Syringic acid ameliorates experimental diabetic nephropathy in rats through its anti-inflammatory, anti-oxidant and anti-fibrotic effects mediated by suppressing Toll like receptor-4 pathway

Yara A. Samra, Omar M. Zabad, Laila A. Eissa

Department of Biochemistry, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt

19th WCIRDC Conference, Hilton Universal City Hotel, Los Angeles, CA

Introduction

Diabetic nephropathy (DN) is an important healthcare challenge because it occurs in about 40% of diabetic people and is a main cause end-stage renal disease. Toll like receptor-4 (TLR-4) signaling is involved in DN incidence and progression through its activation by hyperglycemia and hyperglycemia-induced alterations besides its crucial roles in initiating inflammation and fibrosis in kidney. Syringic acid is a natural phenolic acid. It is naturally available in many fruits and vegetables, including swiss chards, grapes and olives. In this study, renoprotective effects of syringic acid, as anti-inflammatory, anti-oxidant and anti-fibrotic natural compound, against experimental DN were investigated.

Background

Diabetic nephropathy (DN) constitutes the single commonest cause of end-stage renal disease (ESRD) where it accounts for 30–47% of the global incident cases of ESRD. DN pathogenesis is complicated, in which hyperglycemia and its subsequent metabolic and hemodynamic deteriorations collaborate in inducing a chronic sterile inflammation and consequential renal fibrosis culminating in kidney dysfunction and necessity for dialysis. Toll like receptor-4 (TLR-4) is a preserved transmembrane pattern recognition receptor. Its activation in response to hyperglycemia, advanced glycation end products (AGEs) oxidative stress and other stimuli leads to production of various proinflammatory and fibrotic mediators through recruiting nuclear factor kappa B (NFκB) transcription factor. TLR-4 participates in inducing chronic inflammation by generating both interleukin-6 cytokine and CC ligand 2 (CCL2) chemokine, promoting oxidative stress and inducing fibrosis by producing transforming growth factor β1 and collagen. Syringic acid (4-Hydroxy-3, 5- dimethoxybenzoic acid, SA) is a member of phenolic acid family. It is naturally present in wide variety of fruits, vegetables and fungal species, including swiss chards, grapes, dates, olives, honey and mushroom. It has several anti-diabetic and anti-oxidant and anti-cancer effects. In the current study, we investigated the impact of syringic acid on STZ-induced DN model in rats, to find out its roles in halting DN through modulating TLR-4 signaling cascade.

Methods

Diabetes mellitus (DM) was induced by single intraperitoneal (i.p.) injection of STZ (45mg/kg) dissolved in a 0.1 M ice-cold citrate buffer (pH 4.5) after 12-hour-fasting. On the other hand, normal control group were injected with equal volume of the vehicle. All rats with blood glucose level higher than 250 mg/dl were considered diabetic rats then were randomly subdivided into DN and SA groups. Syringic acid group: in which diabetic rats received syringic acid (50 mg/kg) once daily suspended in 0.5% CMC solution by orogastric gavage tube.

After this period, success in mimicking DN pathogenesis and incidence was confirmed through performing kidney function tests and histopathological examination of kidney tissues of 2 DN rats. Blood glucose level, creatinine level, blood urea nitrogen level, urinary total protein level, urine creatinine level, MDA content and SOD activity were estimated by colorimetric assay kits. Serum insulin level, renal TLR-4, renal interleukin-6 and renal TGFβ1 were evaluated by using ELISA kits. Kidney collagen content was calculated by measuring kidney hydroxyproline content. kidney tissues were incubated in 50% KOH overnight, then they were subjected to alkaline hydrolysis using 10 N NaOH. After that, chloramine-Treagent was added to samples and standard hydroxyproline solutions and incubated for 3h. Then, a reddish purple complex was formed by adding freshly prepared Ehrlich's reagent to samples and standard hydroxyproline solutions and keeping them for 20 min. Finally, absorbance of the complex was read at 550 nm. Renal hydroxyproline content was extrapolated from a standard curve.

Results

Impact of syringic acid on fasting blood glucose and insulin levels

Group	Fasting blood glucose level (mg/dl)	Serum insulin level (pg/ml)
Normal control	89.17 ± 2.81	513.9 ± 26.95
Diabetic nephropathy	402.5 ± 25.44*	147.5 ± 10.46*
Syringic acid	218.8 ± 17.75*,$	346.9 ± 19.47*,$

*Table 1: Rats of DN group showed a significant increase in fasting blood glucose level, besides a significant decrease in insulin level as compared to normal control group. Syringic acid showed significant anti-hyperglycemic effects through inducing a significant decline in fasting blood glucose level and a significant rise in insulin secretion when compared with DN rats. * indicates P < 0.05 compared with normal control group, $ indicates P < 0.05 compared with DN group.*

Effects of syringic acid on biochemical parameters of kidney function in urine and serum

