Nifuroxazide, a STAT3 inhibitor, mitigates inflammatory burden and protects against diabetes-induced nephropathy in rats

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\textbf{ABSTRACT}

Diabetic nephropathy (DN) is a serious complication of diabetes mellitus. Moreover, it is amongst the most common causes of end-stage renal failure. Inflammation is a crucial player in both development and progression of DN. JAK2/STAT3 is a pleiotropic cascade reported to regulate diverse inflammatory events. Previous studies reported involvement of JAK2/STAT3 signal transduction pathway in diabetes-associated renal injury. In the current study, the inhibitory effect of nifuroxazide (25 mg/kg/day, orally) against inflammatory condition associating diabetic kidney progression in rats was evaluated. The underlying hypothesis is mainly via the inhibitory effect of nifuroxazide on STAT3 signaling. Results revealed that nifuroxazide effectively inhibited STAT3 activation in diabetic male rats, improved glomerular filtration function, protected against diabetes-induced histopathological and ultramicroscopic structural alterations. Further, nifuroxazide treatment significantly reduced renal macrophage infiltration and fibrosis and decreased mRNA and protein levels of TNF-\textalpha and IL-18 in diabetic renal tissue. The current findings shed the light on nifuroxazide’s efficacy as an alternative anti-inflammatory therapy to hinder the development and progression of DN in diabetic patients mainly via STAT3 inhibition.

1. Introduction

Diabetes mellitus (DM) is a highly prevalent metabolic disease that develops as a result of hyperglycemia, due to deficiency in insulin production and/or action. The increased prevalence of diabetes worldwide is attracting a significant concern. According to International Diabetes Federation, it is estimated that the number of diabetic patients globally will increase from 285 million diabetics as reported in 2010 to 552 million by 2030 [1]. Unfortunately, diabetes and its complications are major causes of morbidity and mortality; moreover, they have huge social and economic impacts. Uncontrolled hyperglycemia is associated with devastating vascular complications that affect both macro- and micro-vasculature [6].

Among the various diabetic complications, diabetic nephropathy (DN) complicates 30\%–40\% of diabetic patients with greater adverse effect on both the quality of life and the patient’s survival. DN is a chronic complication of DM characterized by excessive accumulation of extracellular matrix, glomerular basement membrane thickening, mesangial expansion, hypertrophy of both glomeruli and tubules and ultimately glomerulosclerosis and tuftularistoisstitial fibrosis [15]. Indeed, DN is considered the main cause of end-stage renal disease (ESRD) [25].

The progression of DN to end-stage renal disease is irreversible; therefore, it is mandatory to find effective therapeutic approaches to stop and/or delay progression of diabetes-induced renal damage. Despite significant advances in understanding of pathogenesis, natural history and clinical features of DN, current therapy are still unable to stop the progression of nephropathy in diabetic patients. Hence, it is important to shift research efforts to target early pathogenic insults that contribute to disease progression [10].

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway is a pleiotropic cascade activated through phosphorylation by various triggers including tumor growth factor- \textbeta (TGF-\textbeta), pro-inflammatory cytokines, high glucose, advanced glycation end products (AGEs) and angiotensin II. Activation of this signaling pathway induces expression of multiple genes, including...
pro-inflammatory and pro-fibrotic genes [11]. Typically, this pathway is internally deactivated by internal negative regulators such as members of the suppressor of cytokine signaling (SOCS) and protein inhibitor of activated STAT (PIAS). However, constitutive activation of this cascade is implicated in pathogenesis of many diseases [21]. Indeed, various members of JAK/STAT signaling pathway has been claimed as potential molecular target in various diseases such as atherosclerosis [13] and hypertension [30]. Moreover, Dysregulated JAK2/STAT3 signaling has been reported in various diseases including cancer [12]; [14], Alzheimer [44], cerebral ischemia [16] and arthritis [28].

Notably, several clinical and experimental studies reported involvement of JAK/STAT pathway in various renal disorders [31] [38]; [27]. Among the different members of JAK/STAT cascade, JAK2/STAT3 axis is the best studied and it has been reported to be implicated in progressive renal injury [32]; [5].

