Title:

Upregulation of Nrf2/HO-1 signaling and farnesoid X receptor and attenuation of oxidative stress and inflammation mediate the protective effect of sitagliptin against diabetic nephropathy in rats

Authors and affiliations:

Iman H. Hasan[1]*, Qamraa H. Alqahtani[1], Wedad S. Sarawi[1], Tahani A. ALMatrafi[2], Juman Al-Saab[1], Emad H.M. Hassanein[3], Noha A. Ahmed[4], Mohamed El Mohtadi[5], Mohamed Anany[6], Ayman M. Mahmoud[7]*

[1]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh 11495, Saudi Arabia.

[2]Department of Anatomy, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia

[3]Department of Pharmacology & Toxicology, Faculty of Pharmacy, Al-Azhar University-Assiut Branch, Assiut 71524, Egypt.

[4]Molecular Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef 62514, Egypt.

[5]Department of Biology, Edge Hill University, Ormskirk L39 4QP, UK.

[6]Division of Molecular Internal Medicine, Department of Internal Medicine II, University Hospital Würzburg, Würzburg 97080, Germany.

[7]Department of Life Sciences, Faculty of Science & Engineering, Manchester Metropolitan University, Manchester M1 5GD, UK.

***Corresponding authors:**

Iman H. Hasan
Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh 11495, Saudi Arabia. E-mail: Ihasan@ksu.edu.sa

Ayman M. Mahmoud
Department of Life Sciences, Faculty of Science & Engineering, Manchester Metropolitan University, Manchester M1 5GD, UK. E-mail: a.mahmoud@mmu.ac.uk

Abstract:

Diabetic nephropathy (DN) is a kidney complication associated with diabetes that can lead to renal failure. The dipeptidyl peptidase IV inhibitor sitagliptin (SITA) has shown potential therapeutic benefits for DN. This study investigated the effect of SITA on DN, focusing on its modulation of the farnesoid X receptor (FXR) and nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase 1 (HO-1) signaling and its suppressive efficacy on inflammation and oxidative stress. Thirty-two male rats were divided into four groups: control, SITA-treated, diabetic, and SITA-treated diabetic rats. SITA was administered orally for 8 weeks to diabetic rats induced with streptozotocin, after which samples were collected for analysis. The results indicate that SITA effectively reduced hyperglycemia, weight loss, and kidney injury and fibrosis. SITA also decreased oxidative stress, inflammatory markers, and apoptosis, as demonstrated by reductions in kidney malondialdehyde (MDA), myeloperoxidase, nitric oxide (NO), nuclear factor-kappaB (NF-κB), interleukin (IL)-1β, inducible NO synthase (iNOS), tumor necrosis factor (TNF)-α, Bcl-2-associated X protein (Bax), and caspase-3. These protective effects were associated with Kelch-like ECH-associated protein (Keap)-1 inhibition, increased levels of Nrf2 and FXR, and enhanced antioxidant activity as well as Bcl-2 upregulation. *In silico* analysis showed the binding of SITA with FXR, NF-κB p65, iNOS, Keap-1, caspase-3, and HO-1. In conclusion, SITA mitigates DN by reducing hyperglycemia, inflammation, and oxidative stress, while enhancing antioxidant defenses, FXR and Nrf2/HO-1 signaling.

Keywords: Hyperglycemia; Dipeptidyl peptidase IV inhibitor; Nephropathy; Inflammation; Oxidative stress.

