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Subchronic toxicity studies of the antidiabetic herbal preparation ADD-199 in the rat: absence of organ toxicity and modulation of cytochrome P450

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Abstract

The subchronic toxicity of the aqueous antidiabetic herbal extract ADD-199, prepared from *Maytenus senegalensis*, *Annona senegalensis*, *Kigelia africana* and *Lannea welwitschii*, and administered at a daily dose of 100 or 500 mg/kg body weight over 30 days, was investigated in male Wistar albino rats. Certain haematological, urine and plasma biochemical parameters, and modulation of some hepatic cytochrome P450 (CYP) isozymes were measured as indices of organ specific toxicity or potential for drug interactions. ADD-199 did not affect plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and albumin or creatinine kinase (CK) levels. It also did not affect plasma creatinine and urea levels. Furthermore, ADD-199 neither affected PCV nor blood Hb, RBC, reticulocytes, platelets, lymphocytes and granulocyte levels. It, however, caused significant dose-dependent reductions in WBC counts at day 15 with varying degrees of recovery by day 30. It also reduced the rate of body weight increases after week 3. However, no changes were observed in organ weights at termination. ADD-199 did not significantly affect zoxazolamine-induced paralysis and pentobarbital-induced sleeping times as well as certain CYP isozyme activities in rats. These findings suggest that ADD-199 had no overt organ specific toxicity and did not demonstrate a potential for drug interactions via CYP-mediated metabolism in the rat on subchronic administration.

Keywords: ADD-199; Antidiabetic; Organ; Rat; Cytochrome P450; Toxicity

1. Introduction

Diabetes mellitus (DM), an endocrine disease associated with absolute or relative deficiency of insulin resulting in hyperglycaemia and glucosuria, is prevalent in 20% of the Ghanaian population (Gyesie, 1992). The disease is managed with insulin and oral hypoglycaemic agents like sulphonylureas and biguanides. The biguanide phenformin was removed from the US market in 1977 following reports of relatively high incidence of lactoacidosis (Karam, 1995) and later replaced by metformin. These allopathic drugs are too expensive and thus beyond the reach of DM patients in developing countries. Some patients have, therefore, resorted to the use of herbal preparations made from *Indigofera arrecta* and *Bridelia ferruginea*, which have been shown to be effective in lowering blood glucose levels in man and rats (Iwu, 1980; Addy and Nyarko, 1988) without signs of overt toxicity in laboratory studies (Nyarko et al., 1999).

ADD-199 is an herbal medicine that is prepared from *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), is used by some Ghanaian diabetic patients to manage the disease. A daily dose of 100 mg/kg effectively lowers streptozotocin (STZ)-induced hyperglycaemia

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in mice (Okine et al., 2004). Phytochemical analysis using TLC showed aqueous extract of ADD-199 to contain among others, alkaloids, terpenoids, tannins and flavonoids, with the hypoglycaemic activity being mainly associated with the alkaloidal content (Okine et al., 2004). Some plant extracts containing tannins also contain alkaloids that have toxic side effects in man (Atta-Ur-Rahman and Khurshid, 1989; Lacomblez et al., 1989). Adverse reactions of pure flavonoids and flavonoid-containing herbs include haemolytic anaemias, thrombocytopaenia, hepatitis and acute renal failure (Gandolfo et al., 1992; Lim and Ho, 1994).

Poorly managed diabetes mellitus often leads to complications, which require use of the medications. In view of the chemical constituents identified in ADD-199 and the potential for chronic use of the extract to manage diabetes mellitus, there is need for its further investigation to determine its safety and potential for interaction with other drugs. Chronic use of some medications, including herbal medicines known to contain flavonol compounds may modulate cytochrome P450 (CYP) isozymes activities (Pratt and Taylor, 1990; Gyamfi and Aniya, 1998; Budzinski et al., 2000; Beckmann-Knopp et al., 2000). These may precipitate interactions via metabolism and hence alter the duration of pharmacological effects of components in a multiple drug therapy (Pratt and Taylor, 1990). Therefore, in the present study, we determined the effects of ADD-199 on certain haematological, urinary and plasma biochemical parameters to assess its safety in male Wistar albino rats. We also assessed the potential of the extract for drug interaction via drug metabolism by studying its effects on cytochrome P450-dependent microsomal enzyme activities.

2. Materials and methods

2.1. Herbal extract preparation

The antidiabetic preparation ADD-199 was a kind gift from Dr. K.A. 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 and 1/2 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 sterilised distilled water before use.

2.2. Animals

Male Wistar albino rats, 6–8 weeks old, obtained from the Research animal Unit, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, were used for the study. All animals were fed on normal laboratory chow (GAFCO Ltd., Tema, Ghana) and given sterilised drinking water throughout the period of study. 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).

2.3. Reagents and chemicals

Randox test kits; alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine kinase (CK), creatinine, urea and albumin were purchased from Randox Laboratories Ltd. (Co. Antrim, UK). Dimethylsulphoxide (DMSO) was obtained from Wako Pure Chemical Industries, Japan. Urine reagent strips (URS-10) were purchased from Teco Diagnostics, USA. Bovine serum albumin (BSA), reduced nicotinamide adenine dinucleotide phosphate (NADPH), ethoxyresorufin (ER), *p*-nitrophenol (PNP), pentoresorufin (PR), resorufin (RS) and zoxazolamine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Pentobarbital was obtained from Fischer Scientific Co. (Silver Spring, MD, USA). All other chemicals and reagents were purchased in the purest form available from British Drug Houses (BDH) Ltd. (Poole, UK).

2.4. Animal pre-treatment and blood sampling

Rats were divided into seven groups of six animals/group. Each set of animals received either drinking water or herbal preparation in drinking water (35 ml/rat) daily. Three sets of control animals (Group A) received drinking water only. One set of test animals (Group B) received ADD-199 (100 mg/kg/day) whilst three sets of test animals (Group C) received ADD-199 (500 mg/kg/day). All animals received treatment for 30 days. One set of six animals in each group were weighed on day 0 and then weekly until termination at day 30. For blood parameter studies, blood samples (1 ml each) of these six animals in each treatment group, were taken by tail bleeding, on days 0, 15 and 30 into separate Eppendorf tubes containing EDTA (1.5 mg) and heparin (0.125 mg) for haematological and biochemical analyses, respectively. For cytochrome P450 isozyme studies, the same animals were euthanised by cervical dislocation on last day of bleeding (termination) and their livers excised, weighed and prepared for homogenization.

2.5. Biochemical analyses

Blood samples for biochemical analyses were centrifuged at $4000 \times g$ for 5 min and the plasma collected and stored in Eppendorf tubes at $-20\,^{\circ}C$ and analyzed the following day. Plasma ALT, AST, ALP, CK, urea, creatinine and albumin were determined by spectrophotometric assays according to the Randox kit instruction manual (Randox Laboratories Ltd., Co. Antrim, UK).