Nifuroxazide is an oral nitrofuran antibiotic commonly used as intestinal anti-infective agent. Interestingly, recent studies reported nifuroxazide as a potent and effective STAT3 inhibitor [40] [42]; [47]. In the light of the evidence supporting the role of JAK2/STAT3 signaling in diabetes-induced renal injury and remarkable inhibitory effect of nifuroxazide on phosphorylation of this cascade, the present study was designed to evaluate nifuroxazide’s potential reno-protective effect against diabetes-induced renal injury and DN. Nifuroxazide is hypothesized to inhibit STAT3 cascade in diabetic-induced renal injury and to attenuate inflammation and fibrosis.

2. Materials and methods

2.1. Chemicals

All the chemicals used were of analytical grade. Streptozotocin (STZ) (Sigma Chemicals Co., St. Louis, MO, USA) was immediately prepared before use by dissolution in citrate buffer (0.01 mol/L, pH 4.4) in ice chilled light protected amber bottle. Nifuroxazide was purchased from Sigma Chemicals Co. (St. Louis, MO, USA) and administrated as oral suspension in 0.5% carboxymethylcellulose (CMC).

2.2. Experimental design

STZ was used for experimental induction of diabetes in rats as previously described [9]. Briefly, thirty male Sprague-Dawley rats, weighing 180–230 g, were obtained from the breeding unit of Holding Company for Biological Products and Vaccines, “VACSERA”, Cairo-Egypt and housed in standard animal facility under standard environmental conditions of temperature and light–dark cycle (room temperature 22 ± 2°C and 12-h light–dark cycle) with free access to food and water ad libitum. All the experimental procedures adhered to the guidelines complied with the ethical guidelines of the Research Ethics Committee, Faculty of Medicine, Mansoura University, Egypt.

Following acclimatization, animals received intra-peritoneal injection of STZ (50 mg/kg) after fasting for 12 h, and the normal control received equal volume of citrate buffer instead. Diabetes was confirmed by assessment of fasting blood glucose. Seventy two hours post STZ injection; rats with fastened blood glucose above 250 mg/dl were considered diabetic and were experimentally grouped as follows.

Rats were randomly allocated to three experimental groups (10 rats for each group); normal/vehicle and diabetic controls; rats received daily oral 0.2 ml 0.5% CMC for 8 weeks and nifuroxazide treated group; in this group nifuroxazide was orally administrated (25 mg/kg/day, daily) for 8 week. The dose of nifuroxazide was selected based on previous studies that reported STAT3 inhibitory effect of nifuroxazide [42] [47] [43]; and our pilot study.

After the last nifuroxazide dose, rats in the different experimental groups were individually housed in metabolic cages for urine samples collection. Twenty four hours later, animal scariﬁcation was done using overdose of thiopental sodium and blood was collected. Kidney and body weights were measured and data were used to calculate kidney/body weight index. The left kidney was split lengthwise, one half was first immersed in ice-cold saline and then in buffered formalin 10% for further for histological studies and immunolocalization, and the other half was used for electron microscopy evaluation. The right kidney was flash frozen by immediate immersing in liquid nitrogen and kept at −80 °C for PCR and ELISA assessments.

2.3. Renal function tests

After coagulation, blood samples were centrifuged at 3000 rpm for 10 min to separate serum. Creatinine and blood urea nitrogen were quantified in serum using Diamond Diagnostic; Germany and Biomed, Egypt assay kits respectively, following manufacturer’s instructions. Urinary albumin excretion (albuminuria) was determined using commercially available Exocell (PA, USA) enzyme linked immunosorbent assay (ELISA) kit.

2.4. Assessment of STAT3 activation

The frozen kidneys were homogenized in phosphate buffer saline (pH, 7.4) and the samples were placed on ice for 30 min. Thereafter, the homogenates were centrifuged to remove tissue debris. The supernatants were stored in aliquots for further analyses. Protein content in each sample was assessed using Lowry method [17]. STAT3 activation (phospho-STAT3/STAT3) was assessed in rat renal tissue homogenate using Abcam (Cambridge, MA, USA) assay ELISA kit.

