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Protective effect of vanillin on diabetic nephropathy by decreasing advanced glycation end products in rats

Imad Eddin M. Zabad, Mohamed N. Amin, Mamdouh M. El-Shishtawy*

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

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ABSTRACT

Aims: Diabetic nephropathy (DN) is a common chronic microvascular complication of both types of diabetes mellitus, which leads to renal dysfunction and subsequent need of dialysis and organ transplantation. Advanced glycation end products (AGEs) are metabolic consequence of hyperglycemia and are main contributory factor in the DN pathogenesis through mediating establishment of oxidative status and chronic inflammatory milieu. This study aimed to explore the impact of vanillin on preventing the progression of DN.

Main methods: Experimental DN model was established in rats utilizing streptozotocin. Serum concentration of AGEs and Interleukin-6 (IL-6) and transforming growth factor β1 (TGFβ1) levels in kidney homogenate were assessed using ELISA technique. Also, we evaluated the expression of nuclear factor kappa B (NF-κB) using immunohistochemistry.

Key findings: Treatment with vanillin for 8 weeks significantly ameliorated DN. Vanillin significantly decreased hyperglycemia and improved kidney function reflected by decreased serum levels of blood urea nitrogen, creatinine, and decreased proteinuria. Also, vanillin significantly decreased malondialdehyde content and elevated superoxide dismutase activity in renal tissues. Moreover, vanillin decreased renal expression of NF-κB and renal concentrations of IL-6, TGFβ1 and collagen. In addition, vanillin significantly decreased serum AGEs concentration. Also, vanillin attenuated histological abnormalities in kidney architecture.

Significance: Vanillin, which is a cheap and abundant natural product, exhibited anti-AGEs, antioxidant, anti-inflammatory and anti-fibrotic activities. These activities might be helpful and potent mechanisms in preventing the progression of DN.

1. Introduction

Diabetic nephropathy (DN) is a main and common complication of both types of diabetes mellitus (DM). DN occurs in about 40% of diabetic population which is expected to rise to 629 million diabetic patients by 2045 [1,2]. DN is characterized clinically by albuminuria and pathologically by mesangial expansion, glomerular basement membrane thickening, interstitial fibrosis and glomerular sclerosis [3]. Pathogenesis of DN is heterogeneous where chronic hyperglycemia leads to metabolic and hemodynamic deteriorations, such as advanced glycation end products (AGEs) formation, production of reactive oxygen species (ROS), initiation of the polyol pathway, stimulation of protein kinase C and the renin–angiotensin system, which in turn establish chronic inflammatory cascades culminating in pathological characteristics of DN [4].

AGEs are the result of Maillard's reaction which is non-enzymatic reaction of carbonyl group of reducing sugars with amino groups of proteins, lipids and nucleic acids. The formation of AGEs is accelerated under hyperglycemic conditions, making them a major contributory factor in the pathogenesis of DN [5]. AGEs cause kidney damage by crosslinking of intracellular and extracellular proteins resulting in their conformational and functional changes [6]. AGEs also increase the generation of ROS which are involved in inflammatory response [7]. Moreover, AGEs participate in establishment of chronic inflammatory milieu by binding to receptor for AGEs (RAGE) leading to initiation of nuclear factor kappa B (NF-κB) signaling pathway, which is a master signaling pathway involved in the generation of pro-inflammatory cytokines, chemokines and fibrogenic mediators [8,9]. Hence, AGEs are attractive targets to halt the DN progression.

IL-6 is a member of glycoprotein 130-dependent cytokine family. IL-6 participates in establishment and stabilization of chronic inflammatory environment [10]. Also, IL-6 contributes to renal endothelial dysfunction via the action of angiotensin II [11]. Moreover, IL-6 triggers renal tubular cells to release collagen type 1 resulting in

* Corresponding author at: Biochemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.
E-mail address: mshisht@mans.edu.eg (M.M. El-Shishtawy).

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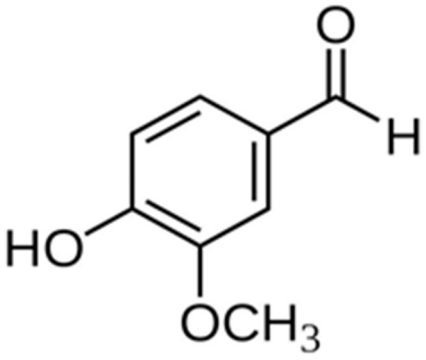


Fig. 1. Structure of vanillin.

tubular atrophy and interstitial fibrosis [12].

Transforming growth factor β1 (TGFβ1) is responsible for the establishment of fibrosis in many organs via Smad-dependent and Smad-independent signaling cascades. Hyperglycemia and oxidative status are important activators of TGFβ1 [13]. Suppression of TGFβ1 halts DN progression [14].

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a naturally occurring phenolic compound (Fig. 1). Vanillin is the main constituent of *Vanilla planifolia* and is a commonly utilized flavoring agent in food, pharmaceutical and cosmetic industries [15]. Vanillin has anti-AGEs activity evidenced by its role in protection against non-enzymatic glycation in human insulin [16]. Also, vanillin is a potent antioxidant compound [17]. Vanillin also has several renoprotective effects including mitigation of cisplatin-induced, potassium bromate-induced, carbon tetrachloride-induced and methotrexate-induced nephrotoxicities [18–21]. Moreover, vanillin ameliorates inflammatory response by reducing level of IL-6 through suppression of NF-κB activation in rodent models of lipopolysaccharides (LPS)-induced mastitis and LPS-induced Parkinson's disease [22,23].

