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Toosendan Fructus ameliorates the pancreatic damage through the anti-inflammatory activity in non-obese diabetic mice

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ABSTRACT

Objectives : The present study was conducted to examine whether Toosendan Fructus has an ameliorative effect on diabetes-induced alterations such as oxidative stress and inflammation in the pancreas of non-obese diabetic (NOD) mice, a model of human type I diabetes.

Methods : Extracts of Toosendan Fructus (ETF) were administered to NOD mice at three doses (50 mg/kg, 100 mg/kg and 200 mg/kg). Mice at 18 weeks of age were measured glucose tolerance using intraperitoneal glucose tolerance test. After 28 weeks of ETF treatment, glucose, total cholesterol (TC), triglyceride (TG), and proinflammatory cytokines in serum, western blot analyses and a histopathological examination in pancreas tissue, and on the onset of diabetes were investigated.

Results : The results showed that levels of glucose, glucose tolerance, TC, TG , interferon-γ, interleukin (IL)-1 β, IL-6, and IL-12 in serum were down-regulated, while IL-4, IL-10, SOD, and catalase significantly increased. In addition, ETF improved protein expression of proinflammatory mediaters (such as cyclooxygenase-2, and inducible nitric oxide synthase) and a proapoptotic protein (caspase-3) in the pancreatic tissue. Also, in the groups treated with ETF (100 mg/kg or 200 mg/kg), insulitis and infiltration of granulocytes were alleviated.

Conclusions : Based on these results, the anti-diabetic effect of ETF may be due to its anti-inflammatory and antioxidant effect. Our findings support the therapeutic evidence for Toosendan Fructus ameliorating the development of diabetic pancreatic damage via regulating inflammation and apoptosis. Our future studies will be focused on the search for active compounds in these extracts.

Key words : Toosendanin fructose, Diabetes, inflammatory cytokine, pancreatic beta cell

Introduction

Non-obese diabetic (NOD) mice, which develop type 1 diabetes (T1D) spontaneously, is very similar to the human disorder, insulin-dependent diabetes mellitus (IDDM). Accordingly, diabetes in NOD mice is the most extensively studied model of autoimmune disease[1,2]. T1D is caused by the destruction of the insulin-producing β-cells through insulitis, which involves the infiltration of leukocytes into pancreatic islets[3,4]. In addition, the development of insulitis lead to the excess accumulation of inflammatory cells, a gradual decrease in β-cells mass, and the development of chronic hyperglycemia[5]. Recently, various research on herbal medicine has been studied as an alternative medicine for prevention and treating diabetes[6].

Toosendan Fructus is a fruit of *Melia toosendan* Sieb. et Zucc. Many compounds are contained in TF including β-sitosterol, toosendanin, kulinone ($C_{30}H_{48}O_2$), methylkulonate ($C_{31}H_{48}O_4$), melianol ($C_{30}H_{48}O_4$), melianodiol, melialactone, andazadiarachtin ($C_{35}H_{44}O_{16}$). Toosendanin in particular

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is a widely known major component. Toosendanin has displayed apoptosis inducing activity[8] and suppressed proliferation of cancer cells[9], anti-botulinum acivity[10], anti-microbial activity[11], inhibitory effect of releasing an acetylcholine[12], activation of drug metabolic enzymes and bile juice release in the liver[13], and anti-hyperlipidemia activity[14]. Recently two limonoids demonstrating anti-inflammation and analgesic properties were reported[15]. In addition, Toosendan Fructus (TF), a typical tranditional medicine, also reported antioxidative and anti-diabetic effect on blood glucose, lipid metabolism, and carbohydrate metabolism-related enzyme activities in the liver of streptozotocin-induced diabetic rats[7]. However, TF has not reported anti-diabetic activity through the anti-inflammatory activity in the pancreatic tissue of NOD mice.

Materials and Methods

1. Plant material

Toosendan Fructus was purchased from Omniherb Co. (Youngcheon, South Korea). Dry plant material was extracted with distilled water. TF (500 g) was powdered with a grinder, sieved through a mesh (pore size 0.5 μm) and boiled gently in 4 times their volume of distilled water for 3 h. The extract of TF (ETF) was then filtered, evaporated on a rotary evaporator (Buchi, Flawil, Switzerland), and lyophilized in a freeze dryer (Eyela FDU-540; Tokyo, Japan). The powdered extract was stored at −20 ℃. The percentage yield was about 16% (w/w). The ETF powder was freshly dissolved in distilled water before use.

2. Animals

A total of 60 mice (Orient, sungnam, Korea) used in the study, each weighing about 18g, were housed (one mouse per cage) under pathogen-free conditions in a temperature-controlled environment with a 12 h light/dark cycle. The commercial diet were supplied by Diet Research (Bethlehem, PA, USA). Mice were divided into 5 groups by stratified random sampling to equalize the mean body weight of the groups. The groups (n = 12 in each group) were designated as follows: group A, normal (non-obese non diabetic) mice treated with distilled water (DW); group B, NOD mice treated with DW; group C, NOD mice treated with ETF at 50 mg/kg body weight/day; group D, NOD mice treated with ETF at 100 mg/kg body weight/day; group E, NOD mice treated with ETF at 200 mg/kg body weight/day for 28 consecutive weeks. The approval and guide line for animal study was DHUARB2013-010.

3. Intraperitoneal glucose tolerance test (IPGTT)

Mice at 19 weeks of age (n = 8) were fasted for 12 h and then injected intraperitoneally with a single bolus of glucose (2 g/kg body weight)[16]. Glucose levels in blood collected from the tail vein were measured 0, 20, 40, 60, 90 and 120 min after glucose administration. Blood was immediately centrifuged (1,800 x g), the serum was separated and stored at −20 ℃ until assayed.

4. Determination of blood glucose, Total cholesterol (TC), and Triglyceride (TG)

For analysis of blood glucose, mice were bled from the orbital sinus following a 4-h fast approximately every 4 weeks from 5 weeks of age. Whole blood was immediately centrifuged to obtain serum. The concentration of glucose in the serum was measured using an enzymatic colorimetric assay (Glucose LiquiColor, Stanbio Laboratory, Boerne, TX, USA) followed by spectrophotometric analysis. Animals with fasting glucose levels greater than 300 mg/dl serum were diagnosed as diabetic. The study was terminated when the mice reached 33 weeks of age. For analysis of TC and TG, serum was collected once at 18 weeks from the tail vein, and analyzed with a commercial ELISA kit (Sigma, MO, USA).

5. Determination of body weight gain (g), and water and food consumption

Weight of each mouse was examined every week from 5 weeks to 33 weeks of age. Water consumption (ml/day [d]) and food intake (g/d) were determined every week. Water consumption was calculated by measuring total consumption (ml) during 1 week in a cage.

6. Assays for serum Cytokines

At 32 weeks of age, overnight-fasted mice were anesthetized to collecte blood samples. Serum insulin concentration was measured by ELISA (Mercodia, Inc., Winston-Salem, NC, USA). Cytokines in the serum were determined using a mouse cytokine array kit (Quansys Biosciences, West Logan, UT, USA), which included IL-1β, IL-4, IL-6, IL-10, IL-12.