1. Introduction

Diabetic kidney disease (DKD), also known as diabetic nephropathy (DN), is a chronic kidney disorder that emerges as a complication in diabetic patients [1]. Key risk factors for DKD

include prolonged periods of poorly controlled blood glucose (BG) levels and hypertension. Persistent hyperglycemia plays a central role in the onset and advancement of DN, despite the multifactorial nature of the disease [2] . Hyperglycemia not only provokes structural alterations in the kidney but also induces mechanical stress and hemodynamic changes in the glomeruli by activating several transcription factors and signaling molecules [2]. Chronic hyperglycemia can impact the glomerular basement membrane by causing non-enzymatic protein glycation, leading to stiffening of the efferent arterioles [1]. This alteration elevates the glomerular filtration rate and promotes ultrafiltration, consequently increasing intraglomerular pressure and triggering progressive hypertrophy of the glomeruli, which culminates in glomerulosclerosis [1]. The chronic microvascular effects of hyperglycemia involve tubuloglomerular atrophy, mesangial matrix expansion, interstitial fibrosis, and thickening of the basement membrane and arterioles. Approximately 40% of diabetic patients may eventually develop DN, making it the leading cause of end-stage renal disease [3, 4]. DKD arises approximately 10 years post-diagnosis in type 1 diabetes mellitus (T1DM) but can occur at various stages in individuals with type 2 diabetes [1].

Oxidative stress (OS) and inflammation are implicated in the complications of diabetes, including nephropathy and cardiomyopathy [5, 6]. Hyperglycemia-driven excess production of reactive oxygen species (ROS) and the resulting OS are recognized as key contributors to the pathophysiological mechanisms underlying diabetes-related vascular complications, including DKD [2, 7]. Elevated ROS levels can detrimentally affect cellular components, and this oxidative burden leads to lipid peroxidation (LPO), protein oxidation, DNA damage, and nuclear factor kappaB (NF-κB) activation, alongside an increase in pro-inflammatory mediators, all of which contribute to cellular injury [8]. OS and inflammation provoke cell death via apoptosis and both processes are therefore fundamental drivers in DN pathogenesis. Evidence from experimental models of DN reveals elevated OS markers, decreased antioxidant

defenses, activation of NF-κB, and upregulated inflammatory and apoptotic factors [5, 9]. Clinically, hyperglycemia in diabetic patients has been correlated with an inflammatory response marked by enhanced macrophage infiltration [10]. Therefore, agents with combined antioxidant and anti-inflammatory properties hold promise for mitigating DN progression and its associated pathologies. In this context, the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and farnesoid X receptor (FXR) has recently been shown to effectively attenuate OS and inflammation in the kidney of diabetic and chlorpyrifos (CPF)-intoxicated rats [9, 11]. In another recent study, the dual activation of FXR and Nrf2 prevented cholestatic liver injury [12]. Activation of Nrf2 represents a key approach to reducing OS and inflammation associated with various metabolic disorders [13]. Nrf2 is a transcription factor (TF) that controls the expression of antioxidant genes, such as heme oxygenase-1 (HO-1) under conditions of elevated ROS [14]. Nrf2 if found sequestered by Kelch-like ECH-associated protein 1 (Keap-1) in the cytoplasm, a binding that is dissociated in response to increased ROS [14]. The protective role of Nrf2 signaling in mitigating redox imbalance and inflammation in diabetes-related complications is well-documented [5, 14, 15]. FXR is expressed abundantly in the kidneys and in several tissues, and provides protective effects against metabolic dysregulation, and redox imbalance [16]. Studies indicate that FXR deficiency is associated with insulin resistance, hyperglycemia, and other metabolic abnormalities and accelerates the progression of DN. In contrast, its activation improves metabolic alterations and attenuates kidney injury by reducing inflammation, OS, and fibrosis [17-19].

Sitagliptin (SITA) is a dipeptidyl peptidase IV inhibitor and an oral anti-hyperglycemic drug. SITA enhances insulin secretion in a glucose-dependent manner and prolongs the half-life time of glucagon-like peptide-1 and other incretin hormones [20]. Its safety and efficacy both in mono- and combination therapies in adult diabetic patients have been suggested and it has also been shown to lower glycated hemoglobin levels [21, 22]. Besides its antidiabetic efficacy,

SITA has shown beneficial effects against diabetes complications and other disorders. Very recently, Li et al [23] reported the potential of SITA to prevent Parkinson's disease and other neurodegenerative disorders. Owing to its antiapoptotic and antifibrotic properties, the beneficial effects of SITA against COVID-19 has been suggested [24]. We have previously reported the protective efficacy of SITA against diabetic cardiomyopathy and investigated the involvement of JAK/STAT signaling [6]. The protective efficacy of SITA against kidney injury induced by chemotherapy [25, 26], and its antifibrosis effects in diabetic rats [27, 28] have also been reported. The role of FXR and Nrf2/HO-1 signaling in mediating the efficacy of SITA on DKD hasn’t been explored yet.