The impact of vanillin on amelioration and prevention of DN progression and its mechanisms of action have to be investigated yet. Hence, this study was carried out to explore the protective effects of vanillin on the DN progression in rats.

2. Materials and methods

2.1. Experimental animals

Fifty adult male albino Sprague Dawley rats (200 ± 20 g) were obtained from "Egyptian Organization for Biological Products and Vaccines, VACSERA" (Giza, Egypt) and were kept at the standard nutritional and environmental conditions throughout the study period. Rats were allowed to acclimatize for one week before starting experiments. All rats were kept at a controlled temperature room (25 °C ± 2) with a constant 12-hour light/dark cycle. Rats were provided with standard food and water ad libitum. The experimental work was designed to meet the ethical principles and guidelines approved by the "Research Ethics Committee" of Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

2.2. Experimental design

Rats were divided into three groups; normal, DN and vanillin groups. Normal group (n = 15), were given orally the drug vehicle 0.5% (w/v) carboxy methyl cellulose (CMC), once daily. For DN group (n = 20), diabetes was established by streptozotocin (STZ), single intraperitoneal injection (i.p.) of 45 mg/kg (Sigma Aldrich Chemicals Co., St. Louis, MO., USA, Cat. No. S0130) dissolved in freshly prepared chilled citrate buffer (pH 4.5) after overnight fasting [24] then rats were given orally the vehicle 0.5% (w/v) CMC once daily. While vanillin group (n = 15) were treated as DN rats but given orally vanillin, 100 mg/kg (Sigma Aldrich Chemicals Co., St. Louis, MO., USA, Cat. No. V1104), suspended in 0.5% (w/v) CMC once daily [18,25–28]. Two days after STZ injection, the induction of DM was confirmed by the measurement of blood glucose level (from tail vein) and rats with blood glucose levels above 250 mg/dl were considered diabetic and DN was confirmed by histopathological changes (three rats). Vanillin administration was started 48 h after injection of STZ and continued for 8 weeks.

2.3. Sample collection and processing

At the end of the eighth week, 24-h urine samples were collected from all rats using metabolic cages. They were then centrifuged at 2000 rpm at 4 °C for 1 min and were used immediately for the evaluation of urine volume/day, urine creatinine and urine protein concentration. After overnight fasting, rats were weighed (body weight) and blood samples were collected from the retro-orbital venous plexus under light ether anesthesia. Serum was then separated by centrifugation at 3000 rpm at 4 °C for 5 min and was stored at −20 °C. After decapitation of rats, kidneys were rapidly isolated, rinsed in chilled saline and weighed to obtain kidney/body weight index. Left kidney was preserved at −80 °C for tissue homogenate preparation and right kidney was slit then fixed in 10% buffered formalin for further histopathological and immunohistochemical investigations.

Kidney homogenate was prepared by homogenization of kidney tissue (100 mg) in 1 ml chilled 0.5% potassium chloride followed by sonication for 60 s. The homogenate was then centrifuged for 10 min at 3000 rpm and 4 °C, then supernatant was separated and preserved for further biochemical analysis [29].

2.4. Biochemical analysis

Fasting blood glucose [30], blood urea nitrogen (BUN) [31] and serum creatinine [32] concentrations were estimated using commercially available assay kits (Biodiagnostic, Giza, Egypt) in accordance to instructions of the manufacturer.

Urinary protein [33] and creatinine [32] levels were assessed in 24-h urine samples using commercially available assay kits (Spinreact Co., Spain) and (Biodiagnostic, Giza, Egypt), respectively) in accordance to instructions of the manufacturers. Creatinine clearance was then calculated according to Larsen [34].

Superoxide dismutase (SOD) activity [35] and malondialdehyde (MDA) [36] content were evaluated in kidney homogenate using commercially available assay kits (Biodiagnostic, Giza, Egypt)) in accordance to instructions of the manufacturer.

Kidney hydroxyproline content was quantitatively assessed using kidney tissue for quantification of kidney collagen content [37], as follows: 100 mg kidney tissue was incubated in 6 ml 5% KOH at 37 °C for 24 h. Then, it was hydrolyzed with 10 N NaOH and incubated with chloramine T reagent at 25 °C for 3 h. Finally, Ehrlich's reagent was added to the reaction components followed by incubation at 65 °C in water bath for 20 min. The absorbance of the resultant purple complex was read at 550 nm. Kidney collagen content was deduced by multiplication of kidney hydroxyproline value by 7.46, where hydroxyproline accounts for 13.5% of collagen structure [38,39].

Concentrations of IL-6 and TGFβ1 in kidney homogenate were assessed using commercial enzyme linked immunosorbent assay (ELISA) kits (Affymetrix eBioscience, San Diego, California, USA, Cat. No. BMS625 and BMS623-3), respectively, and serum AGEs concentration was, also, assessed by ELISA kits (cloud-clone Co., Houston, USA, Cat. No. CEB353Ra), according to instructions of the manufacturer.

