Homeopathy (2013) 102, 160–171

http://dx.doi.org/10.1016/j.homp.2013.05.002, available online at http://www.sciencedirect.com

ORIGINAL PAPER

Effect of homeopathic preparations of *Syzygium jambolanum* and *Cephalandra indica* on gastrocnemius muscle of high fat and high fructose-induced type-2 diabetic rats

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Background: **Homeopathy is a holistic method of treatment that uses microdoses of natural substances originating from plants, minerals, or animal parts. *Syzygium jambolanum* and *Cephalandra indica* are used in homeopathy for treatment of type-2 diabetes. However, the molecular mechanisms responsible for such effects are not known.**
Methods: **Homeopathic preparations of *S. jambolanum* and *C. indica* in mother tincture, 6c and 30c were used to examine the molecular mechanism of antidiabetic effects in the skeletal muscle of rats with high fat and fructose-induced type-2 diabetes mellitus. After 30 days treatment, fasting blood glucose, serum insulin and insulin signaling molecules in the skeletal muscle (gastrocnemius) were measured.**
Results: **Diabetic rats showed a significant decrease in serum insulin and lipid profile as well as low levels of insulin receptor (IR), v-akt murine thymoma viral oncogene homolog (Akt), p-Akt^{ser473} and glucose transporter-4 (GLUT4) protein expression ($p < 0.05$) with a significant increase in fasting blood glucose level ($p < 0.05$) compared to the control group. Treatment with homeopathic remedies significantly increased the serum insulin and expression of these proteins ($p < 0.05$) with a significant decrease in fasting blood glucose ($p < 0.05$) compared to diabetic rats.**
Conclusions: **In the present study homeopathic preparations of *S. jambolanum* and *C. indica*, including ultramolecular dilutions exhibit antidiabetic effects, improving insulin action through activation of insulin signaling molecules in skeletal muscle of type-2 diabetic rats.** *Homeopathy* (2013) **102**, 160–171.

Keywords: Insulin signaling; Homeopathic preparation; *Syzygium jambolanum* and *Cephalandra indica*; Gastrocnemius muscle

Introduction

Diabetes mellitus (DM) is the most common endocrine and metabolic disorder, currently affecting over 170 million people worldwide and potentially over 365 million in the year 2030.[1] Type-2 DM is rapidly emerging as one of the greatest global health challenges of the 21st century. The major pathophysiological event contributing to the development of type-2 DM is the resistance of target tissues to insulin, which is usually associated with abnormal

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Received 21 October 2012; revised 30 April 2013; accepted 7 May 2013

insulin secretion.[2,3] Metformin is recommended as the first line oral antidiabetic drug, and a number of options are available for use as the second line oral therapies when metformin is ineffective, which include sulphonylureas, dipeptidyl peptidase-4 (DPP-IV) inhibitors, and thiazolidinediones (TZD). However, these drugs are associated with significant side effects and tolerance problems.[4] Therefore, there is great interest in developing new treatments which can provide long-lasting control with minimal side effects and better safety profiles.

Homeopathy is a form of complementary medicine that uses microdoses or ultra high dilutions of natural substances originating from plants, minerals or animal parts.[5,6] It was introduced and first practiced by the German physician Samuel Hahnemann (1755−1843). It is based on the 'principle of similars' "*similia similibus curentur*". Substances known to cause symptoms similar to a particular disease are given to patients in highly diluted form to stimulate auto-regulation and self-healing processes.[7,8] In classical homeopathy a single homeopathic remedy is selected, based on a patient's total spectrum of symptoms.[9] The proportion of patients obtaining homeopathic care quadrupled in a seven year period in the USA during the 1990s.[10]

The process of dilution with succussion is referred to as 'potentisation'. The potentized medicine is thought to retain properties of the original source substance when a chosen medicine is applied homeopathically to a particular patient according to the 'law of similars'.[11−14] Chikramane *et al.*[15] demonstrated the presence of nanoparticles of the starting materials and their aggregates even at extremely high dilutions.