This study aimed to investigate whether the attenuation of oxidative stress, inflammation, and renal damage by SITA in a diabetic rat model is linked to the upregulation of Nrf2/HO-1 signaling and FXR. Utilizing a combined *in vivo* experimental approach and *in silico* molecular docking analysis, we evaluated the hypothesis that SITA confers protection against DN by modulating Nrf2/HO-1 signaling and FXR and attenuating oxidative and inflammatory responses. By defining the involvement of Nrf2/HO-1 signaling and FXR, this research may offer an insight into the mechanism of action of SITA, potentially strengthening the rationale for its use in mitigating DN progression beyond glycemic control.

2. Materials and methods

2.1. Animals and experimental design

Thirty-two 10 week-old male Sprague-Dawley rats were housed at a standard temperature and humidity on a 12-h light/dark cycle in polypropylene cages (42.5 cm × 26.6 cm × 18.5 cm) enriched with corn cob. The rats (3 per cage) were given access to food and water *ad libitum*. The study was approved by the ethics committee of King Saud University (IRB: SE-19-155). Streptozotocin (STZ; Sigma, USA; Cat. no.: S0130) (55 mg/kg body weight) dissolved in cold citrate buffer (0.1 M, pH 4.5) was injected intraperitoneally (i.p.) to overnight fasted rats to

induce T1DM, while citrate buffer was given to the control rats. A commercial kit (Spinreact, Spain; Cat. no. 1001190) was used to measure BG after 72 h and animals with fasting BG values of at least 250 mg/dl were selected. Normal rats were allocated into Group I (Control) and Group II (SITA) that received 0.9% saline and 10 mg/kg SITA (Sigma, USA; Cat. no. SML3205) dissolved in 0.9% saline, respectively. Diabetic rats were allocated into Group III (STZ) and Group IV (STZ + SITA) that received 0.9% saline and 10 mg/kg SITA, respectively. Each group included 6 rats and SITA and 0.9% saline were supplemented daily for 8 weeks via oral gavage. For 24-h urine collection, rats were temporarily housed in individual metabolic cages equipped with wire-mesh floors and urine collection funnels.

Following treatment, rats were fasted overnight, and blood was collected via cardiac puncture under ketamine/xylazine anesthesia. After immediate dissection, the kidneys were removed and weighed. Samples from the kidney were collected on RNALater (ThermoFisher, USA; Cat. no. AM7020) while others on 10% neutral-buffered formalin (NBF). Other samples were processed via homogenization (10% *w*/*v*) in Tris–HCl buffer (10 mM; pH 7.4), centrifuged, and the supernatant was stored at -80°C.

2.2. Biochemical assays

Levels of creatinine and blood urea nitrogen (BUN) in serum and microalbumin in urine were assayed using Biodiagnostic (Egypt; Cat. no. CR 1250 and UR 2110) and Spinreact (Spain; Cat. no. 1107170) kits, respectively. Levels of reduced glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO) and activities of superoxide dismutase (SOD) and catalase were determined in the kidney homogenate supernatant using Biodiagnostic kits (Egypt; Cat. no. TA2511, MD2528, NO2533, SD2521, and CA2517, respectively). The activities of kidney HO-1 and myeloperoxidase (MPO) were measured according to Abraham et al. [29] and Krawisz et al. [30], respectively. NF-κB p65, TNF-α and IL-1β (ELabscience, China; Cat. no. E-EL-R0674, E-EL-R2856 and E-EL-R0012, respectively), and caspase-3 (Cusabio, China;

Cat. no. CSB-E08857r) were measured in the kidney homogenate supernatant. All assays were conducted strictly following the manufacturers' instructions.