2.5. Histopathological and immunohistochemical examination

After fixation of right kidney in 10% buffered formalin, they were

processed by dehydration using ethanol and subsequent clearing in xylene, and were finally fixed in paraffin wax. Three sets of 5-μm thick sections were sliced. The first set was stained with hematoxylin and eosin (H&E). The second set was stained with Masson's Trichrome stain to evaluate the severity of fibrosis, which was assessed semi-quantitatively and scored from 0 to 3, as follows: 0 = normal; 1 = mild fibrosis; 2 = moderate fibrosis; 3 = severe fibrosis [40].

The third set was deparaffinized in xylene, rehydrated using graded ethanol, then quenched in 0.3% H_2O_2/methanol solution for 10 min at room temperature. Slides were microwaved in 0.01 mmol/l citrate buffer (pH 6.0) for 20 min, then were overnight incubated with primary monoclonal mouse anti-rat antibody of NF-κB p65 (Santa Cruz Biotechnology, USA; 1:100 dilution) at 4 °C. Slides were rinsed with phosphate buffered solution (PBS) three times, incubated with biotinylated anti-mouse IgG secondary antibodies at 37 °C for 30 min, rinsed with PBS and incubated with avidin-biotin-peroxidase complex. Slides were visualized with 3,3′-Diaminobenzidine. Lastly, slides were counterstained with hematoxylin and examined using light microscope. Normal rat serum was used instead of the primary antibody for preparation of negative control procedure. Immunoreaction of NF-κB was assessed semi-quantitatively and scored from 0 to 4 as described previously [41]. Briefly, a score of 0 = absent staining; 1 = weak staining with focal distribution; 2 = moderate staining with focal distribution; 3 = strong staining with focal distribution or weak and diffuse; and 4 = strong and diffuse. All sets of slides were examined in blinded manner.

2.6. Statistical analysis

The results are showed as mean ± standard error of the mean (SEM). Analysis of parametric data was executed using one way analysis of variance (ANOVA) and Tukey-Kramer's post hoc test, whereas, analysis of non-parametric data, which included scores of histopathology and immunohistochemistry, was executed using Kruskal-Wallis test and Dunn's multiple comparison post-hoc test. GraphPad Prism V 6.01 (GraphPad Software Inc., San Diego, CA, USA) was utilized to carry out statistical analysis and graphing. In all tests, statistical significance is considered as: significant for $P < .05$, very significant for $P < .01$ and extremely significant for $P < .001$.

3. Results

3.1. Vanillin decreases fasting blood glucose level in diabetic rats

Rats of DN group exhibited significant elevation in fasting blood glucose level in comparison with normal group ($P < .001$). Vanillin administration significantly decreased fasting blood glucose level in comparison with DN group ($P < .01$), but fasting blood glucose level was still significantly higher than that of normal group ($P < .001$), (Table 1 & Fig. 2, A).

3.2. Vanillin improves kidney functions in diabetic rats

DN group revealed significant elevation of serum creatinine and BUN levels in comparison with normal group ($P < .001$). Vanillin administration reduced significantly both serum creatinine and BUN in comparison with DN group ($P < .001$), but serum creatinine level still higher than that of normal group ($P < .001$), (Table 1 & Fig. 2, B).

DN group revealed significant decrease in creatinine clearance with significant increase in proteinuria concentration in comparison with normal group ($p < .001$). Vanillin administration significantly increased creatinine clearance and decreased proteinuria concentration in comparison with DN group ($P < .001$), but they were still significantly different from the normal group (P < .001), (Table 1 & Fig. 2, C).

Kidney/body weight index was significantly increased in DN group in comparison with normal group ($P < .001$). Vanillin administration significantly reduced kidney/body weight index in comparison with DN group (P < .001), but it was still significantly different from that of normal group ($P < .01$), (Table 1 & Fig. 2, D).

3.3. Vanillin has antioxidant and anti-inflammatory effects on diabetic nephropathy

DN group revealed significant reduction in SOD activity with concomitant elevation in MDA content in renal homogenate in comparison with normal group ($P < .001$). Vanillin administration significantly increased SOD activity and decreased MDA content in comparison with DN group. SOD activity was still significantly lower than that of normal group (Table 2 & Fig. 3, A).

DN group revealed significant increase in hydroxyproline and collagen contents, in kidney homogenate, in comparison with normal group ($P < .001$). Vanillin administration significantly decreased hydroxyproline and collagen contents compared to DN group (P < .001). They were still significantly different from normal group ($P < .01$), (Table 2 & Fig. 3, B).

In kidney homogenate, DN group revealed significant increase in IL-6 and TGFβ1 contents, as well as, serum AGEs concentration was significantly elevated, in comparison to normal group (P < .001). Vanillin administration led to significant reduction in IL-6, TGFβ1 contents and AGEs concentration in comparison to DN group (P < .01 & P < .001), respectively. IL-6 concentration was still significantly different from normal group (P < .01), (Table 2 & Fig. 3, C).

3.4. Vanillin ameliorated histological abnormalities and immunohistochemical investigation of the kidney

Kidney sections from normal group revealed normal architecture of glomeruli and tubules, using H&E (x400, bar = 50) stain, (Fig. 4, A).

