Journal of Traditional and Complementary Medicine 6 (2016) 184–192

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original article

Blood glucose level and lipid profile of alloxan-induced hyperglycemic rats treated with single and combinatorial herbal formulations

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ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form
26 November 2014
Accepted 1 December 2014
Available online 24 February 2015

Keywords:
Diabetes mellitus
Dyslipidemia
Herbal formulations
Hyperglycemia
Phytochemicals

ABSTRACT

The current study sought to investigate the capacities of single and combinatorial herbal formulations of leaf extracts of *Acanthus montanus*, *Asystasia gangetica*, *Emilia coccinea*, and *Hibiscus rosasinensis* to reverse hyperglycemia and dyslipidemia in alloxan-induced diabetic male rats. Phytochemical composition of the herbal extracts, fasting plasma glucose concentration (FPGC), and serum lipid profile (SLP) of the rats were measured by standard methods. The relative abundance of phytochemicals in the four experimental leaf extracts was in the following order: flavonoids > alkaloids > saponins > tannins. Hyperglycemic rats (HyGR) treated with single and combinatorial herbal formulations showed evidence of reduced FPGC compared with the untreated HyGR and were normoglycemic (FPGC < 110.0 mg/dL). Similarly, HyGR treated with single and combinatorial herbal formulations showed evidence of readjustments in their SLPs. Generally, HyGR treated with triple herbal formulations (THfs) exhibited the highest atherogenic index compared with HyGR treated with single herbal formulations (SHfs), double herbal formulations (DHfs), and quadruple herbal formulation (QHf). The display of synergy or antagonism by the composite herbal extracts in ameliorating hyperglycemia and dyslipidemia depended on the type and number of individual herbal extract used in constituting the experimental herbal formulations. Furthermore, the capacities of the herbal formulations (SHfs, DHfs, THfs, and QHf) to exert glycemic control and reverse dyslipidemia did not follow predictable patterns in the animal models.

1. Introduction

Hyperglycemia and dyslipidemia, among other disorders, are metabolic syndromes associated with a dysfunctional endocrine system clinically referred to as diabetes mellitus (DM).[1–3] DM is described and classified on the basis of intrinsic and extrinsic causative factors, which has been exhaustively explained elsewhere.[4–7] Although the etiology of DM is multifaceted, the prevalence of the disease worldwide is often linked to genetic/physiologic factors, sedentary lifestyle, and obesity,[8–11] of which poor dietary habits such as high consumption of sugars and saturated fats in addition to low intake of polyunsaturated fatty acids (PUFAs) have been implicated to be major contributory factors toward the progression of the disease.[12,13]

The earliest and common diagnostic indices of DM are hyperglycemia and glucosuria. In that regard, the unusual metabolism of carbohydrates in DM, and associated profound adjustments of glycolytic pathways[14,15] engender the activation of alternative polyol metabolic pathways with resultant intracellular accumulation of sorbitol[16] and auto-oxidation of glucose.[17] These distortional metabolic events have been implicated in the etiology of diabetic peripheral neuropathy, retinopathy, and cataracts.[9,18] Patterns of dyslipidemia in DM and connecting primary risk factors have been described in earlier reports.[2,19,20] Atherosclerosis-induced coronary heart disease (CHD), stroke, and hypertension are major causes of increasing rate of fatalities among patients with DM.[21,22]

The dilapidating action of DM qualifies it as a disease of major public health concern and epidemiological survey showed that it is the seventh leading cause of death worldwide.[23] Additionally, projections showed that the disease will become the foremost

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2014.12.005

cause of morbidity and mortality within the next 25 years, especially in Africa and Asia.[5,9,24] In addition to the option of DM management that involves intramuscular administration of insulin to individuals with diabetes, there are several synthetic antidiabetic medicinal preparations of notable capacity to act as agents of glycemic control.[25–27] However, from a toxicological standpoint, alternative herbal formulation remedies are sometimes preferred to synthetic antidiabetic drugs because of its minimal or no side effects.[28,29] Furthermore, because the uses of traditional plant medicines are cost-effective mitigation strategies, ethnomedicinal practices are being increasingly applied worldwide for the alleviation and management of DM.[9,18,24,30–33] Decoctions from parts or whole plants of *Acanthus montanus*, *Asystasia gangetica*, *Emilia coccinea*, and *Hibiscus rosasinensis* have been effectively applied for the treatment and management of numerous pathologic conditions.[34–39] Most ethnomedicinal practitioners presume that administration of combinatorial extracts of different plant species serves to potentiate the efficacy of herbal concoctions[40] and may exhibit competitive therapeutic potentials when compared with that of orthodox medicines.[41] Accordingly, the current study sought to investigate the capacities of single and combinatorial herbal formulations of leaf extracts of *A. montanus*, *A. gangetica*, *E. coccinea*, and *H. rosasinensis* to reverse hyperglycemia and dyslipidemia in alloxan induced diabetic male rats.