Syzygium jambolanum (Jamun) and *Cephalandra indica* (Ivy gourd) in herbal form have been investigated for antidiabetic effects both in preclinical and human studies.[16−20] These plants are used in homeopathy for treating patients with diabetes related symptoms. However, there have been no experimental studies evaluating its pharmacological activity in the field of homeopathy. The molecular mechanisms behind the antidiabetic activity of homeopathic preparations of these plants are not known. The aim of the present study is to investigate the effect of homeopathic preparations of *S. jambolanum* and *C. indica* on insulin signaling molecules in gastrocnemius muscle of type-2 diabetic rats. This mechanistic approach is essential for scientific validation of the antidiabetic property of these homeopathic preparations.

Materials and methods

Materials

All chemicals and reagents used in the present study were of analytical grade and they were purchased from Amersham Biosciences Ltd, UK; Sigma Chemical, St. Louis, MO; Sisco Research Laboratories, Mumbai, India; Bio-Rad Laboratories Inc, Hercules, USA. Glucose estimation kit was purchased from CPC diagnostics, Spain. The β-actin monoclonal antibody was purchased from Sigma chemicals. Polyclonal insulin receptor (IR), v-akt murine thymoma viral oncogene homolog (Akt) and phospho-Akt^{ser473}, and glucose transporter-4 (GLUT4) antibodies were purchased from Santa Cruz Biotechnology Inc, CA, USA. Horseradish peroxidase-conjugated goat anti-mouse and goat anti-rabbit antibodies were obtained from GeNei, Bangalore, India.

Homeopathic remedies

Homeopathic remedies (*S. jambolanum mother tincture* Batch number: 7670 and *C. indica mother tincture* Batch number: 7619) were procured from Hahnemann publishing Co. Pvt. Ltd (Hapco), Kolkata, India. *C. indica* mother tincture was prepared from fresh *C. indica* leaves and *S. jambolanum* mother tincture was prepared from fresh seeds of *S. jambolanum* (Linn.). The above two mother tinctures were prepared with drugs strength 1/10th i.e. 10 g dry drug substances were used for preparation of each 100 ml of the tincture. The centesimal or c scale was prepared by the procedure of homeopathic serial dilutions and succussions, diluting 1 part of mother tincture by a factor of 100 at each stage up to the 30c.

Induction of type-2 diabetes

Healthy adult male albino rats of Wistar strain, weighing 180−210 g were used in the present study. Rats were made diabetic (type-2) by high fat diet containing cholesterol 2 g, cholic acid 1 g, coconut oil 30 mL, standard rat feed 100 g, and 25% fructose through drinking water. After 30 days,[21] a single intraperitoneal injection of streptozotocin in relatively low dose (35 mg/kg body weight) was given. High fat diet and fructose feeding were continued till the end of the study (30 days). Control rats were fed with normal pelleted rat feed and water was made available *ad libitum*. In this model, rats develop insulin resistance but not frank hyperglycemia or diabetes. Low dose of streptozotocin was given to generate a slight trauma to beta cells of pancreas to mimic the condition of chronic hypoinsulinemic insulin resistant condition. Animals were maintained as per the National Guidelines and Protocols approved by the Institutional Animal Ethical Committee (IAEC No: 03/020/09 dated 01-04-2009).

Experimental design

Rats were divided into the following groups.

Group 1: Control (plain control − vehicle treated − 20 μl/100 g body weight, twice daily through oral intubation)
Group 2: Type-2 diabetic rats (plain control − vehicle treated − 20 μl/100 g body weight, twice daily through oral intubation)
Group 3: Diabetic (type-2) rats treated with *S. jambolanum MT* (mother tincture) (Sj MT 20 μl/100 g body weight, twice daily through oral intubation) for 30 days after 5 days of streptozotocin treatment
Group 4: Diabetic (type-2) rats treated with *S. jambolanum*-6 centesimal (Sj-6c-20 μl/100 g body weight, twice