Available online at www.sciencedirect.com
ScienceDirect
Journal of Ethnopharmacology 113 (2007) 91–99
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

# Protective effect of Hachimi-jio-gan against the development of pancreatic fibrosis and oxidative damage in Otsuka Long-Evans Tokushima Fatty rats

Noriko Yamabe, Takako Yokozawa *

*Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*

Received 22 November 2006; received in revised form 26 March 2007; accepted 1 May 2007
Available online 10 May 2007

**Abstract**

In our previous study, the polyherbal drug Hachimi-jio-gan was reported to possess a protective effect against the progression of diabetic nephropathy by attenuating glucose toxicity and renal damage with a type 2 diabetic model, Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Based on these findings, this study was undertaken to reveal the effect of Hachimi-jio-gan on pancreatic damage focusing on fibrosis and oxidative stress in type 2 diabetes. OLETF rats were orally administered Hachimi-jio-gan for 32 weeks, and we assessed the changes in the serum glucose level every 8 weeks, as well as those of body weight, and food and water consumption every 4 weeks. In addition, pancreatic wet weight, insulin content, and Western blot analyses of transforming growth factor-$\beta_1$, fibronectin, and nuclear factor-κB-related inflammatory enzymes, such as inducible nitric oxide synthesis and cyclooxygenase-2, were also performed in the pancreas. As a consequence, long-term treatment with Hachimi-jio-gan had a hypoglycemic effect, reducing pancreatic atrophy and fibrosis, and ameliorating the oxidative status. Therefore, this may provide evidence that Hachimi-jio-gan is a therapeutic target for preventing the development of pancreatic damage concomitant with hyperglycemia in type 2 diabetes.


*Keywords:* Hachimi-jio-gan; Pancreas; Fibrosis; Oxidative stress; Type 2 diabetes

## 1. Introduction

The pancreas is a complex of exocrine and endocrine glands that controls many homeostatic functions; thus, pancreatic fibrosis caused by chronic pancreatitis, pancreatic cancer, and diabetes leads to serious disorders in carbohydrate and lipid metabolism. Particularly, in the development of type 2 diabetes, chronic hyperglycemia can exert deleterious effects on β-cell function as well as hyperlipidemia (Moran et al., 1997; Gleason et al., 2000), and the mechanisms of glucotoxicity involve several transcriptional factors and are, at least in part, mediated by the generation of chronic oxidative stress through the glycation reaction (Kaneto et al., 1996; Laybutt et al., 2002). On the other hand, β-cell dysfunction caused by glucotoxicity is reported to be potentially reversible with the restoration of metabolic control (LeRoith, 2002). Therefore, an effective remedy to attenuate the decline in pancreatic function by suppressing fibrosis and oxidative stress with the restoration of glucose metabolism may help to prevent the development of diabetic complications, while attempts to stimulate insulin secretion and improve insulin action with drug therapies are temporarily helpful but, in fact, are ultimately unable to prevent progressive β-cell dysfunction.

For centuries, traditional plant drugs, which are less toxic and free from side-effects than general synthetic drugs, have been used for the treatment of diabetes, and in Japan, Hachimi-jio-gan, which is a polyherbal drug composed of eight medicinal plants, is known as a treatment for diabetes, but also hypertension, nephritic syndrome, and glomerulonephritis. In our previous study, we demonstrated that Hachimi-jio-gan has a beneficial effect on the progression of diabetic nephropathy in a type 2 diabetic model, Otsuka Long-Evans Tokushima Fatty (OLETF) rats, by attenuating glucotoxicity and renal damage (Yamabe and Yokozawa, 2006); however, the mechanisms responsible for the effects of Hachimi-jio-gan on glucotoxicity in other internal organs have remained obscure.

* Corresponding author. Tel.: +81 76 434 7670; fax: +81 76 434 5068.
*E-mail address:* yokozawa@inm.u-toyama.ac.jp (T. Yokozawa).

0378-8741/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2007.05.010

Table 1
Composition of Hachimi-jio-gan

| Botanical name | Common name | Family name | Part used | Content (%) |
|---|---|---|---|---|
| *Rehmannia glutinosa* Libosch. var. *purpurea* Makino | Rehmanniae Radix | Scrophulariaceae | Root | 27.27 |
| *Cornus officinalis* Sieb. et Zucc. | Corni Fructus | Cornaceae | Fruit | 13.64 |
| *Dioscorea japonica* Thunb. | Dioscoreae Rhizoma | Dioscoreaceae | Rhizome | 13.64 |
| *Alisma orientale* Juzep. | Alismatis Rhizoma | Alismataceae | Rhizome | 13.64 |
| *Poria cocos* Wolf | Hoelen | Polyporaceae | Sclerotium | 13.64 |
| *Paeonia suffruticosa* Andrews | Moutan Cortex | Paeoniaceae | Bark | 11.36 |
| *Cinnamomum cassia* Blume | Cinnamomi Cortex | Lauraceae | Bark | 4.54 |
| *Aconitium carmichaeli* Debx | Aconiti Tuber | Ranunculaceae | Tuber | 2.27 |