2.5. Assessment of renal inflammatory cytokines

mRNA and protein levels of tumor necrosis factor alpha (TNF-α) and interleukin-18 (IL-18) were quantified in rat renal tissue. For gene expression assessment, total RNA was isolated and purified over an Rneasy mini column. Then, RNA was reverse transcribed into a cDNA and quantified using an ultraviolet spectrophotometer and assessed by electrophoretic examination. Real time was performed using (Applied Biosystem ABI 7000, USA). RNA was reverse transcribed into a cDNA. Each cDNA mixture was diluted for PCR amplification using gene expression products assay (Applied Biosystem ABI, USA) and appropriate primers (Table 1). RT-PCR gave the quantification of genes to be extracted, and analysis was conducted using the Step one plus software. Relative expression analysis was calculated by these equations: The relative gene expression = \(2^{-\Delta \Delta Ct}\).

For assessment of protein levels TNF-α and IL-18, commercially available ELISA (Cloud-Clone Corp, Carlsbad, CA, USA) kits were used for quantification of renal tissue content of both markers following the manufacturer’s instructions.

2.6. Histopathological observation of rat kidneys

The dissected left kidneys were cut into small pieces after its dissection out, kept in fixative (10% neutral buffered formalin) for about 1 week, washed, dehydrated with alcohol of different grades, then cleared by xylene and embedded in paraffin wax to form hard block. 5-
μm thick sections from hard block were mounted on glass slides and subjected to the following staining procedures: Hematoxylin and Eosin stain for detection of any histopathological changes, Masson's Trichrome stain for evaluation of collagen and extracellular matrix (ECM) accumulation and immunohistochemical (IHC) stain for detection of CD68 and tumor growth factor-β (TGF-β). For IHC, the slides were mounted with 3–5 μm thick sections then deparaffinized with xylene and different grades of ethanol. Citrate buffer (pH = 6) was used to mediate antigen retrieval, blocking the samples was done using 1% bovine serum albumin at 21 °C for 10 min. The slides incubated with primary antibody (Anti-CD68 antibody-ab31630-5 μg/ml with dilution 1/800) and (anti-TGF-β antibody-ab190503- 5 μg/ml with dilution 1/500) at 21 °C for 2 h. For detection of the primary antibody, secondary antibody (goat anti-mouse IgG polyclonal conjugated with biotin) was used at a dilution 1/200 and visualized using an ABC system. The sections were counterstained using Mayer's Hematoxylin and DPX was used as mounting medium. Negative control sections were done by using phosphate buffer saline instead of primary antibody [33]. Area and area percent of fibrosis and TGF-β were measured as previously described [9] using Leica Qwin 500 image analyzer computer system.

2.7. Electron microscopy

Small specimens (1 mm³) of the dissected left kidneys were immediately fixed in glutaraldehyde (2.5%), buffered with 0.1 M cacodylate buffer, pH 7.4) at 4 °C for 3 h. This was followed by post fixation in 1.0% osmium tetroxide for 1 h and dehydration with ethanol gradient series. Using 1:1 propylene: epoxy resin, the specimens were infiltrated and left overnight, then left in epoxy resin for another night. The specimens were trimmed, sectioned at 1 μm (semitihin sections) and 60–70 nm (ultrathin section). The produced ultrathin sections were placed on copper grids for staining with lead citrate and uranyl acetate. The sections were then examined at different magnification by transmission electron microscope (JEOL 2100 electron microscope, Tokyo, Japan) at Electron Microscopy Unit, Faculty of Agriculture, Mansoura University, Al Mansoura, Egypt.

2.8. Statistical analysis

Data are expressed as Mean ± SE. Group’s statistical differences were evaluated using one-way ANOVA and results will be considered significant at p < .05. Once the differences exist among the means of different groups, post-hoc Bonferroni correction test will be used.