Table 1
Effect of vanillin on fasting blood glucose level, parameters of kidney function and kidney/body weight index.

Groups	Normal	DN	Vanillin
Fasting blood glucose level (mg/dl)	94.70 ± 2.96	419 ± 24.13***	289.10 ± 32.04$^{\#\#,***}$
Serum creatinine (mg/dl)	0.51 ± 0.014	1.03 ± 0.015***	0.71 ± 0.023$^{***,\#\#\#}$
BUN (mg/dl)	18.59 ± 1.12	55.9 ± 3.87***	23.77 ± 1.85$^{\#\#\#}$
Creatinine clearance (ml/min)	0.0687 ± 0.003	0.018 ± 0.0012***	0.0482 ± 0.0035$^{***,\#\#\#}$
Proteinuria (mg/day)	65.89 ± 5.56	174.1 ± 3.53***	106.7 ± 6.58$^{***,\#\#\#}$
Kidney/body weight index (X10^3)	3.61 ± 0.11	6.7 ± 0.39***	5.03 ± 0.24$^{\#\#\#,***}$

DN, diabetic nephropathy; BUN, blood urea nitrogen. Data are mean ± SEM; $n = 14$. Groups were compared using ANOVA & Tukey-Kramer's post-hoc test. Probability values were set as:

** p < .01 vs. normal group.
*** $p < .001$ vs. normal group.
$^{\#\#}$ p < .01 vs. DN group.
$^{\#\#\#}$ p < .001 vs. DN group.

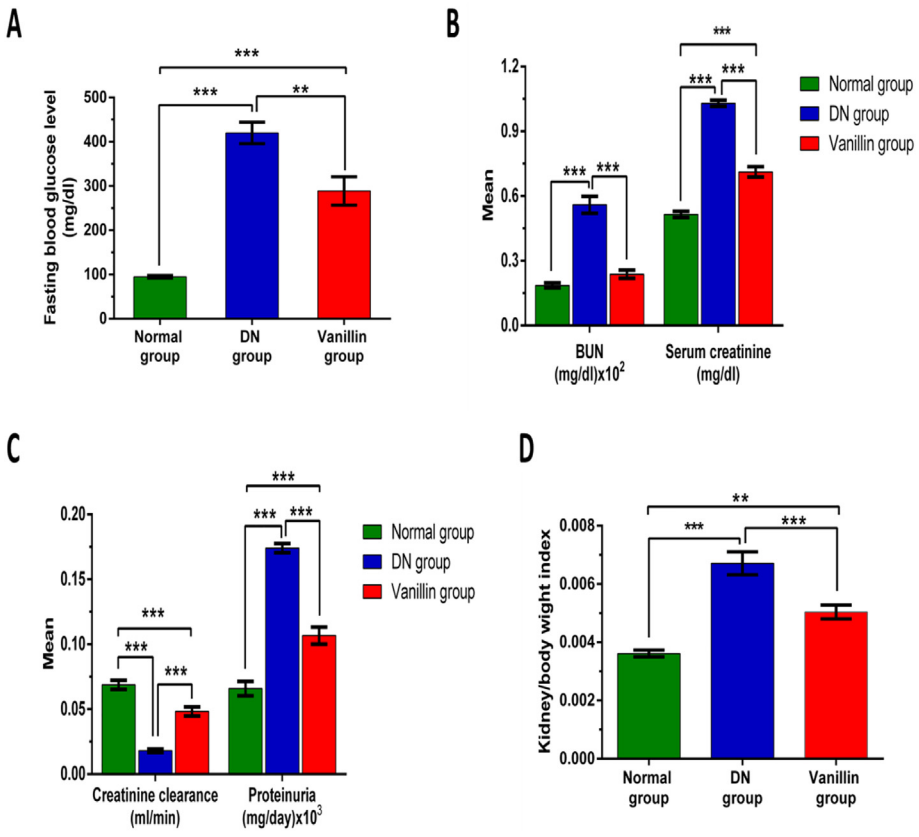


Fig. 2. Effect of vanillin on biochemical parameters and kidney/body weight index.
DN, diabetic nephropathy; Data as mean ± SEM, $n = 14$. A: Fasting blood glucose level (mg/dl); B: Mean values for serum creatinine (mg/dl) and blood urea nitrogen {BUN (mg/dl) $\times 10^2$}; C: Kidney/body weight index; D: Mean values for creatinine clearance (ml/min) and proteinuria {(mg/day) $X10^3$}. Groups were compared using ANOVA & Tukey-Kramer's post-hoc test; probability values were set as: very significant for $P < .01$ (**) & extremely significant for $P < .001$ (***).

DN group showed greatly swollen Bowman's capsule eosinophilic proteinaceous material (black arrows) with marked tubular dilation and degeneration (yellow arrows), tubular cast formation (red arrows) and tubular necrosis (black arrows). Vanillin group showed moderate tubular dilation (yellow arrows) with slightly swollen Bowman's capsule eosinophilic proteinaceous material (black arrows).

Also, kidney sections from normal group showed no fibrosis using Masson trichrome stain, (×100, bar = 100). DN group showed moderate blue stained perivascular collagen deposition (yellow arrows). Vanillin group showed mild collagen deposition (yellow arrows), (Fig. 4, B).