2.3. Histopathology and immunohistochemistry (IHC):

Following fixation in 10% NBF for 24 h, the kidney samples were processed for embedding in paraffin. 5-µm thick-sections were cut, stained with hematoxylin and eosin (H&E) and Masson's trichrome and examined using a light microscope (Olympus BX40, Olympus Corp., Japan). Other sections were processed for IHC staining to determine changes in Nrf2 and FXR. Briefly, the sections were dewaxed, rehydrated, and then treated with 0.05 M citrate buffer (pH 6.8) and 0.3% hydrogen peroxide (H_2O_2). After blocking, primary antibodies (Biospes, China; Cat. no. YPA1865 and YPA1581 for Nrf2 and FXR, respectively) were added overnight at 4°C, and the sections were washed and the secondary antibody (Biospes, China; Cat. no. BSA1031) was added. 3,3′-diaminobenzidine (Sigma, USA; Cat. no. D12384) in H_2O_2 was used for color development and counterstaining was carried out using hematoxylin. ImageJ (NIH, USA) was used to measure intensity of the developed color (6/rat).

2.4. *qRT-PCR*

Changes in kidney Bax, Bcl-2, caspase-3, iNOS, Nrf2, FXR, NF-κB p65, and Keap-1 mRNA were assayed using qRT-PCR. RNA was isolated using Trizol (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA, Cat. no. 15596026) and RNA samples with A260/A280 value ≥ 1.8 after purification were processed for cDNA synthesis via reverse transcription. cDNA was amplified using SYBR Green (ThermoFisher Scientific, USA; Cat. no. 4309155) and primer pairs in Suppl. Table I. The $2^{-\Delta\Delta Ct}$ method [31] was employed for analysis using β-actin as a control.

2.5. *In silico* molecular docking

The affinity of SITA towards FXR (PDB ID: 7D42), NF-κB p65 (PDB ID: 5UO1), iNOS (PDB ID: 3EAI), caspase-3 (PDB ID: 1NME), HO-1 (PDB ID: 1DVE), and Keap-1 (PDB ID:

5CGJ) was investigated using PyRx virtual screening software (version 0.8) [32]. The target protein were prepared using Autodock Tools (ADT; v1.5.6) and PyMOL (v2.3.2) and LigPlot (v2.2.8) [33] were used for visualization of binding mode and protein-ligand interactions, respectively.

2.6. Statistical analysis

All the data were expressed as the mean ± SEM. Statistics was performed using GraphPad Prism 8 software. The comparisons between the different groups were performed by one-way ANOVA, following with the post hoc Tukey's test. P values less than 0.05 were considered statistically significant.

3. Results

3.1. SITA ameliorates hyperglycemia and kidney damage in diabetic rats

Initial and final BG levels were markedly elevated in diabetic rats (Fig. 1A; $P<0.001$). The same animals exhibited significant decrease in body weight (BW) (Fig. 1B,C) and increased kidney weight (KW)/BW ratio (Fig. 1D) ($P<0.001$). Biochemical findings represented in Figures 2A-C revealed significant increase in creatinine, BUN, and microalbumin in diabetic animals ($P<0.001$). The findings in Fig. 3 showed degeneration of the epithelial lining of renal tubules, atrophied irregular renal corpuscle, and deposition of fibers in the interstitium in diabetic rats which also showed fiber deposition surrounding blood vessels. SITA effectively alleviated hyperglycemia, BW, KW/BW ratio (Fig. 1), kidney function markers (Fig. 2), and prevented kidney tissue damage (Fig. 3).

3.2. SITA mitigates kidney OS in diabetic rats

Diabetic rat kidney showed remarkable elevation in MDA (Fig. 4A) and MPO (Fig. 4B) along with declined GSH (Fig. 4C), SOD (Fig. 4D), and CAT (Fig. 4E) as compared to the control ($P<0.001$). SITA ameliorated MDA levels, MPO activity and antioxidants when supplemented to diabetic rats whereas had no effect on normal animals.

