Journal of Ethnopharmacology 133 (2011) 402–411

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Randomized and double-blinded pilot clinical study of the safety and anti-diabetic efficacy of the *Rauvolfia-Citrus* tea, as used in Nigerian Traditional Medicine

Joan I.A. Campbell-Tofte[a,*], Per Mølgaard[a], Knud Josefsen[b], Zostam Abdallah[a], Steen Honoré Hansen[c], Claus Cornett[c], Huiling Mu[c], Erik A. Richter[d], Henning Willads Petersen[e], Jens Christian Nørregaard[f], Kaj Winther[g]

[a] *Dept. of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universtitetsparken 2, 2100 Copenhagen, Denmark*
[b] *Bartholin Institute, Rigshospitalet, Section 3733 Ole Maaløes Vej 5, 2200 Copenhagen, Denmark*
[c] *Dept. of Pharmaceutics & Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark*
[d] *Dept. of Exercise & Sport Sciences, Faculty of Natural Sciences, University of Copenhagen, Nørre Allé 51, 2200 Copenhagen, Denmark*
[e] *Pharmacy Service, Dept. of Veterinary Disease Biology, University of Copenhagen, 1870 Frederiksberg, Denmark*
[f] *Eye Clinic, Frederiksberg University Hospital, Nordre Fasanvej 57, 2000 Frederiksberg, Denmark*
[g] *Dept. of Clinical Biochemistry, Frederiksberg University Hospital, Nordre Fasanvej 57, 2000 Frederiksberg, Denmark*

ARTICLE INFO

Article history:
Received 28 May 2010
Received in revised form
18 September 2010
Accepted 6 October 2010
Available online 16 October 2010

Keywords:
Clinical research
Type 2 diabetes
Rauvolfia vomitoria
Citrus aurantium
Herbal remedies
Ethnopharmacology

ABSTRACT

Aim of the study: The aim of this randomized and double blinded pilot clinical trial was to investigate the anti-diabetic efficacy of the *Rauvolfia-Citrus* (RC) tea in humans. We have earlier shown that a combination of calorie-restriction and chronic administration of the RC tea to the genetic diabetic (BKS-*db*) mice resulted in the normalization of blood sugar, reduction in lipid accumulated in the mice eyes and prevention of the degeneration of the otherwise brittle BKS-*db* pancreas. The tea is made by boiling foliage of *Rauvolfia vomitoria* and fruits of *Citrus aurantium* and is used to treat diabetes in Nigerian folk medicine.
Materials and methods: The RC tea was produced using the Nigerian traditional recipe and tested in the traditional dosage on 23 Danish type 2 diabetes (T2D) patients. The participants were divided into two equivalent groups after stratification by sex, age and BMI, in a 4-month double-blinded, placebo-controlled and randomized clinical trial. Most of the study subjects (19/23) were using oral anti-diabetic agents (OADs). Mean disease duration was 6 ± 4.6 years, mean age was 64 ± 7 years and mean BMI was 28.7 ± 3.8 kg/m^2. Prior to starting the treatment, the participants received individual dietician consultations.
Results: At the end of the 4-month treatment period, the treated group showed an 11% decrease in 2-h postprandial plasma glucose relative to the 3% increase in the placebo group ($p = 0.004$). The improvement in blood glucose clearance with RC tea treatment was reflected in a 6% reduction in HbA_{1c} ($p = 0.02$) and in a 10% reduction in fasting plasma glucose ($p = 0.02$), when comparing the post 4-month treatment to pre-treatment baseline values. Though the basal levels of phosphorylated acetyl CoA carboxylase enzyme in skeletal muscle were significantly reduced in the treated group ($p = 0.04$), as compared to the placebo, only the pattern of reductions in the tissue fatty acids (FAs) differed in the two groups. While all types of FAs were reduced in placebo, only saturated (SFA) and monounsaturated (MUFA) FAs were reduced with treatment. Interestingly, a modest increase in the polyunsaturated FAs fraction was observed in the RC treated group. In addition, the reduction in SFA and MUFA with RC tea treatment came solely from the triglyceride fractions, as there was an increase in the skeletal muscle phospholipids.
Conclusions: Chronic administration of the RC tea to overweight T2D on OADs caused significant improvements in markers of glycaemic control and modifications to the fatty acid profile of skeletal muscle, without adverse effects or hypoglycaemia. Further exploration of the anti-diabetic effects of the RC tea is warranted.

