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The antidiabetic activity of the herbal preparation ADD-199 in mice: a comparative study with two oral hypoglycaemic drugs

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Abstract

The antidiabetic and antioxidant effects of the herbal preparation ADD-199 were investigated in STZ-induced diabetic C_3H mice and results were compared with two allopathic hypoglycaemic drugs, glibenclamide and metformin. Plasma glucose, insulin and lipids as well as liver glycogen, lipids and lipid peroxidation were measured following treatment for 8 weeks. The results indicated that plasma insulin levels in normal controls at termination were about 76 μmol/L compared to trace levels in untreated diabetic mice. Glibenclamide and ADD-199 increased insulin levels in diabetic mice up to 70% of levels in untreated non-diabetic mice whilst metformin had no effect. Basal plasma glucose levels in diabetic controls (18.8 mM) were reduced to 14.0 mM by 100 mg/kg ADD-199 in <2 weeks compared to 4 and 6 weeks for glibenclamide and metformin, respectively. This hypoglycaemic effect of ADD-199 appeared to be associated with the alkaloidal content of the extract. Treatment with ADD-199 or the hypoglycaemic agents reversed the observed elevation in plasma lipids but increased hepatic glycogen, triacylglycerol and cholesterol levels. Treatment also increased glucose uptake by isolated diaphragms and attenuated hepatic lipid peroxidation. These antihyperglycaemic and antioxidant actions of ADD-199 at a dose of 100 mg/kg/day are comparable to those of the maximum daily therapeutic doses of glibenclamide (0.25 mg/kg) and metformin (50 mg/kg). These could explain the basis for use of this plant extract to manage diabetes mellitus (DM).

Keywords: Antidiabetic; Antioxidant; ADD-199; Streptozotocin-induced diabetic mice; *Maytenus senegalensis*; *Annona senegalensis*; *Kigelia africana*, *Lannea welwitschii*

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycaemia, hypertriglyceridaemia and hypercholesterolaemia, resulting from defects in insulin secretion or action or both (Georg and Ludvik, 2000; Nyholm et al., 2000). One of the late complications of uncontrolled DM is the formation of advanced glycosylated end products (AGE) (Vlassara and Palace, 2002). Some of these end products can react with other proteins and are also capable of causing increased permeability and thickening of blood vessel walls with loss of elasticity (Dominiczak et al., 1990). Protein glycation and glucose autooxidation may generate free radicals in the diabetic patient, which in turn catalyses lipid peroxidation (Onorato et al., 2000). The antioxidant status of the diabetic is compromised and is unable to protect against oxyradical damage (Bonnefont-Rousselot et al., 2000).

Two groups of oral hypoglycaemic drugs, sulphonylureas and biguanides, have been used in the treatment of DM. They act by lowering blood glucose levels thereby delaying or preventing the onset of diabetic complications. The mechanism

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of action of biguanides (e.g., metformin) are not fully understood but may involve the enhancement of insulin receptors (Uehara et al., 2001; Zangeneh et al., 2003) to increase the absorption of sugars whilst the mechanism of action of sulphonylureas (e.g., glibenclamide) involves the augmentation of insulin secretion and consequently are effective only when residual pancreatic β-cell activity is present (British National Formulary (BNF), 1995; Guney et al., 2002).

Medicinal plants are used in several countries, including Ghana, to manage DM and are thought to be less toxic than allopathic hypoglycaemic drugs like the biguanides, sulphonylureas or insulin therapy. Plant medicines are also easily available and affordable to many, especially in developing countries like Ghana. Indeed, there are over 400 medicinal plants that are claimed to have antidiabetic properties (Bailey and Day, 1989). In fact, many plant species like *Momordica charantia* (Day et al., 1990; Ali et al., 1993), *Cucurbita ficifolia* and *Psacalium peltatum* (Roman-Ramos et al., 1992) and *Indigofera arrecta* (Addy and Nyarko, 1988; Nyarko et al., 1993) are shown to lower blood glucose. The WHO expert committee (1985) recommends scientific investigation of hypoglycaemic agents of plant origin for the treatment of DM.