205 3.3. SITA attenuates kidney inflammation in diabetic rats

206 The effect of SITA on the inflammatory response in the kidney of diabetic rats was evaluated
207 via assessment of changes in NF-κB p65 and pro-inflammatory mediators. Diabetes was
208 associated with elevated NF-κB p65 levels (Fig. 5A,B) along with increased kidney TNF-α
209 (Fig. 4C) and IL-1β (Fig. 4D). iNOS mRNA (Fig. 4E) and NO levels (Fig. 4F) were remarkably
210 increased in the diabetic kidney when compared with the control group ($P<0.001$). Treatment
211 with SITA effectively decreased NF-κB p65 and pro-inflammatory mediators (TNF-α, IL-1β
212 and iNOS), and NO in dibetic rats ($P<0.001$).

213 *In silico* investigations revealed the affinity of SITA to bind NF-κB p65 and iNOS with binding
214 energies -7.5 and -8.2 kcal/mol, respectively (Fig. 6 and Table 1). The complexes of SITA with
215 NF-κB p65 and iNOS showed polar bonding with two amino acid residues of both proteins and
216 hydrophobic interactions with five and three residues, respectively.

217 3.4. SITA prevents kidney apoptosis in diabetic rats

218 Kidney Bcl-2 mRNA was decreased in diabetic rats whereas Bax and caspase-3 were
219 upregulated significantly as compared to control rats (Fig. 7A-D). SITA upregulated Bcl-2 and
220 decreased Bax and caspase-3 in the diabetic rat kidney ($P<0.001$). Investigation of the binding
221 of SITA with caspase-3 revealed 8 hydrophobic interactions and -5.3 kcal/mol binding energy
222 (Fig. 7E and Table 1).

223 3.5. SITA upregulates Nrf2/HO-1 signaling and FXR in diabetic rats

224 Keap-1 (Fig. 8A) and Nrf2 (Fig. 8B) mRNA levels were significantly increased and decreased,
225 respectively in the kidney of diabetic rats as compared to control rats ($P<0.001$). IHC staining
226 of Nrf2 (Fig. 8C-D) and HO-1 activity (Fig. 8E) determination showed significant
227 downregulation in diabetic rats as compared to control rats. SITA downregulated Keap-1 and
228 boosted Nrf2 and HO-1 in the kidney of diabetic rats while had no effect on normal rats. The
229 effect of SITA on Keap-1 and HO-1 was further investigated using *in silico* molecular docking

(Fig. 9 and Table 1). SITA exhibited -10.2 and -9.4 kcal/mol binding energies with Keap-1 and HO-1, respectively. SITA (Fig. 9A) and the Nrf2 activator RA839 (Fig. 9B, Suppl. Table II) showed 7 and 17 hydrophobic interactions and 5 and 3 polar bonding, respectively, with Keap-1. The complex of SITA with HO-1 showed polar bonding and hydrophobic interactions with one and ten residues, respectively (Fig. 9C and Table 1).

Data represented in Fig 10 A-C showed significant downregulation of FXR mRNA and protein in the kidney of diabetic rats as compared to control rats ($P<0.001$). SITA upregulated FXR remarkably in diabetic rats whereas showed no effect on normal rats. Twelve and two hydrophobic and polar interactions, respectively, between SITA and FXR were shown *in silico* and the binding energy is -9.2 kcal/mol (Fig. 10D and Table 1). Twelve of the residues in SITA/FXR complex were noticed in the complex formed by the FXR activator tropifexor (Suppl. Fig. I, Suppl. Table II).

4. Discussion

Nephropathy is one of the serious complications of diabetes characterized by functional and structural changes in the kidney [1]. Despite the progression in understanding the pathophysiology of DN, the condition is still undertreated due to the lack of effective sustainable treatments. The implication of OS and inflammation in DKD has been acknowledged and agents that attenuate these processes showed beneficial effects against the disease progression [9]. This study revealed that SITA protects the kidney against diabetes-induced injury by ameliorating hyperglycemia, OS, inflammation, and apoptosis, an effect that is associated with upregulation of FXR and Nrf2/HO-1 signaling.