3. Results

3.1. Effect of nifuroxazide on body and kidney weights, kidney/body weight index and urine volume

As observed in Table 2, body weight of diabetic rats was significantly decreased by approximately 45% and kidney/body weight index was markedly increased by 1.45 fold in comparison to normal group. Daily administration of nifuroxazide for eight weeks resulted in marked increase in rats’ body weights and decrease with almost restoration of normal kidney/body weight index. Regarding urine volume, 8 weeks post diabetes induction urine volumes significantly increased by approximately 3.2 fold compared to normal control. Nifuroxazide treatment significantly reduced urine volume by approximately 49.4% compared to diabetic control.

3.2. Effect of nifuroxazide on kidney functions

Diabetic rats showed a significant elevation of creatinine, blood urea nitrogen (BUN) and albuminuria in serum by about 7.1, 11.4 and 4.9 folds, respectively compared to normal group. Oral nifuroxazide for eight weeks significantly reduced these parameters by about 67%, 68% and 40%, respectively compared to diabetic group, Fig. 1.

3.3. Effect of nifuroxazide administration on renal content of STAT3

As shown in Fig. 2, diabetes resulted in significant increase in STAT3 activation in renal tissue as represented by increased phospho-STAT3/STAT3 ratio compared to normal group (5.6 fold). Nifuroxazide treatment significantly reduced renal ratio of phospho-STAT3/STAT3 compared to diabetic group (by 50%).

3.4. Effect of nifuroxazide treatment on gene and protein levels of inflammatory cytokines; TNF-α and IL-18 in diabetic rat kidneys

Diabetic rats showed increase of mRNA levels of TNF-α and IL-18 in renal tissues by 9.2 and 10.9 fold, respectively, in comparison to normal group. Nifuroxazide treatment significantly reduced mRNA levels of TNF-α and IL-18 in renal tissue by 69.5% and 62.4%, respectively compared to diabetic control, Fig. 3A. Regarding protein levels, significantly higher levels of both TNF-α and IL-18 were detected in kidney homogenates of diabetic rats compared to normal control. Daily administration of nifuroxazide significantly decreased renal levels of both TNF-α and IL-18 compared to diabetic control Fig. 3B.

3.5. Effect of nifuroxazide treatment on renal histopathology

Histopathological findings in H&E stained renal specimen of diabetic kidneys were shrunken glomerular capillaries with widening of capsular space and infiltration of inflammatory cells. Moreover, examination revealed inflammatory cells infiltration around dilated tubules. Moreover, presence hyaline casts was observed inside the lumen of the tubule. These alterations were effectively decreased with nifuroxazide treatment. Fig. 4. Immunostaining for CD68 demonstrated extensive staining for CD68 in diabetic kidney sections. Nifuroxazide administration notably reduced the CD68 positive staining areas. Fig. 7.

3.6. Effect of nifuroxazide treatment on renal fibrosis

As shown in Fig. 8, diabetes induction for 8 weeks up-regulated collagen deposition as evidenced by increased Masson's trichrome staining in renal tissue of diabetic rats compared to control group especially around distal and convoluted tubules. Area % of fibrosis was significantly increased in specimens isolated from diabetic animals compared to these from control group. However, nifuroxazide treatment retracted fibrosis with marked reduction of fibrous tissue as indicated by reduced Masson's trichrome staining in renal specimens of nifuroxazide-treated diabetic rats.

Moreover, immunostaining for TGF-β in renal tissue of diabetic rats was much higher than that in control group. The immune reaction markedly increased around distal and convoluted tubules. In contrast, TGF-β immunostaining was markedly reduced in diabetes + nifuroxazide group compared to diabetic group. Fig. 9.
3.7. Electron microscopy study of renal specimen

Ultrathin sections of kidneys from diabetic control rats showed focal thickening of glomerular basement membrane, podocyte with irregular nuclear membrane, major processes and minor processes and sub-podocytic space widening. Vacuoles and lysosomes were found in cytoplasm of the proximal convoluted tubules. In addition, proximal convoluted tubules showed microvilli loss, disarranged basal mitochondria and elongated nucleus. These ultramicroscopic changes were markedly attenuated by nifuroxazide, where ultrathin sections of kidneys from treated group showed more or less normal appearance of glomeruli and tubules, Figs. 5 and 6.