As shown in Fig. 5, Kidney sections from normal group showed weak positive staining of NF-κB. DN group showed strong positive staining (sometimes focal or diffuse). Vanillin group showed weak positive staining of NF-κB (x100, bar = 100).

4. Discussion

DN is a multifactorial serious complication of DM, which is mediated by metabolic and hemodynamic deteriorations and oxidative status culminating in inflammation and consequent fibrosis, thereby resulting in renal dysfunction. In this study, experimental DN was established in STZ-induced diabetic rat model.

We selected vanillin, which is a natural, abundant and cheap product, to avoid the adverse effects, invasiveness and running cost of daily treatment with insulin injections and other hypoglycemic drugs.

Hyperglycemia is the master initiator of progression of diabetic complications via induction of metabolic and hemodynamic deteriorations, vanillin exhibited significant hypoglycemic effect. Although, effect of vanillin on modulation of hyperglycemia has not been studied yet, we propose that the hypoglycemic effect of vanillin, at least in part,

Table 2
Effect of vanillin on serum advanced glycation end products and kidney contents of oxidative status parameters, interleukin-6, transforming growth factor β1, hydroxyproline and collagen.

Groups	Normal	DN	Vanillin
Serum AGEs concentration (ng/ml)	0.42 ± 0.013	0.73 ± 0.017***	0.47 ± 0.2###
Renal SOD activity (U/mg)	117.6 ± 4.45	46.7 ± 1.49***	92.42 ± 3.02***,###
Renal MDA content (nmol/g)	58.72 ± 3.76	124.5 ± 3.35***	71.54 ± 4.68###
Renal IL-6 concentration (ng/g)	6.55 ± 0.35	10.85 ± 0.32***	8.12 ± 0.39###,**
Renal TGFβ1 concentration (ng/g)	124.1 ± 10.47	217.8 ± 12.00***	156.5 ± 12.02##
Renal hydroxyproline content (μg/g)	35.51 ± 2.59	174.2 ± 8.55***	75.84 ± 5.13###,**
Renal collagen content (μg/g)	264.9 ± 19.38	1300 ± 63.76***	565.8 ± 38.28###,**

DN, diabetic nephropathy; AGEs, advanced glycation end products; SOD, superoxide dismutase; MDA, malondialdehyde; IL-6, interleukin-6; TGFβ1, transforming growth factor β1; Data are mean ± SEM; n = 14. Groups were compared using ANOVA & Tukey-Kramer's post-hoc test.
Probability values were set as:
** $p < .01$ vs. normal group.
*** $p < .001$ vs. normal group.
$p < .01$ vs. DN group.
$p < .001$ vs. DN group.