Diabetic rats in this study exhibited hyperglycemia and abnormal renal function markers, including creatinine, BUN, and microalbumin. Hyperglycemia is a result of β-cells destruction and impaired insulin secretion because STZ is particularly toxic to these cells and promotes oxidative DNA damage [34]. Hyperglycemia was associated with a decrease in BW and

increase in KW/BW ratio. These findings align with previous studies showing BW loss and increased KW/BW ratio along with hyperglycemia and altered levels of kidney function markers in diabetic rodents [5, 9, 35]. Additionally, examination of the stained tissue revealed glomerular atrophy, irregular corpuscles, tubular epithelium degeneration and interstitial fibrosis as previously reported [9]. The progression of DN involves distinctive changes such as interstitial fibrosis, atrophic changes and renal dysfunction [3, 36], all have been observed in this study. BW loss observed in the diabetic rats is directly ascribed to the surplus utilization of lipids and proteins to provide energy in the lack of ability to use glucose [37]. The structural abnormalities, such as collagen deposition, could explain the changes in KW which could also be attributed to the upregulation of fibronectin, collagen, and growth factors [38]. SITA effectively ameliorated hyperglycemia, kidney hypertrophy and injury along with amelioration of creatinine, BUN and microalbumin. These findings highlight the nephroprotective efficacy of SITA which has been previously demonstrated in animals challenged with chemotherapy [25, 26]. In uninephrectomized rats challenged with doxorubicin, SITA administration for 6 weeks ameliorated tubulointerstitial injury and fibrosis [25], and its administration for 14 days prevented cyclosporine-induced kidney injury and ameliorated serum urea and creatinine [26]. In addition to its antidiabetic efficacy, the nephroprotective mechanism of SITA could be linked to its role in attenuating OS and inflammation as reported in animal models of chemotherapy-induced nephrotoxicity [25, 26]. In this study, markers of OS were evident through increased MDA, NO, and MPO activity, accompanied by reduced GSH and antioxidant enzyme activities. Inflammation in diabetic kidneys was characterized by elevated levels of NF-κB p65, iNOS, TNF-α, and IL-1β. The pathogenesis of DN is heavily influenced by OS and inflammatory mechanisms [2, 7, 39]. Persistent hyperglycemia leads to excessive production of ROS, driving LPO and NF-κB activation, which collectively contribute to cellular injury [8]. Upon activation by ROS, NF-κB leads to increased secretion of IL-1β and TNF-α, and

promotes iNOS, thereby explaining the rise in NO production. In our recent study [9], we have provided further evidence of inflammation in DN manifested by upregulation of CD68 which is an indicator of macrophage infiltration and has been demonstrated in renal autopsy samples from diabetic patients [40]. This interaction between OS and inflammation fosters apoptotic pathways. For instance, superoxide, a prominent ROS, can combine with NO to form the highly reactive oxidant peroxynitrite that damages DNA and induces cell death [41]. In this study, diabetic rats showed declined Bcl-2 and increased Bax and caspase-3 expression in the kidney. Inflammatory mediators and ROS stimulate pro-apoptotic Bax expression and disrupt mitochondrial membrane potential [42] and together with the impairment of mitochondrial respiratory function, Bax results in DNA damage and increases mitochondrial membrane permeability and cytochrome c release into the cytosol and subsequent activation of caspase-3 [43]. Activated caspase-3 promotes DNA fragmentation and cytoskeleton breakdown, culminating in apoptosis.