2. Materials and methods

2.1. Collection and preparation of herbal samples

Fresh leaves of *Acanthus montanus* (Nees) T. Anderson (ACMO), *Emilia coccinea* G. Don (EMCO), and *Hibiscus rosasinensis* L. (HIRO) were collected from uncultivated lands in Umuamacha Ayaba Umaeze, Osisioma Ngwa Local Government Area (LGA), Abia State, Nigeria, whereas fresh leaves of *Asystasia gangetica* L. T. Anderson (ASGA) were collected from Ubowuala, Emekuku, Owerri North Local Government Area (LGA), Imo State, Nigeria. The four herbs were identified and authenticated by Dr. M. Ibe, School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri, Nigeria. All the leaves were collected between the months of July 2009 and August 2009.

The leaves of individual plants were washed with continuous flow of distilled water for 15 minutes and allowed to dry at laboratory ambient temperature (24 ± 5 °C). A 500 g portion of each of the herbal samples were weighted using a triple beam balance (OHAU 750-50: Burlington, NC, USA) and dried in an oven (WTC BINDER, 7200 Tuttlingen, Germany) at 60 °C until a constant weight was achieved. The dried leaves were packaged in dark polyethylene bags and kept in cold room (7 ± 3 °C) for 24 hours prior to pulverization. Next, the separate dried leaves were pulverized using a Thomas-Willey milling machine (Thomas Wiley® Mini-Mill; ASTM D-3182; India), after which the ground samples were stored in air-tight plastic bottles with screw caps pending extraction.

2.2. Extraction of herbal samples

A 40 g portion of each **of the** pulverized dried samples of *A. montanus*, *A. gangetica*, *E. coccinea*, and *H. rosasinensis* were subjected to repeated Soxhlet extraction cycles for 2 hours using 96% C_2H_5OH (BDH, UK) as solvent to obtain a final volume of 500 mL of each herbal extract. These volumes of the herbal extracts were concentrated and recovered in a rotary evaporator for 12 hours at 60 °C under reduced pressure. The extracts were dried in a desiccator for 24 hours, wrapped in aluminum foil, and stored in air-tight plastic bottles with screw caps at ≤ 4 °C. The yields were calculated to be as follows: *A. montanus* = 16.35% (*w/w*), *A. gangetica* = 16.69% (*w/w*), *E. coccinea* = 17.99% (*w/w*), and *H. rosasinensis* = 17.23% (*w/w*). The separate extracts were reconstituted in phosphate buffered saline (PBS) solution (extract vehicle), osmotically equivalent to 100 g/L PBS (90.0 g NaCl, 17.0 g $Na_2HPO_4.2H_2O$, and 2.43 g $NaH_2PO_4.2H_2O$), before appropriate doses were administered to the experimental animals. Portions of the individual herbal extracts were also measured for their phytochemical contents.

2.3. Phytochemical composition of herbal extracts

Flavonoids content was measured by the methods of Boham and Kocipai.[42] The concentration of alkaloids of the herbal extracts was measured by the methods of Harborne.[43] Measurement of saponin content of the herbal extracts was performed according to the methods of Harborne,[43] as reported by Obadoni and Ochuko.[44] The Van-Burden and Robinson[45] method as reported by Belonwu et al[46] was used to measure concentration of tannins of the herbal extracts.

2.4. Experimental animals

Male albino (Wistar) rats (School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri, Nigeria) weighing between 150–160 g were maintained at room temperatures of 24 ± 5 °C, 30–55% of relative humidity on a 12-hour light/12-hour dark cycle, with access to water and standard commercial feed (SCF; Ewu Feed Mill, Edo State, Nigeria) *ad libitum* for a 2-week acclimatization period. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

2.5. Induction of diabetes/experimental design

Hyperglycemia was induced in the experimental rats by single intraperitoneal (i.p.) injection of 90 mg/kg body weight of alloxan monohydrate (Sigma-Aldrich, St. Louis, MO, USA) in PBS solution (pH = 7.4). The animals with fasting plasma glucose concentration (FPGC) > 110 mg/dL for 5 consecutive days were considered hyperglycemic and selected for the study. A total of 102 male Wistar rats were allotted into 17 groups of six rats each. The animals were deprived of food and water for an additional 16 hours prior to the commencement of treatment as described elsewhere.[47] The animal groups were designated on the basis of treatments received at regular intervals of 2 days for 30 days. Herbal treatments of the hyperglycemic rats (HyGR) were described as single herbal formulations (SHf): (HrACMO, HrASGA, HrEMCO, and HrHIRO), double herbal formulations (DHf): (HrAGAM, HrAGEC, HrAGHR, HrAMEC, HrAMHR, and HrECHR), triple herbal formulations (THf): (HrAGEH, HrAMAE, HrAMAH, and HrAMEH), and quadruple herbal formulation (QHf): (HrAAEH).

- NORM: Normal rats received SCF + water *ad libitum* + 1.0 mL/kg of PBS.
- DIAB: HyGR received SCF + water *ad libitum* + 1.0 mL/kg of PBS.
- HrACMO: HyGR received SCF + water *ad libitum* + *A. montanus* (20 mg/kg in PBS; i.p.).
- HrASGA: HyGR received SCF + water *ad libitum* + *A. gangetica* (20 mg/kg in PBS; i.p.).
- HrEMCO: HyGR received SCF + water *ad libitum* + *E. coccinea* (20 mg/kg in PBS; i.p.).
- HrHIRO: HyGR received SCF + water *ad libitum* + *H. rosasinensis* (20 mg/kg in PBS; i.p.)
- HrAGAM: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus* (20 mg/kg in PBS; i.p.).