4. Discussion

DN is considered a leading cause of end-stage renal disease and an independent risk factor for cardiovascular diseases [36]. The current therapeutic approaches for DN management are not sufficient. Therefore, exploring new therapies that target the primary mechanisms related to pathogenesis of DN is of great demand.

Nifuroxazide is a widely used anti diarrheal agent; however, it has been recently reported as potent STAT3 inhibitor in various diseases [42]; [43]. The present study sheds light on the reno-protective effect of nifuroxazide against diabetes-associated renal impairment in diabetic rats. Herein, nifuroxazide showed both STAT3 inhibitory, anti-inflammatory and anti-fibrotic effects. In addition, nifuroxazide restored glomerular filtration function and attenuated diabetes-induced renal histopathological and ultramicroscopic structural alterations.
A single intraperitoneal injection of STZ has been well documented to induce diabetes that mimics the human counterpart [20]. STZ induces necrosis of pancreatic beta cells diminishing its capacity to secrete insulin. In addition, histopathological and ultra-microscopic alterations related to DN have been reported to develop in the rat kidneys at around 8 weeks [7]. Herein, 8 weeks after STZ injection, both biochemical and histopathological characteristics of diabetes-induced renal injury were observed. In addition, the results observed in the current study revealed increased serum creatinine and BUN in diabetic control rats. These biochemical changes reflected the incidence of progressive renal damage [2].

Moreover, rats in diabetic group suffered significant albuminuria, which is widely acknowledged as the earliest marker of DN that develops as a result of glomerular filtration barrier damage [23]. Nifuroxazide treatment effectively reduced serum creatinine, BUN and albuminuria in diabetic rats suggesting an ameliorative effect of nifuroxazide against diabetes-induced renal injury with ability to improve glomerular filtration functions.

Amongst the characteristics of STZ-induced diabetes are the marked loss of body weight and polyuria. These symptoms are attributed to hyperglycemia, muscle tissue damage and loss of body proteins [20]. In the present study, diabetic rats control showed significant reduction in body weight and significant increase in urine volume. Nifuroxazide treatment markedly reduced body weight loss and urine output to almost normal levels, furthermore, suggesting an ameliorative effect of nifuroxazide on the course of hyperglycemia, diabetes mellitus symptoms and diabetic kidney progression.

Increased kidney weight in proportion to the body weight is acknowledged as an index of renal hypertrophy [20]. Various mechanisms have been reported to be implicated in development of renal

Fig. 3. Effect of nifuroxazide treatment on inflammatory cytokines in diabetic rat kidney. Oral administration of nifuroxazide (25 mg/kg/day, oral) reduced both mRNA levels of TNF-α and IL-18 (3A) and protein levels of TNF-α and IL-18 in diabetic renal tissues (3B). *** Significant difference compared to control group at p < .001. ** Significant difference compared to diabetic group at p < 0.01. # Significant difference compared to diabetic group at p < .05.
hypertrophy in diabetes such as alterations in the production of one or more of the local growth factors and increased accumulation of renal extracellular matrix components (ECM) leading to glomerular hypertrophy and nephromegaly [24]. Several studies reported increased kidney/body weight in STZ-induced diabetes experimental models [3] and [26]. In line, the results of the current study showed diabetes-induced imbalance in kidney/body weight index. The results revealed significant increase in kidney/body weight index in diabetic control rats, an effect which was significantly attenuated with nifuroxazide treatment.