Abbreviations: FPG, fasting plasma glucose; FA, fatty acid; IMPDs, investigational medicinal products; NKML, Nordisk Metodikkomite for Levnedsmidler; OADs, oral anti-diabetic agents; ACC-p, phosphorylated acetyl CoA carboxylase; AMPK, 5′AMP-activated protein kinase; PPG, postprandial plasma glucose; RC, *Rauvolfia-Citrus*; T2D, type 2 diabetes.

* Corresponding author at: Department of Clinical Biochemistry, Co-ordinating Research Unit, Frederiksberg University Hospital, Nordre Fasanvej 57, 2000 Frederiksberg, Denmark. Tel.: +45 38163448; fax: +45 38164719.
E-mail address: Joan.Campbell-Tofte@frh.regionh.dk (J.I.A. Campbell-Tofte).

0378-8741/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2010.10.013

1. Introduction

It is well documented that type 2 diabetes (T2D) is associated with the accumulation of intracellular fat in peripheral tissues, such as the eye, muscle and the pancreas (Kautzky-Willer et al., 2003; Sharma et al., 2004; Campbell-Tofte et al., 2007). The relationship between this ectopic lipid accumulation and disorders in metabolism caused by interference with insulin secretion and signalling has been demonstrated (Perseghin et al., 2002; Roden, 2005). While most of the currently used oral anti-diabetic agents (OADs) work by reducing fasting plasma glucose (FPG) and glycosylated haemoglobin (HbA_{1c}) to acceptable levels, the disease is not cured nor is the development of the complications associated with long term diabetes prevented (Temple, 1999; Koski, 2006; Hadler, 2008). Hence, there is the need to find more effective T2D therapy.

In northern Edo state of Nigeria, a decoction made from boiling foliage of *Rauvolfia vomitoria* with fruits of *Citrus aurantium,* is believed to have curative effects on diabetes, if the patients avoid alcohol and adhere to a calorie-restricted diet. We have previously published evidence that a combination of calorie-restriction with a 6-week administration of the herbal remedy – the *Rauvolfia-Citrus* (RC) tea to genetic diabetic (BKS-*db*) mice resulted in (a) normalization of blood glucose levels, (b) increased serum triglyceride, (c) reductions in the fat content of the mice eyes; and (d) protection of the "brittle" pancreas, that is otherwise characteristic of these T2D model mice with poorly controlled diabetes (Campbell et al., 2006).

As was observed in the BKS-*db* experiments, treatment of a T2D patient with the RC tea in a Danish human case study had no acute effect on blood glucose (Campbell-Tofte, unpublished). Rather, while taking the tea daily for 4 months, a normalization of blood pressure and reduction in fasting plasma glucose occurred within the second and third months, respectively. The patient maintained a calorie-restricted diet during the treatment. We hypothesize that unlike most oral anti-diabetic medications that act by regulating blood glucose levels, the RC therapy might interact with some aspects of cellular metabolism that subsequently causes the normalization of blood glucose. The changes in serum lipid and tissue fatty acid contents seen in the BKS-*db* experiments, would suggest that these interactions may be on lipid metabolism.

In this study, a double blinded, placebo-controlled and randomized pilot clinical trial was carried out to assess the safety and efficacy of the traditional RC tea for the treatment of T2D in patients already on OADs. Another objective of the study was to find out if the tea had the tissue-lipid-lowering effect in human, as observed with administration of the RC tea to the BKS-*db* mice (Campbell et al., 2006). Serum markers of liver and kidney function, as well as sugar and lipid metabolic parameters were measured at every clinic visit. In addition, fatty acid profiles and expression of key metabolic enzymes in skeletal muscle were compared in biopsies taken before and after the treatment.