SITA demonstrated significant protective effects against OS, inflammation, and kidney injury. These protective actions were reflected by reduced levels of markers associated with oxidative and inflammatory stresses, including MDA, NO, MPO, NF-κB p65, pro-inflammatory mediators, Bax, and caspase-3, alongside an increase in antioxidant levels and Bcl-2 expression. Previous pre-clinical studies have pointed to the efficacy of SITA in mitigating OS and inflammation in the kidney and other tissues [6, 25, 26]. SITA afforded a protective role against myocardial oxidative damage in diabetic animals [6]. SITA mitigated LPO, IL-6 and IL-1β, suppressed JAK/STAT signaling and enhanced antioxidants in the heart of diabetic rats [6]. In a rat model of cyclosporine nephrotoxicity, SITA ameliorated MDA, GSH, SOD, CAT, Bax and TNF-α [26]. In a doxorubicin nephropathy model, SITA mitigated inflammation and ROS generation as shown by decreased IL-1β and the mRNA levels of NADPH oxidase subunits [25]. In a rat model of high-fat diet-induced T2DM, SITA ameliorated renal function

and prevented TGF-β1/Smad-mediated kidney fibrosis [27]. Similarly, SITA has been shown to attenuate inflammation and fibrosis by suppressing TGF-β, collagen deposition, and TNF-α in diabetic rats [28]. *In silico* findings added further support to the anti-inflammatory and anti-apoptosis efficacy of SITA. The results pinpointed the affinity of SITA to bind NF-κB p65 which can lead to suppression of its transcriptional activity. Moreover, SITA exhibited affinity towards numerous amino acid residues in iNOS and caspase-3.

To investigate the nephroprotective mechanism of SITA, its influence on the Nrf2 and FXR was evaluated in the kidney of rats and an *in silico* investigation was also conducted. The diabetic rat kidneys exhibited upregulation of Keap-1 and a concurrent decrease in Nrf2. Nrf2, HO-1, and FXR, indicating suppression of the Nrf2/HO-1 pathway and FXR expression. The suppression of Nrf2 was observed in rats with DN as previously demonstrated [5, 9, 44]. These findings are also consistent with previous research demonstrating that hyperglycemia and other metabolic disturbances in diabetes and obesity correlate with reduced FXR levels, which in turn contribute to the progression of DN [9, 18, 19, 45, 46].

SITA downregulated Keap-1 and enhanced the expression of Nrf2, HO-1, and FXR, results directly associated with the alleviation of metabolic disturbances, OS and inflammation. Nrf2 induces the transcription of antioxidant and cytoprotective enzymes, thereby reducing ROS and mitigating oxidative damage, and directly inhibits NF-κB [14, 47]. In this study, the increased expression of FXR was linked to improved BG levels and mitigation of inflammatory and oxidative stresses. FXR modulates BG regulation by influencing insulin release and sensitivity and gluconeogenesis [48-50]. Conversely, FXR deficiency leads to hyperglycemia, which is associated with decreased insulin release [49], with research on β-cells indicating that FXR modulates insulin secretion [51]. Activation of FXR has been shown to protect against mitochondrial damage and excess ROS associated with ischemia/reperfusion in murine kidney [52]. Nrf2 mediates the beneficial role of FXR against OS, with the silencing of Nrf2 nullifying

the beneficial consequences of FXR activation [52]. FXR activation also confers protection to the kidney in diabetes by reducing oxidative damage, fibrosis, and glomerulosclerosis [17]. Recently, Nrf2 and FXR have been shown to jointly mitigate liver injury caused by cholestasis [12]. Therefore, the combined action of Nrf2 and FXR likely contributes, at least in part, to the nephroprotective effects of SITA.

A key finding of this study is the demonstration that the protective role of SITA against DN involves the concerted upregulation of both Nrf2/HO-1 signaling and FXR. The integrated approach, combining *in vivo* experimental validation with *in silico* molecular docking to predict the interactions of SITA with key targets, including NF-κB p65, iNOS, caspase-3, Keap-1, HO-1, and FXR, provides insights into its mechanism of renoprotection. However, certain limitations should be acknowledged. The study utilized a single dose and duration of SITA treatment; exploring dose-response relationships and longer-term effects would provide further insights. While the STZ-induced diabetic rat model is well-established for studying DN pathogenesis and drug effects, the obtained findings should be investigated in clinical settings.

