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EXPERIMENTAL STUDY

Nelumbo Nucifera leaf extract attenuated pancreatic β-cells toxicity induced by interleukin-1β and interferon-γ, and increased insulin secretion of pancreatic β-cells in streptozotocin-induced diabetic rats

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Abstract

OBJECTIVE: To evaluate the effect of Nelumbo Nucifera leaf water extract (NNLE) on insulinoma (RIN) cells induced by interleukin-1β (IL-1β) and interferon-g (IFN-γ), and injured pancreatic β-cells induced by Streptozotocin (STZ) in rats.

METHODS: The anti-oxidative effects of NNLE were assessed using 1,1-diphenyl-2-picryl hydrazyl (DPPH) and nitric oxide (NO) scavenging assays. The inhibitory effect of NNLE on α-glucosidase and DPP (dipeptidyl peptidase)-IV was measured *in vitro*. Pancreatic β-cell protective and insulin secretory effects were assessed, using IL-1β and IFN-γ-induced rat RIN cells. STZ-induced diabetic rats were treated with 50, 100, and 400 mg/kg NNLE for 4 weeks. The effects of NNLE on blood glucose (BG), body weight (BW), and lipid profiles were measured.

RESULTS: NNLE inhibited DPPH, NO, α-glucosidase, and DPP-Ⅳ which were directly linked to the function of β-cells. Furthermore, NNLE protected RIN cells from toxicity induced by IL-1β and IFN-γ, decreased NO production, and increased insulin secretion. NNLE caused a significant reduction in blood glucose, triglyceride (TG), total cholesterol (TC), blood urea nitrogen (BUN), and creatinine in STZ-induced diabetic rats. Furthermore, it significantly decreased BW loss in STZ-induced diabetic rats.

CONCLUSION: Our results suggest that NNLE reduced the toxicity in insulinoma cells and increased insulin secretion in pancreatic β-cells in STZ-induced diabetic rats.

Key words: Nelumbo Nucifera; Diabetes mellitus; Insulin; Interleukin-1beta; Interferon-gamma; Insulin-secreting cells; Streptozotocin; Glucose

INTRODUCTION

Diabetes is caused by impaired insulin production and / or decreased tissue response to the insulin. The number of people with diabetes are increasing worldwide; About 366 million people have diabetes, and 552 million people are expected to have diabetes in 2030.[1] According to the International Diabetes Federation, every

six second a person dies from diabetes. In diabetes, insulin secreting pancreatic β-cells are injured by the selective destruction of the islets of Langerhans.[2] Infiltrated immune cells, in and around the islets of Langerhans, play an important role during the early stage of the pathogenesis of insulin dependent diabetes mellitus (IDDM).[3] Cytokines such as interleukin-1β (IL-1β) and interferon-γ (IFN-γ) are the effector molecules that play a central role during the initial destruction of pancreatic β cells; the combination of IL-1β and IFN-γ upregulates an inducible form of nitric oxide synthase expression, and subsequently NO production, which impairs insulin secretion, causing diabetes.[4] Recently, it has been reported that the protection of pancreatic β-cells play a major role in glucose homeostasis by preserving, expanding, and improving their function.[5] Moreover, recent diabetic research has been focused on how to protect and improve functional β- cells to regulate glucose homeostasis in diabetes.[6] Therefore, there is an urgent need for the development of β- cell protective and safe hypoglycemic agents that could target the intimate mechanisms of beta cell damage and change the anti-diabetic therapy scenario in near future.

Several medicinal plants with high flavonoid and polyphenol contents are reported to have not only blood glucose lowering effects, but also pancreatic β-cell protective effects.[7] Nelumbo Nucifera, commonly named lotus, is an aquatic plant belonging to the family Nelumbonacea, widely distributed in China, Japan, and India. Nelumbo Nucifera is an agricultural crop and is cultivated for food and drink for thousand of years in South Korea. Every part of the lotus, including leaves, flowers, seeds, and rhizomes, have been reported to have several medicinal values;[8] many Korean food recipes contain lotus as a healthy ingredient. Furthermore, tea, noodles, juice, etc., prepared from the lotus, have gained popularity in South Korea. Recently, Huang and his colleagues reported that 100% methanol extract of the lotus leaves reversed the glucose intolerance in high-fat-diet-induced obese mice.[9] Similarly, Liu *et al* [10] reported the hypolipidemic and α-glucosidase inhibitory effects of the total flavonoids from Nelumbo Nucifera leaves. It has been reported that the water extract of Nelumbo Nucifera leaf contains 27.2% total flavonoids and 9.3% of total phenolic acids.[11] However, to the best of our knowledge, no one has reported on its effect on pancreatic β-cells against cytokine mediated toxicity, and biochemical parameters in pancreatic β-cells-injured diabetic rats. Therefore, in this study, we hypothesized to find whether the water extract of Nelumbo Nucifera leaves could protect pancreatic beta cells in insulinoma (RIN) induced by IL-1β and IFN-γ in rats, and increase the insulin secretion in pancreatic beta cells in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Cell culture and reagents

RIN cells were purchased from the American Type Culture Collection (Rockville, MD, USA) and grown at 37 ℃ under a humidified 5% CO_2 atmosphere in RPMI 1640 medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA) 2 mM glutamine, 100 units/mL of penicillin, 100 μg/mL of streptomycin, and 2.5 μg/mL of amphotericin B. IL-1β and IFN-γ were purchased from R & D Systems (Minneapolis, MN, USA), and rat insulin ELISA kit was purchased from Mercodia Developing Diagnostics (Mercodia, Uppsala, Sweden). 2, 2-diphenyl-1- picrylhydrazyl (DPPH), sodium nitropruside (SNP), rat intestinal acetone powder, p-Nitrophenyl α-D-gluco-pyranoside, and streptozotocin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Substrate for DPP-IV enzyme, H-Gly-Pro-AMC, was purchased from AnaSpec Inc. USA. All chemicals and reagents were purchased from Sigma (St. Louis, MO, USA), unless otherwise specified.

Preparation of Nelumbo Nucifera leaf extract (NNLE)

Nelumbo Nucifera leaves (2 kg) were dried and extracted with 4000 mL distilled water for 24 h at room temperature. The extraction was repeated three times. The residue was removed by filtration and the filtrate was evaporated followed by freeze drying. The yield of the extract was about 12% of the starting material. The concentration of total phenolic acids and the total flavonoid content in the NNLE was measured as described previously.[11]

Measurement of DPPH, NO, DPP-IV, and α-glucosidase

DPPH, NO, DPP-IV, and alpha glucosidase scavenging activities were measured as described previously.[12,13]

Cell viability, NO production, and insulin secretion

Cell viability, NO production, and insulin secretion were measured as described previously.[14]

Experimental animals and induction of diabetes

Six weeks old adult male albino Wistar rats were purchased from the Central Lab Animal Inc. (Seoul, Korea). They were kept at standard living conditions (Room temperature of 25 ℃, 45%-50% relative humidity and 12 h dark/light cycle) in the Animal Research Center of Mokpo National University. All the animals were provided with standard pellet diet and water ad libitum. The rats were acclimatized to the laboratory conditions for 1 week prior to the commencement of the experiment. Procedures involving animal care were conducted in conformity with the institutional guidelines of Mokpo National University, South Korea. Diabetes was induced in overnight-fasted rats by a single intravenous injection of STZ (50 mg/kg BW),