1. ABSTRACT

Studies on the role of Nrf2 activator-naringenin in experimental diabetes

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder with multiple etiological factors resulting from a defect in insulin secretion, insulin action, or both, which in turn leads to chronic hyperglycemia with disturbances in the metabolism of carbohydrates, fats, and proteins. The incidence and burden due to diabetes are increasing at an alarming rate globally with an estimated 422 million individuals currently suffering from this disease, and this number is expected to increase to 642 million by 2040 as per 2016 International Diabetes Federation report. Chronic hyperglycemia and unusually high oxidative stress is the major characteristic feature of diabetes. Nuclear factor E2-related factor 2 (Nrf2), a master regulator of intracellular redox homeostasis, controls the expression of antioxidant and detoxification genes thereby interrupting the link between oxidative stress and diseases including diabetes. Targeted activation of Nrf2 either by inhibiting its negative regulator kelch-like ECH associated protein-1 (keap1) or by promoting the expression through transcriptional activation has been reported to protect pancreatic beta cells thereby mitigate the complications of diabetes. Hence, an attempt was made to check whether naringenin, a known activator of Nrf2, protects pancreatic beta cells from streptozotocin (STZ) induced damage thereby help in decreasing the complications of diabetes.

OBJECTIVE

The objectives of the present study include:
1) Determine the antioxidant potential of naringenin using in vitro antioxidant assay methods
2) Study the effect of naringenin on Nrf2 activation in MIN6 pancreatic β-cell line
3) Evaluate the ability of elevated Nrf2 to mitigate STZ-treatment induced pancreatic β-cell apoptosis in MIN6 cells
4) Assess the efficacy of Nrf2-upregulating naringenin for protecting mice from STZ-induced diabetes

METHODOLGY

The free radical scavenging activity of naringenin was evaluated by measuring its effect on (a) hydroxyl; (b) superoxide; (c) hydrogen peroxide; (d) nitric oxide; and (e) DPPH radicals as well as for inhibiting the peroxidation of lipids. The ability of naringenin to activate Nrf2 was assessed using Nrf2-Keap1 complementation system in MIN6 cells. Immunoblot analysis was performed to detect the Nrf2 in cytosolic and nuclear extracts. Anti-apoptotic activity of naringenin was evaluated by estimating the levels of caspase-3 and Annexin V expression. Anti-diabetic potential of naringenin was assessed by estimating (a) blood glucose; (b) lipid profile; (c) oxidative stress markers TBARS, lipid hydroperoxides, SOD, catalase, GST, GPX, and the levels of reduced glutathione in the pancreas, liver and kidney homogenates prepared from animals treated with multiple low dose streptozotocin (MLD-STZ) (50mg/kg b.w) for 5 consecutive days (i.p). In addition, the potential of naringenin to modulate the activities of key carbohydrate metabolizing enzymes and glycogen content in liver was also assessed.
RESULTS

Naringenin showed potent free radical scavenging activity in _in vitro_ cell-free system. Naringenin effectively neutralized (a) hydroxyl radicals; (b) superoxide; (c) hydrogen peroxide; (d) nitric oxide radical; (e) DPPH and inhibited the oxidation of unsaturated fatty acids in a dose dependent manner. In cells, naringenin could not only activate Nrf2 by promoting its release from Keap1 complex but also promoted the translocation of cytosolic Nrf2 into nucleus. Nuclear Nrf2 activated target genes possessing ARE sequences such as GST and NQO1, thereby inhibited cellular apoptosis as evidenced by the reduction in caspases-3 expression. In animals, administration of naringenin, for 45 days, significantly decreased STZ-induced blood glucose levels, and normalized the lipid profile. Furthermore, naringenin administration decreased the oxidation of unsaturated lipids and augmented the levels of antioxidants in liver tissues. Histochemical analysis of pancreas showed healthy islets compared to STZ-treated animals. Immunohistochemical analysis measuring the number of insulin-positive cells in pancreas revealed that naringenin could improve the number of insulin positive cells. In addition, naringenin modulated the levels of key enzymes of carbohydrate metabolism, such as hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase and liver glycogen in such a way that promote glycolysis and inhibit gluconeogenesis. In summary, naringenin inhibits STZ-induced experimental diabetes by upregulating Nrf2 and its target genes NQO1 and GST, hence, is a potential candidate for developing a naturally occurring anti-diabetic formulation.

CONCLUSION

In conclusion, results of this study demonstrate the potential of naringenin for promoting the expression of anti-oxidant regulator Nrf2 and its target genes, while modulating the levels of carbohydrate metabolizing enzymes to treat diabetes. Hence, future studies should consider testing naringenin in higher animals to determine its potential for inhibiting experimental and spontaneous diabetes.

KEY WORDS: Diabetes, Streptozotocin, Naringenin, Nrf2, pancreatic β-cells, Apoptosis.