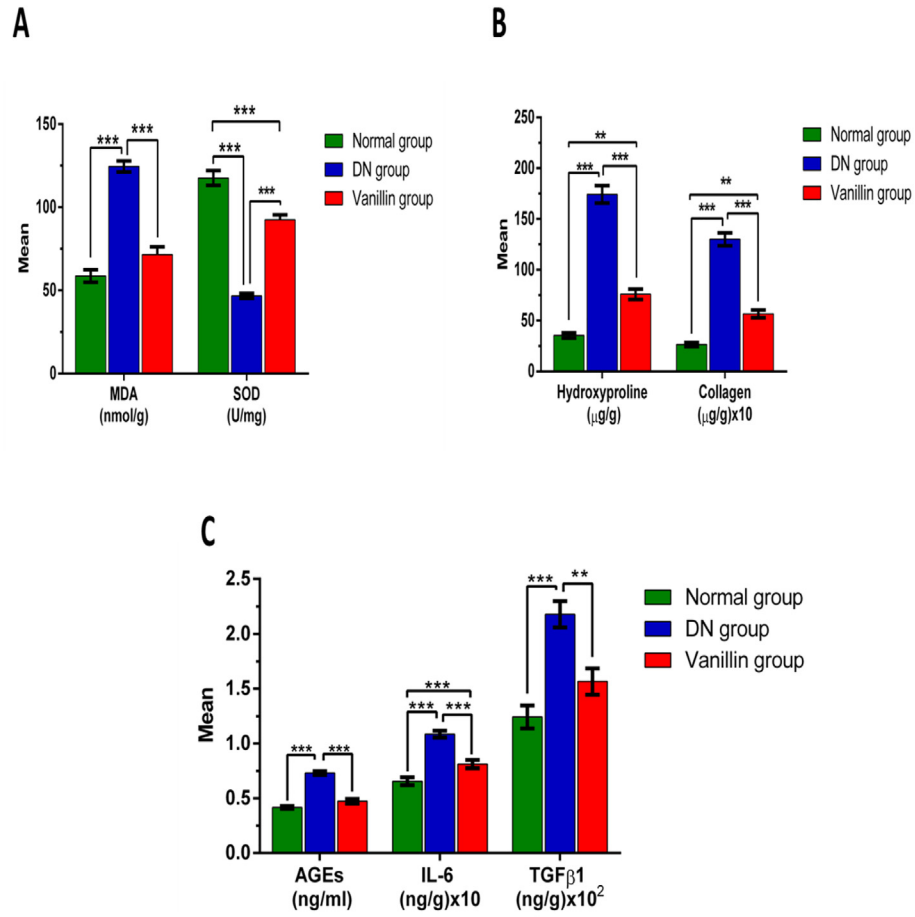
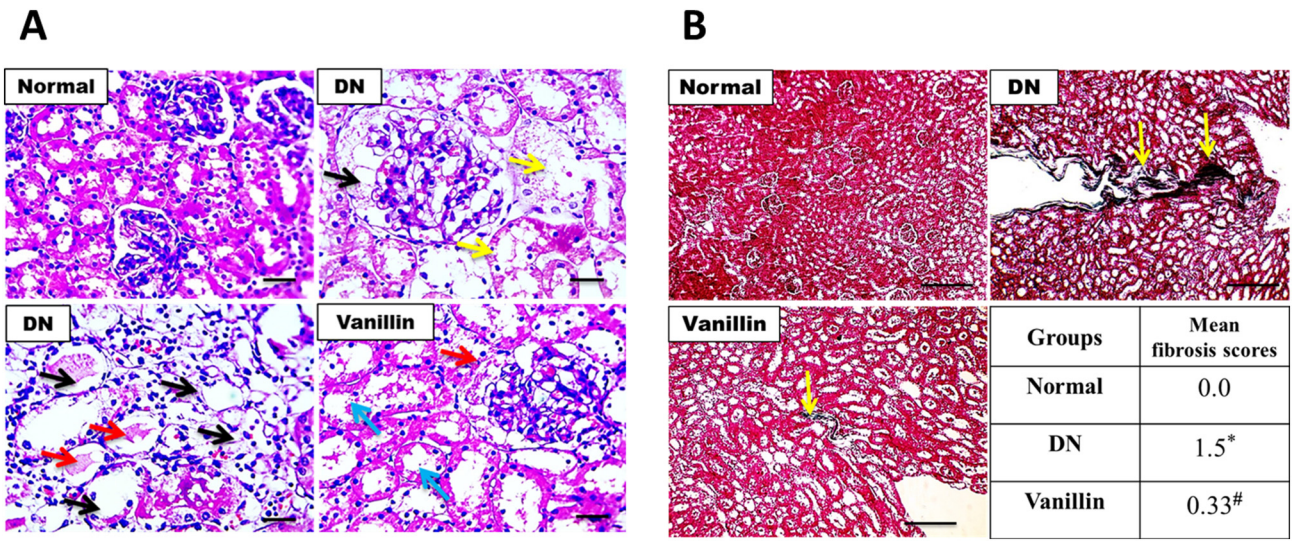


Fig. 3. Effect of vanillin on advanced glycation end-products, interleukin-6, transforming growth factor β1 and Oxidative status.
DN, diabetic nephropathy. Data as mean ± SEM, n = 14. A: Mean values for renal malondialdehyde {MDA (nmol/g)} and renal superoxide dismutase {SOD (U/mg)}; B: Mean values for hydroxyproline and collagen in kidney(μg/g); C: Mean values for advanced glycation end-products in serum {AGEs (ng/ml)}, interleukin-6 in kidney {IL-6 (ng/g) ×10} and transforming growth factor β1{TGFβ1 (ng/g) $\times 10^2$}. Groups were compared using ANOVA & Tukey-Kramer's post-hoc test; probability values were set as: very significant for P < .01 (**) & extremely significant for P < .001 (***).

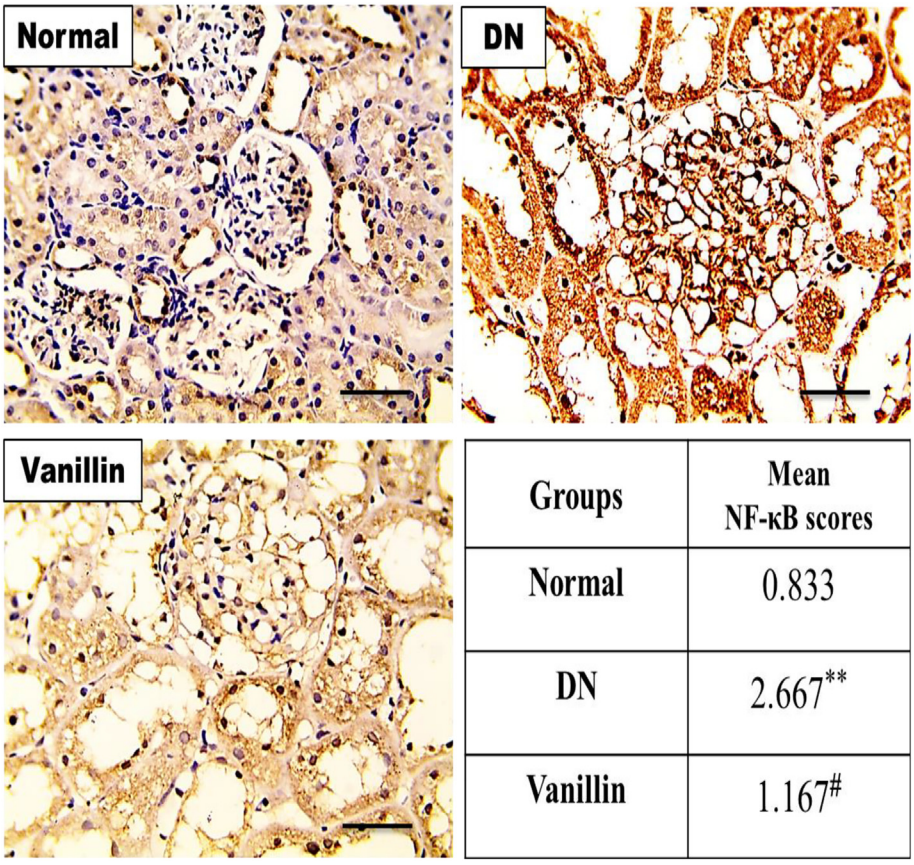
is mediated by anti-AGEs effect of vanillin, which protects insulin from AGEs [16].