Fig. 4. Effect of nifuroxazide treatment (25 mg/kg/day, oral) on kidney histological changes. Histopathological examination of rat renal tissue sections stained with H&E (400×). Control group showed glomerular capillaries (G) surrounded by Bowman’s capsule. Capillary space between the parietal and visceral layers (S) is seen. In between the renal corpuscles, there are multiple proximal convoluted tubules (P) and distal convoluted tubules (D). In addition, normal cortical labyrinths (C) and medullary rays (arrow heads) are shown. Diabetic group showed shrunken glomerular capillaries (AG) and widening of the capsular space (S). Some of inflammatory cells are seen (IC). The tubules showed infiltration of inflammatory cells, dilation and presence of hyaline cast inside the lumen of the tubule (arrow heads). Nifuroxazide group showed a normal corpuscle (S), few widened renal tubules (arrow heads) and few infiltration of inflammatory cells (arrow).

Fig. 5. Effect of nifuroxazide treatment (25 mg/kg/day, oral) on renal ultramicroscopic structure. (A) Control group showed glomerulus with normal basement membrane (crossed arrow), minor process (arrows) with foot plates (curved arrows). (B) STZ group showed focal thickening of glomerular basement membrane (crossed arrow), widening of subpodocytic space (asterisks), podocytes with irregular nuclear membrane (N), major processes (MA) and minor processes (MI). (C) In nifuroxazide group, there is more or less normal glomerular basement membrane (crossed arrow), more or less normal podocyte (Po) with subpodocytic space (*), (TEM ×3000).
Phosphorylation of STAT3 leads to dimerization and translocation to nucleus. Activation of this signaling pathway induces expression of multiple genes, including pro-inflammatory genes [11]. Among different members of JAK/STAT cascade, STAT3 is the most extensively studied one and have been reported to be implicated in progressive renal injury [32] and [5]. It is reported that signal transduction through STAT3 contributes to high glucose-induced overproduction of ECM proteins in glomerular mesangial cells [37]. Moreover, diverse approaches, including STAT3 gene knockdown, JAK2 inhibition markedly reduced diabetic-induced renal lesions [19]; [35]. Nevertheless, expression of members of STAT pathway markedly increased in renal biopsies from diabetic patient [4]. Collectively, these evidences suggest that activation of STAT3 protein by high glucose is a key player in progression of DN. Therefore, STAT3 signaling pathway represents an appealing drug target for management of DN. In this context, the current study reported STAT3 activation in renal tissues of diabetic control rats. This activation was significantly inhibited by nifuroxazide treatment; a well-documented STAT3 inhibitor. This inhibitory effect was associated with reversal of diabetes-induced renal pathological and ultramicroscopic alterations, including thickened glomerular basement membrane, shrunken glomerular capillaries, widening of capsular space, tubular dilation and podocyte injury. 

Fig. 6. Effect of nifuroxazide treatment (25 mg/kg/day, oral) on renal ultramicroscopic structure. (A) Control group: The cells of proximal convoluted tubule rest on a basement membrane (crossed arrow). They have rounded and euchromic nuclei (N), scattered mitochondria (M), lysosomes (L) and longitudinal oriented basal mitochondria (arrow). The luminal border shows numerous microvilli (MV). B) Diabetic group: The cells of proximal convoluted tubule show vacuoles (V) and lysosomes (L) in their cytoplasm and resting on a thickened basement membrane (crossed arrow) with loss in their cytoplasm on the apical surface (thick arrow), disarranged basal mitochondria (arrow) and elongated nucleus (arrow head). C) Nifuroxazide group: The cells of proximal convoluted tubules are more or less of normal appearance with euchromatic nuclei (N) scattered mitochondria (M), apical microvilli (MV) lysosomes (L) and more or less normal basement membrane (crossed arrow), (TEM ×3000).

Fig. 7. Effect of nifuroxazide (25 mg/kg/day, oral) on CD68 staining in diabetic renal tissues.

Inflammation is a hallmark of DN. current evidence implies that immune-inflammatory mechanisms are key players in pathogenesis and progression of DN with exuberant production of pro-inflammatory molecules [22]. On the other hand, targeting JAK/STAT pathway members have been claimed as promising strategy for treatment of both acute and chronic inflammatory conditions. Specifically, targeting STAT3 has been reported to suppress inflammation in various inflammatory diseases such as colitis [34], sepsis [45] and arthritis [41].