ADD-199 is a herbal preparation used in Ghana, with anecdotal claims of effectiveness in the management of DM. It is made from the following plant species: *Maytenus senegalensis* (Lam.) Exell. (Celastraceae), *Annona senegalensis* Per var senegalensis, Robyns and Ghesquiere (Annonaceae), *Kigelia africana* (Lam.) Benth. (Bignoniaceae) and *Lannea welwitschii* (Hiein) Engl. (Anacardiaceae). These plants have been used in the treatment or management of various ailments. For example, the bark or root of *Maytenus senegalensis* has been used for dyspepsia and wounds; the root of *Annona senegalensis* for stomach problems; the fruit of *Kigelia africana* for wounds, anemia and stomach ailments and the bark of *Lannea welwitschii* for abdominal pain and skin ulcers (Agbovie et al., 2002). However, there is no available empirical data as to their use, either singly or in combinations, in the management of DM. We report here detailed comparative scientific study of the antidiabetic activity of this preparation and two allopathic oral hypoglycaemic drugs, glibenclamide and metformin, aimed at establishing the efficacy and possible mode of action of ADD-199.

2. Materials and methods

2.1. Chemicals and reagents

Glucose, triacylglycerol and cholesterol kits were obtained from Randox Laboratories Ltd. (Co. Antrim, UK). Enzyme-linked immunosorbent assay for insulin was from Boehringer (Mannheim, Germany). Streptozotocin (STZ), bovine serum albumin (BSA) and amyloglucosidase were purchased from Sigma Chemical Co., (MO, USA). Glibenclamide and metformin (99.8% purity) were obtained from Margokin Pharmacy (Accra, Ghana). All other chemicals used were obtained in the highest purity grade available from FLUKA, Switzerland.

2.2. Preparation of ADD-199 extract

The antidiabetic preparation ADD-199 was a kind gift from Dr. Koranteng, a local herbalist. The preparation was made from four different plant species obtained from their natural habitat: bark of *Maytenus senegalensis* (“wotsi”), root of *Annona senegalensis* (“bardugda”), fruit of *Kigelia africana* (“nufutine”) and bark of *Lannea welwitschii* (“okumnini”). Equal quantities of air-dried samples of each species were ground and mixed with 10 times the equivalent volume of water and boiled for 1.5 h. The mixture was sieved through a fine mesh and allowed to cool down. The extract was freeze-dried (giving 42.7 ± 6.2 mg extract/g crude material) and stored in a cool dry place. It was reconstituted in water before use.

2.3. Chromatographic separation and qualitative analysis

A certain quantity (2 g) of freeze-dried aqueous extract of ADD-199 was refluxed with ethyl acetate (EtoAc) at 125 °C for 1 h. The EtoAc extracts was filtered and then concentrated to 2 mL. Similar extractions were performed separately with petroleum ether (PE) or chloroform ($CHCl_3$). Thin layer chromatography on silica gel was performed with the four extracts using acetone: petroleum ether (1:4) and iso-octane:diethyl ether:acetone (3:1:1) as solvent systems. The spots were visualized under UV-light (366 nm). The fluorescence of each spot was noted and the R_f value determined. The spots were scraped into the solvent used in its extraction, vortex-mixed and centrifuged to sediment the silica gel. Qualitative tests were performed to identify the classes of phytochemicals present in the spots (Harborne, 1984; Volhard, 1956).

2.4. Animal treatment and blood sampling

C_3H/He inbred male mice, 7 weeks old, were obtained from the Research Animal Unit, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana (Legon, Ghana) for the study. The animals were fed ad libitum on pelleted animal feed obtained from Ghana Agro Food Co. Ltd. (GAFCO), Tema, Ghana. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication no. 85–23, revised in 1985).

Animals were divided into six groups of five animals per group and blood samples collected for determination of baseline glucose levels. Diabetes mellitus was induced in five groups by injecting each mouse intraperitoneally, 60 mg/kg/day of streptozotocin freshly prepared in (0.01 M) citrate buffer pH 4.5, daily for three consecutive days. Ani-