2. Methods

2.1. Ethical issues

The trial was conducted according to the guidelines of the Declaration of Helsinki and reported using the recommendations for reporting randomized clinical trials, as defined in the Consolidated Standards of Reporting Randomized Clinical Trials (CONSORT) statement (Moher et al., 2001). The study protocol was reviewed and approved by the Biomedical Research Ethics for the Capital Region of Denmark (registration No.: H-KA-20060092) and by the Danish Medicines Agency (journal No.: 2614-3). The trial protocol was registered with EudraCT with Reg. number nr. 2006-003642-42 and with the Danish Data Protection Agency (journal No.: 2008-41-2025). All participants returned signed informed consent forms which are kept at the Department of Clinical Biochemistry, Frederiksberg Hospital, Denmark. An import certificate addressed to the Nigerian Federal Ministry of Agriculture and Natural Resources was obtained from the Danish Plant Directorate for the transfer of the plant materials used in preparing the RC tea.

2.2. Study participants

Twenty-six T2D patients (11 females and 15 males), aged between 52 and 79 years were recruited from 50 responders to newspaper advertisements published in and around Copenhagen from May to July 2008. Of the 21/26 participants on OADs, 18 used metformin, 6/21 were taking the sulfonylurea/repaglinide family of OADs, while 19/26 were on anti-hypertensive drugs (mostly ACE-inhibitors – 10/19). Some of the participants (11/18) took metformin in combination with another OAD or anti-hypertensive medication, and a cholesterol-reducing agent.

The following exclusion criteria were used: T2D patients on insulin, suffering from known allergies, with a recent thrombolic episode, uncontrollable hypertension or with known alcohol/drug abuse.

Prior to entering the study, the participants received individual consultations with a dietician on how best to restrict and calculate their daily calorie intake. The participants were equipped with a diary and apparatus for measuring blood glucose and blood pressure at home. They were instructed to abstain from alcohol or keep to a maximum of 1 unit per day, and to record in the diaries, their daily food and alcohol intake, measurements of blood glucose and blood pressure, as well as intake of other medicines.

2.3. Methods of randomization and patient flow

As the study is a pilot clinical trial, the Ethical Committee approved the inclusion of a maximum of 24 participants. The first 20 participants who enrolled for the study were allocated into 4 blocks, consisting of 2 females and 3 males balanced for BMI and age as follows: 63 ± 5 years and $27 \pm 2\,kg/m^2$, 60 ± 11 years and $29 \pm 6\,kg/m^2$, 63 ± 4 years and $30 \pm 5\,kg/m^2$ and 65 ± 6 years and $29 \pm 4\,kg/m^2$, respectively. The blocks were later randomly allocated to RC treatment or placebo by the Pharmacy. Six participants (3 men and 3 women) who later joined the study were either placed into the existing blocks, where their BMI and age matched those who had dropped out, or were randomly assigned to the treated or placebo groups by the Pharmacy as illustrated in the participant flow diagram – Fig. 1.

Double-blindness was ensured as the participants had no contact with the Pharmacy, who kept the study code and the group allocations. Apart from one married couple who got the same IMPD, the study subjects had not met each other prior to participating in the trial.

2.4. Investigational medicinal products

The investigational medicinal products (IMPDs) are the RC tea and a placebo. Foliage of *Rauvolfia vomitoria*, and fruits of *Citrus aurantium* were collected in and around Auchi, Edo state, Nigeria between September 2007 and February 2008. The foliage was dried at ambient temperature in the shade. Voucher specimens of the plant materials are kept at the Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen (FARMA).

2.4.1. Preparation of the IMPDs

The RC tea was prepared by boiling *Rauvolfia vomitoria* foliage and *Citrus aurantium* fruits (400 g:2 kg for 10 l), as already described