Regarding the key biochemical parameters for the development of DN, vanillin produced a significant decrease in serum creatinine, BUN and proteinuria with significant raise in creatinine clearance in vanillin group as compared to DN group. From histopathological perspective, our findings are consistent with the biochemical findings. Vanillin significantly ameliorated glomerular impairment and tubular degeneration. In addition, vanillin significantly reduced renal fibrosis confirmed with less deposition of collagen in Masson's trichrome stained renal sections. Moreover, vanillin significantly decreased the kidney/body weight index as compared to DN group reflecting its anti-hypertrophic effect. These findings are in agreement with the previously documented renoprotective effect of vanillin in cisplatin-



Groups	Mean fibrosis scores
Normal	0.0
DN	1.5*
Vanillin	0.33#

Fig. 4. Effect of vanillin on histopathological examination of the kidney.
DN, diabetic nephropathy. A: Histopathological investigation of the kidney using H&E stain (×400, bar = 50), *n* = 6; B: Histopathological investigation of the kidney and fibrosis scoring using Masson's trichrome stain (×100, bar = 100), *n* = 6.



Groups	Mean NF-κB scores
Normal	0.833
DN	2.667**
Vanillin	1.167#

Fig. 5. Effect of vanillin on nuclear factor kappa B in the kidney of rats as evaluated by immunohistochemistry.
DN, diabetic nephropathy. NF-κB expression in the kidney using immunohistochemistry stain (×100, bar = 100), n = 6. Groups were compared using Kruskal-Wallis test followed by Dunn's multiple comparison post-hoc test; probability values were set as: $^{**}p < .01$, $\#p < .05$, versus normal control and DN groups, respectively.

induced renal injury [18]. Thus, these observations confirm that vanillin protects against DN or halts its progression.

AGEs are a type of post-translational modification of proteins resulting in loss of their functions. The increased formation of AGEs induced by hyperglycemia is a main contributory factor in the DN pathogenesis. AGEs participate in establishment of intracellular oxidative status and chronic inflammatory environment via interaction with RAGE which mainly initiate NF-kB cascade culminating in production of proinflammatory cytokines and fibrogenic mediators [5]. In our study, vanillin administration significantly decreased AGEs concentration in serum of diabetic rats in comparison to DN group. This finding is in agreement with the previously documented anti-AGEs effect of vanillin in protection against AGEs-induced amyloid aggregation in insulin [16]. This result proposes that vanillin is beneficial for the prevention of DN by reducing AGEs formation.

Binding of AGEs with RAGE stimulates the generation of reactive oxygen species (ROS). AGEs also induce generation of ROS via persistent respiratory chain protein glycation, thus contributing to DN. ROS have several cytotoxic effects, where they can initiate numerous inflammatory and fibrogenic signaling cascades. Therefore, antioxidants should be considered as a treatment strategy for AGEs-RAGE-related disorders such as DN [42]. SOD activity and lipid peroxidation in term of MDA content are two key indices in the oxidative balance system. In our study, vanillin administration led to significant increase in SOD activity with significant decrease in MDA content in renal homogenate as compared to DN group, which might partially explain its renoprotective mechanism. These findings are in agreement with the previously documented antioxidant effect of vanillin in rotenone-induced Parkinson's disease in rats [17].

AGEs can activate NF-κB signaling cascade either directly via binding to RAGEs or indirectly via generation of ROS. NF-kB is a specific transcription factor that plays a major role in production of proinflammatory cytokines and fibrogenic mediators. Typically, NF-κB presents in the cytoplasm bound to inhibitory κB (IkB), which dissociates from NF-κB upon activation by stimuli such as AGEs, then NF-κB translocates to the nucleus and induces overexpression of proinflammatory cytokines, such as IL-6 [9,43]. In our study, vanillin administration significantly decreased histological scores for NF-κB expression by immuno-histochemistry as compared to DN group, which may be responsible for its anti-inflammatory effect and delaying DN development. This finding is in agreement with the previously documented anti-inflammatory effect of vanillin on the central nervous system [23].

Inflammation is a key feature of DN. IL-6 is a member of glycoprotein130-dependent cytokine family. IL-6 is responsible for stabilization of chronic inflammatory milieu throughout the course of DN. IL-6 promotes differentiation of CD4+ T cells into pro-inflammatory T helper 17 cells and enhances their proliferation and their resistance to death through initiation of signal transducer and activator of transcription factor 3 (STAT3) signaling cascade. IL-6 also promotes differentiation of renal monocytes into macrophages which are essential contributors in establishment of inflammatory environment and production of fibrogenic mediators [29]. In our study, vanillin administration significantly decreased IL-6 level in renal homogenate in comparison to DN control. This finding is in agreement with the previously documented anti-inflammatory effect of vanillin in LPS-induced mastitis in mice [22].