OLETF rats were used as a model of human type 2 diabetes, and their histopathological changes in the pancreas were classified into three stages: (1) degenerative changes and necrosis of islets (after 12 weeks of age); (2) fibrosis and enlargement of islets (after 20 weeks of age); and (3) replacement of islets (after 40 weeks of age) (Kawano et al., 1992), and were reported as a useful model for elucidation of the pathogenesis of chronic pancreatitis (Ashizawa et al., 2006). Recently, Nakayama et al. (2005) revealed that increased oxidative stress in islets was correlated with the course of β-cell damage in OLETF rats. Moreover, pancreatic morphological changes in OLETF rats coexist with glomerular sclerotic changes seen in diabetic nephropathy, and TGF-β plays an important role in the development of pancreatic fibrosis (Yoshikawa et al., 2002). This study may provide the first report suggesting the potential effect of the polyherbal drug Hachimi-jio-gan on fibrosis and oxidative damage in the pancreas in type 2 diabetes.

## 2. Materials and methods

### 2.1. Materials

The following reagents were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan: bovine serum albumin (BSA), 2-amino-2-hydroxymethyl-1,3-propadiol (tris (hydroxymethyl) aminomethane), Tween 20, glycerol, phenylmethyl sulfonyl fluoride (PMSF), protease inhibitor cocktail, and skim milk powder. Precision plus protein standards and the Bio-Rad protein assay kit were purchased from Bio-Rad Laboratories, Japan. Rabbit anti-human insulin polyclonal antibody, rabbit anti-human nuclear factor-κB (NF-κB) p65 polyclonal antibody, rabbit anti-human inhibitor binding protein κB-α (IκB-α), mouse anti-mouse NOS2 monoclonal antibody (primary antibody for inducible nitric oxide synthesis (iNOS)), mouse anti-human cyclooxygenase-2 (COX-2) monoclonal antibody, rabbit anti-human transforming growth factor-$\beta_1$ (TGF-$\beta_1$) polyclonal antibody, goat anti-rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibody, and goat anti-mouse IgG HRP-conjugated secondary antibody were purchased from Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA. Rabbit anti-human fibronectin polyclonal antibody was purchased from DAKO Cytomation, Denmark. Anti-mouse β-actin antibody was purchased from Sigma–Aldrich, St. Louis, MO, USA. ECL Western blotting detection reagents were purchased from Amersham Bioscience, Piscataway, NJ, USA.

### 2.2. Preparation of Hachimi-jio-gan extract

The water extract of Hachimi-jio-gan used in this study was produced by Tsumura Juntendo Inc., Tokyo, Japan, and the composition of Hachimi-jio-gan is shown in Table 1. In brief, 8 medicinal plants with their contents weighed were boiled gently in 10 times their volume of water for 60 min, filtered, and the filtrate was spray-dried to obtain the extract at a yield of about 10%, by weight, of the original preparation. For analysis of the components of Hachimi-jio-gan, the aqueous extract (0.5 g) was extracted with 20 ml methanol under ultrasonication for 30 min. The solution was filtered through a membrane filter (0.45 μm) and then subjected to high-performance liquid chromatography (HPLC) analysis using a TSK-GEL ODS-80TS column (Ø 4.6 mm × 250 mm, Tosoh, Japan) with a LC $10AD_{vp}$ pump and a SPD-$M10A_{vp}$ absorbance detector. The elution solvents were (A) 0.05 M AcOH-$AcONH_4$ and (B) $CH_3CN$, and the column was eluted with a linear gradient of, by volume, 90% A and 10% B changing over 60 min to 100% B. The flow rate was 1.0 ml/min and the effluent from the column was monitored and processed into three-dimensional data by an SPD-M10A array detector. All assigned peaks were identified by comparing their UV spectral data with those co-injected authentic samples using Class LC-10 Version 1.62 software (Shimadzu, Japan).

### 2.3. Animals and experimental design

Male OLETF and Long-Evans Tokushima Otsuka (LETO) rats were kindly supplied by the Tokushima Research Institute (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan). All rats were kept in wire-bottomed cages with controlled temperature (about 25 °C) and humidity (about 60%), and a 12-h light:12-h dark cycle, and were given free access to laboratory pellet chow (CE-2, CLEA Japan Inc., Tokyo, Japan, comprising 24.0% protein, 3.5% lipids, and 60.5% carbohydrate) and water. We prepared 5 groups of rats of 22 weeks old: a non-diabetic LETO group given water (no treatment) ($n = 5$; LETO control), a group of diabetic OLETF rats given water (no treatment) ($n = 10$; OLETF control), and three groups of diabetic OLETF rats given Hachimi-jio-gan (50, 100, or 200 mg/kg body weight/day) orally by gavage once a day ($n = 10$; OLETF + HJG50, 100, or 200). The amount (ml) of oral administration was calculated as 1/200 of each body weight (g). During the administration period of 32 weeks, food and water consumption were checked, rats being kept in the individual cages, every 4 weeks, and every 8 weeks,