 million, representing 8.3% of the global adult population and this number is projected to rise to 552 million people or 9.9% of adults by 2030 (Whiting et al., 2011). DM is considered the fourth or fifth leading cause of death in most industrialized countries accounting for about 4.6 million deaths globally and there is substantial evidence that its prevalence is equally rising at an exponential rate in developing countries (Whiting et al., 2011). In fact, it is estimated that 80% of people with DM reside in low- and middle-income countries and the greatest number of these being between 40 and 59 years of age. Also, DM and its associated complications are known to be associated with a huge social and economic burden. For example, in the year 2011 alone, the total healthcare cost on DM was put at over $465 billion (Guariguata et al., 2011).

Despite the great interest shown by the scientific community and concerted efforts directed towards discovery, development and evaluation of new drugs from herbal plants and their byproducts for the disease management, few of these antidiabetic herbal remedies have been investigated so far (Liu et al., 2004; Vuksan and Sievenpiper, 2005; Matsui et al., 2006; Fröde and Medeiros, 2008). One of the herbal remedies that have not been exhaustively investigated is the water decoction made from leaves and seeds of *Phyllanthus amarus*.

Abbreviations: AI, atherogenic index; CRI, coronary artery index; FIGR, fasting insulin–glucose ratio; FIRI, fasting nsulin resistance index; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol; PAE, leaf and seed aqueous extract of *Phyllanthus amarus*; TC, total cholesterol; TG, triglyceride

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Phyllanthus amarus Schum. and Thonn. (family: Euphorbiaceae) is an annual tropical shrub that grows mostly on uncultivated land and often regarded as weeds. It is widely distributed across the Central and Southern India where it is known as "Bahupatra and Bhuiamla", respectively. It is also widely distributed across Sierra Leone, Ivory Coast, Kenya, Angola and Southwest and Southern Nigeria (Adeneye et al., 2006; Iranloye et al., 2011). Amongst the Yoruba (Southwest Nigeria), Igbo (Southeast Nigeria) and Asaba (Niger Delta) people, it is locally known as "Iyin-Olobe", "ngwu" and "buchi oro", respectively (Adeneye et al., 2006; Iranloye et al., 2011). Different extracts of the plant have been reported to be effective in the management of human diseases such as liver and renal stone diseases (Ott et al., 1997; Kiemer et al., 2003; Adeneye and Benebo, 2008), menstrual disorder, infections (Kokwaro, 1976), pain and inflammation (Kassuya et al., 2003; Iranloye et al., 2011), skin ulcers, typhoid fever (Iwu, 1993; Raphael et al., 2002), diabetes mellitus, hyperlipidemia and hypertension (Srividya and Periwal, 1995; Kaliwal et al., 2012) and anemia (Adeneye and Arogundade, 2007), cancers (Joy and Kuttan, 1998; Sripanidkulchai et al., 2002) and amnesia (Joshi and Parle, 2007).

In a previous study, the hypoglycemic and hypocholestrolemic effect of 150–600 mg/kg of the aqueous leaf and seed extract of *Phyllanthus amarus* in normal mice was reported (Adeneye et al., 2006). The current study is designed towards further evaluating the effectiveness and mechanism(s) of action of 150–600 mg/kg of the aqueous leaf and seed extract of the *Phyllanthus amarus* in type 2 DM model using 10% sucrose-induced hyperglycemic rats.

2. Materials and methods

2.1. Plant collection

Two kilograms of the fresh whole plants of *Phyllanthus amarus* was collected from an abandoned arable land within the Ojo Campus of Lagos State University, Ojo, Lagos State, Nigeria, within the month of February, 2009. Plant identification and authentication were done as previously reported (Adeneye et al., 2006). The harvested plant materials were gently but thoroughly rinsed in tap water after which they were completely air-dried under shade and at room temperature (23–26 °C) in the laboratory for 2 weeks. The plant seeds and leaves were then separated from the whole plants and then pulverized into fine powder using the Laboratory Hammer-mill at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Idi-Araba, Lagos State, Nigeria. The pulverized plant sample was then kept in air-tight and water-proof containers and kept in the refrigerator at 4 °C until needed for extraction.

2.2. Extraction process

Two hundred grams of the pulverized sample material was completely extracted in 1 L of distilled water for 3 h using Soxhlet extractor obtained from the Experimental Laboratory of the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Idi-Araba, Lagos State, Nigeria. Soxhlet extraction of the pulverized plant material yielded a deep greenish-brown filtrate which was completely air-dried at 40 °C in a digital aerated oven leaving behind a deep brown, sweet-smelling solid residue. The process was repeated for four more times to give a yield of 22.5 ± 1.0%. The residues were all pooled into a dry, clean air- and water-tight containers and stored in the refrigerator at 4 °C to prevent decomposition of the extract.

2.3. Experimental animals

A total of 70 young adult male Wistar rats were obtained from the Animal House of University of Ilorin, Ilorin, Kwara State, Nigeria, in two batches of 30 and 40 rats in the months March and May 2009, respectively, after ethical approval has been obtained from the Ethical Committee of Lagos State University College of Medicine, Ikeja, Lagos, Nigeria. The rats were acclimatized for 14 days, fed on standard rat chow and tap water *ad libitum*. The rats were housed in a standard rat cages in the Rat Colony of the Animal House, Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria and maintained at standard laboratory conditions (12/12 h light-dark periodicity, temperature: 23–26 °C) as prescribed by the United States National Institutes for Health Publication (1985). Seven days prior to commencement of the experiment, rats were randomly divided into different treatment groups such that the weight differences within and between treatment groups do not exceed ±20% of the average weight of the rat population.

2.4. Oral treatments of normal rats with PAE

In this first phase of the experiment, 25 normal Wistar rats were divided into five treatment groups of five rats in each group and treated on daily for 30 days. Group I rats have 10 ml/kg/day of distilled water given; Group II rats were given 1 mg/kg of glibenclamide dissolved in distilled water while Groups III–V rats were administered 150 mg/kg/day, 300 mg/kg/day and 600 mg/kg/day of PAE all dissolved in distilled water, respectively. All treatments were through the oral route.

2.5. Induction of sucrose-induced hyperglycemia and dyslipidemia and oral treatment with PAE

In this second phase of the experiment, Wistar rats were divided into six groups of five rats per group. Between 08:00 h and 09:00 h, the rats were orally pre-treated with 1 mg/kg of glibenclamide and 150–600 mg/kg/day of PAE before being freely fed with 10% sucrose dissolved in their drinking water for 30 days as follows:

Group I = 10 ml/kg of distilled water.
Group II = 10% sucrose drink + 10 ml/kg of distilled water.
Group III = 10% sucrose drink + 1 mg/kg of glibenclamide in distilled water.
Group IV = 10% sucrose drink + 150 mg/kg of PAE.
Group V = 10% sucrose drink + 300 mg/kg of PAE.
Group VI = 10% sucrose drink + 600 mg/kg PAE.

2.6. Measurement of body weight

Body weights of the treated rats were measured on the first, 15th and 31st day of the experiment with a mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight difference on the 15th and 31st day in reference to the initial weight per group was calculated.

2.7. Determination of FBG

Whole blood was collected by tail tipping method at between 07:00 h and 09:00 h as described by Adeneye and Adeyemi (2009) and FBG was determined by glucose oxidase method of Trinder (1969) using a One Touch Basic Blood Glucose Monitoring System® (Life Scan Inc., Milpitas, California, USA). The blood glucose monitor was calibrated and validated at the beginning of, midway into and at the end of the experiment. However, the FBG was determined on the first, 15th and 31st day of the experiment, respectively.