Renal fibrosis is defined as deposition of abnormal quantities of extracellular matrix (ECM) components, primarily collagen, in renal tissues resulting in degeneration of kidney architecture. TGFβ1 induces renal fibrosis through both direct generation of ECM components and stimulation of trans-differentiation of renal cells into the main ECM producing cells, myofibroblasts, via both epithelial-mesenchymal transition and endothelial-mesenchymal transition [13]. In our study, vanillin administration significantly decreased renal TGFβ1 level as compared to DN group. This result is supported by kidney collagen

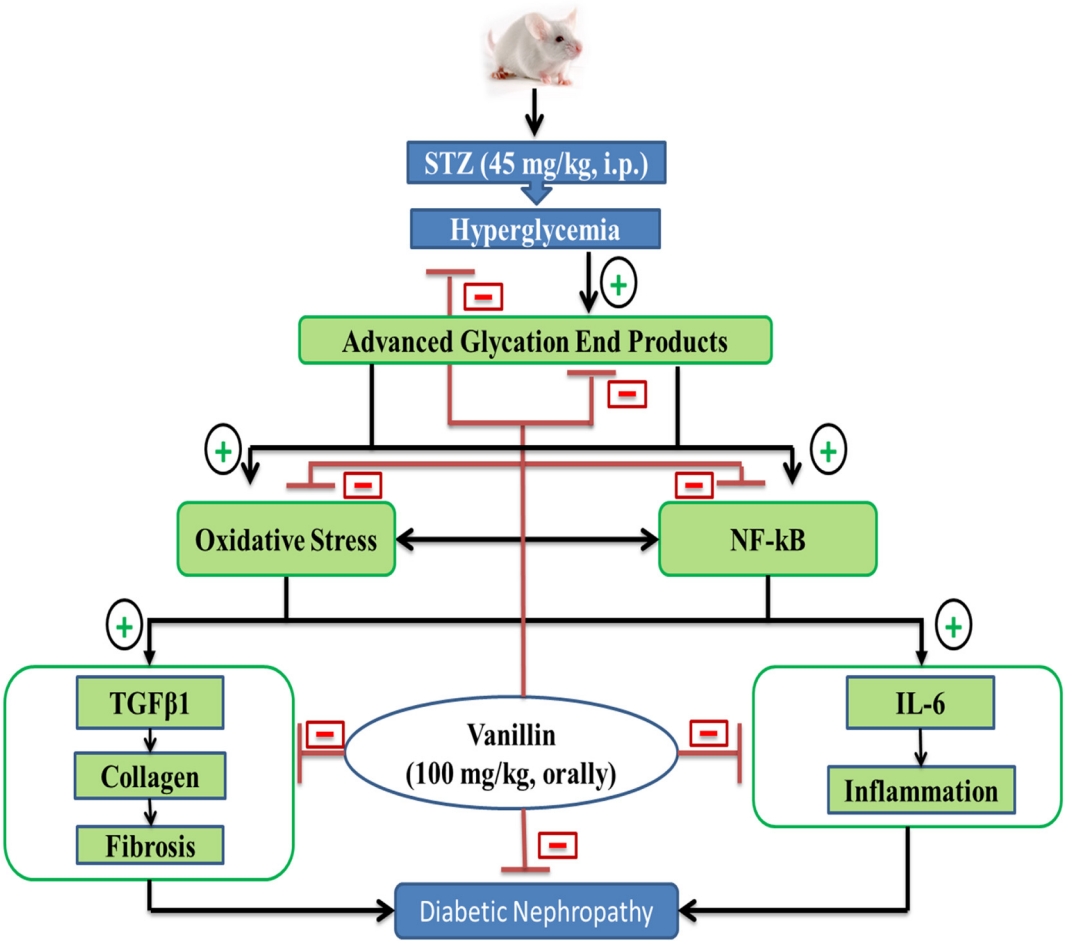


Fig. 6. Conceptual frame of the study results.
STZ, streptozotocin; NF-κB, nuclear factor kappa B; IL-6, interleukin-6; TGFβ1, transforming growth factor β1, ⊕; increase, ⊖; decrease.

content, which was also significantly decreased by vanillin administration as compared to DN group.

The findings of our experiment revealed that vanillin functionally, biochemically, and histopathologically halted the progression of DN, as shown in Fig. 6.

In conclusion, treatment with vanillin, a cheap and abundant natural product, exhibited a potent reno-protective action against experimental DN at dose of 100 mg/kg. Vanillin administration led to reduction in hyperglycemia-induced formation of AGEs and attenuated the consequent events including oxidative status (increase SOD and decrease MDA), inflammatory cascade (NF-κB and IL-6) and fibrosis (TGFβ1 and collagen). Our study suggests that vanillin administration in the early stages of DN needs to be clinically investigated for future utilization of vanillin in such conditions, as shown in Fig. 6.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.
Author disclosure statement
No competing financial interests exist.

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