Group	Creatinine clearance (ml/min.)	Protein in urine (mg/day)	Serum creatinine (mg/dl)	Blood urea nitrogen (mg/dl)
Normal control	0.064 ± 0.0031	64.17 ± 5.43	0.53 ± 0.01	19.16 ± 1.29
Diabetic nephropathy	0.017 ± 0.0015*	167.2 ± 4.54*	1.01 ± 0.02*	60.12 ± 3.16*
Syringic acid	0.041 ± 0.0036*,$	103.7 ± 7.52*,$	0.77 ± 0.03*,$	26.46 ± 1.42$

*Table 2: Rats of DN group showed a significant rise in urinary total protein, BUN and serum creatinine levels in comparison to normal control rats. However, DN group showed a significant decline in creatinine clearance when compared to normal control rats. Contrarily, syringic acid significantly declined urinary total protein, BUN and serum creatinine levels in comparison to DN group. However, syringic acid administration induced a significant rise in creatinine clearance as compared with DN rats. * indicates P < 0.05 compared with normal control group, $ indicates P < 0.05 compared with DN group.*

Effects of syringic acid on oxidant/antioxidant parameters

Group	Renal MDA content (nmol/gm tissue)	Renal SOD activity (U/mg tissue)
Normal control	46.98 ± 3.37	118.9 ± 3.86
Diabetic nephropathy	105.2 ± 1.99*	49.2 ± 2.14*
Syringic acid	68.19 ± 4.57*,$	85.65 ± 3.39*,$

*Table 3: DN group showed a significant decline in renal SOD activity with a concomitant significant increase in MDA content as compared to normal control rats. Contrarily, syringic acid administration led to a significant decrease in renal MDA content and a significant rise in renal SOD activity when compared to DN group. * indicates P < 0.05 compared with normal control group, $ indicates P < 0.05 compared with DN group.*

Effects of syringic acid on toll like receptor-4 (TLR-4) and interleukin (IL-6) concentration in kidney homogenate:

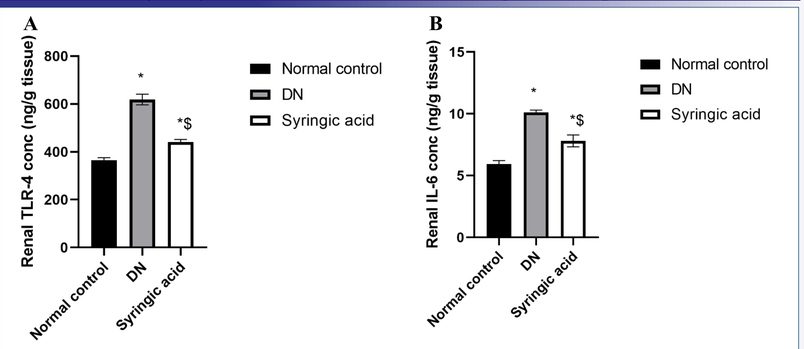

*Figure 1: A) Kidney content of TLR-4 significantly increased in DN group in comparison to normal group, whereas it significantly decreased after syringic acid administration as compared to DN group. B) Interleukin-6 content significantly increased in DN group as compared with normal control group. Contrarily, syringic acid administration significantly decreased it as compared to DN group. *,$: significantly different in comparison to normal control group and diabetic nephropathy group, respectively (p < 0.05).*

Impact of syringic acid on transforming growth factor β1 content in kidney homogenate:

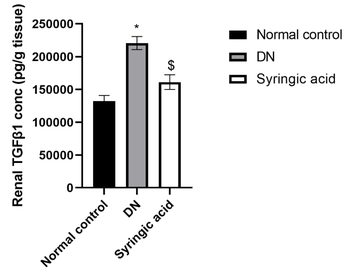

*Figure 2: DN group showed a significant increase in renal TGFβ1 content as compared with normal control group. Contrarily, syringic acid administration led to a significant decline in renal TGFβ1 in comparison to DN group. *,$: significantly different in comparison to normal control group and diabetic nephropathy group, respectively (p < 0.05).*

Impact of syringic acid on kidney contents of hydroxyproline and collagen:

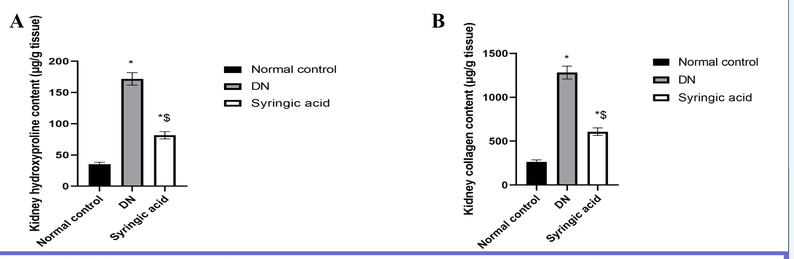

*Figure 3: A,B) Kidney contents of hydroxyproline and collagen significantly rose in DN group in comparison to normal group. On the other hand, both of them significantly declined after syringic acid administration as compared to DN group. *,$: significantly different in comparison to normal control group and diabetic nephropathy group, respectively (p < 0.05).*

Conclusion

The findings of this study proved that syringic acid is capable of preventing DN progression via its immune-modulatory, anti-inflammatory, anti-oxidant and anti-fibrotic effects.