Moreover, it has been reported that STAT3 inhibition suppressed tissues’ macrophage infiltration with subsequent cytokines production [34]. Interestingly, inhibition of STAT3 activation abrogated the
Fig. 8. Effect of nifuroxazide (25 mg/kg/day, oral) on renal fibrosis. Histopathological examination of rat renal tissue sections stained with Masson’s Trichrome stain.

Fig. 9. Effect of nifuroxazide (25 mg/kg/day, oral) on TGF-β staining in diabetic rat renal tissues.
activation of macrophage and production of inflammatory cytokines in mouse model of renal fibrosis [32]. In agreement with these previous studies, the current results revealed that nifuroxazide markedly attenuated inflammatory events in diabetic renal tissues.

Nifuroxazide administration in the current study, reduced renal expression of TNF-α and IL-18 on both genetic and protein levels. Moreover, macrophage infiltration in diabetic renal tissue was significantly reduced as evidenced by reduced CD-68 immunostaining. Macrophage infiltration is a key event in the pathogenic mechanism of DN progression, either directly by interaction with renal cells or indirectly by enhancing the release of fibrotic and inflammatory mediators [39].

The inflammatory response that characterizes DN is attributed to excessive production of pro-inflammatory cytokines. Hence, various anti-inflammatory agents have been reported to target excessive production of inflammatory cytokines in DN [18]; [9]. Anti-inflammatory agents have been proposed to offer a protective effect against the development and the progression of renal injury-associated diabetes mellitus. Among these various inflammatory cytokines, TNF-α and IL-18 are strongly linked to incidence of DN with diverse actions that are potentially involved in diabetes-induced renal injury. Suppression of TNF-α and IL-18 has been previously reported to alleviate inflammation in diabetes-induced renal injury model [8]. Herein, nifuroxazide was also found to dampen expression of TNF-α and IL-18 and to inhibit macrophage infiltration, highlighting its anti-inflammatory role in diabetic rats.

Beside production of inflammatory cytokines, macrophages are rich source of TGF-β. Indeed, macrophage depression led to reduction of collagen expression in glomeruli of diabetic kidney [29]. On the other hand, TGF-β has been linked to development and progression of nephropathy in various models of diabetes [46]. TGF-β stimulates expression, accumulation and cross-linking of ECM proteins. In addition, it inhibited ECM-degrading enzymes leading to glomerulosclerosis and interstitial fibrosis. These studies suggested that TGF-β plays a critical role in ECM accumulation and fibrosis in DN. Our results indicated that nifuroxazide treatment reduced TGF-β expression and collagen accumulation in diabetic rat kidney.

Further investigations have to be done to indicate if nifuroxazide reno-protective effect observed in DN is mediated primarily via acting as STAT3 inhibitor or it has also direct action on high glucose-induced inflammatory and fibrotic events in renal tissue.

In conclusion, nifuroxazide, inhibited STAT3 activation, abrogated inflammatory cascade in rats’ diabetic kidneys and prevented the development of diabetes-induced nephropathy (Fig. 10). A recent study reported safety of nifuroxazide in tumor-bearing mice. In this study, Yang and coworkers observed no adverse effects including toxic death, skin ulceration and body weight loss, moreover, no blood system’s abnormality and no pathologic changes were observed in the heart, liver, spleen and kidney by microscopic examination compared with the vehicle-treatment group after nifuroxazide treatment [42]. Moreover, [21], reported safety of nifuroxazide in human. Indeed, nifuroxazide is marketed as an orally active safe medication with well-known and documented pharmacokinetics and safety profiles; therefore the current study provides new opportunity and therapeutic venue for prophylaxis against diabetes mellitus associated diabetic complications using an already approved safely marketed drug; nifuroxazide.

Given approval of nifuroxazide for human use, it can be presumed that nifuroxazide can be easily preceded for further clinical trials to evaluate its therapeutic efficacy against DN progression in humans. This approach will save time and money expended for discovery of new drug.

Fig. 10. Proposed mechanism for nifuroxazide protective effect against diabetes-induced renal injury.