5. Conclusion

This study provides new insights into the protective mechanisms of SITA against DKD. SITA alleviated hyperglycemia and kidney damage, fibrosis, OS, inflammation, and cell death. These protective effects were linked to the activation of FXR and Nrf2/HO-1 signaling, which led to increased antioxidant defenses and a reduced inflammatory response. Molecular docking studies suggested that SITA interacts with FXR, HO-1, Keap1, NF-κB, iNOS, and caspase-3, further supporting its therapeutic potential. In summary, SITA shows promise in mitigating DN by activating Nrf2/HO-1 signaling, enhancing FXR, and reducing OS, inflammation, and apoptosis. Future studies are recommended to further investigate other underlying mechanisms.

Acknowledgment:

The authors extend their appreciation to the Ongoing Research Funding Program (ORF-2025-573), King Saud University, Riyadh, Saudi Arabia.

Declaration of Competing Interest:

All authors declare no conflict of interests in relation to the manuscript.

Funding:

This study was supported by the Ongoing Research Funding Program (ORF-2025-573), King Saud University, Riyadh, Saudi Arabia.

Availability of data and materials

The manuscript and supplementary material contain all data supporting the reported results.

References:

[1] C. Mora-Fernández, V. Domínguez-Pimentel, M.M. de Fuentes, J.L. Górriz, A. Martínez-Castelao, J.F. Navarro-González, Diabetic kidney disease: from physiology to therapeutics, The Journal of physiology 592(18) (2014) 3997-4012.
[2] F.A. Wagener, D. Dekker, J.H. Berden, A. Scharstuhl, J. van der Vlag, The role of reactive oxygen species in apoptosis of the diabetic kidney, Apoptosis : an international journal on programmed cell death 14(12) (2009) 1451-8.
[3] B. Najafian, C.E. Alpers, A.B. Fogo, Pathology of human diabetic nephropathy, Diabetes and the Kidney 170 (2011) 36-47.
[4] S. Iacobelli, J.P. Guignard, Maturation of glomerular filtration rate in neonates and infants: an overview, Pediatr Nephrol 36(6) (2021) 1439-1446.
[5] S.A. Antar, W. Abdo, R.S. Taha, A.E. Farage, L.E. El-Moselhy, M.E. Amer, A.S. Abdel Monsef, A.M. Abdel Hamid, E.M. Kamel, A.F. Ahmeda, A.M. Mahmoud, Telmisartan attenuates diabetic nephropathy by mitigating oxidative stress and inflammation, and upregulating Nrf2/HO-1 signaling in diabetic rats, Life Sci 291 (2022) 120260.
[6] N.M. Al-Rasheed, N.M. Al-Rasheed, I.H. Hasan, M.A. Al-Amin, H.N. Al-Ajmi, A.M. Mahmoud, Sitagliptin attenuates cardiomyopathy by modulating the JAK/STAT signaling pathway in experimental diabetic rats, Drug Des Devel Ther 10 (2016) 2095-107.
[7] D.M. Bandeira, L.J.S. Da Fonseca, D.S. Guedes, L.A. Rabelo, M.O. Goulart, S.M.L. Vasconcelos, Oxidative stress as an underlying contributor in the development of chronic complications in diabetes mellitus, International journal of molecular sciences 14(2) (2013) 3265-3284.
[8] K. Kachhawa, M. Varma, P. Kachhawa, D. Agrawal, M. Shaikh, S. Kumar, Study of dyslipidemia and antioxidant status in chronic kidney disease patients at a hospital in South East Asia, Journal of Health Research and Reviews 3(1) (2016) 28-30.
[9] I.H. Hasan, S.Y. Shaheen, A.M. Alhusaini, A.M. Mahmoud, Simvastatin mitigates diabetic nephropathy by upregulating farnesoid X receptor and Nrf2/HO-1 signaling and attenuating oxidative stress and inflammation in rats, Life Sci 340 (2024) 122445.
[10] D. Nguyen, F. Ping, W. Mu, P. Hill, R.C. Atkins, S.J. Chadban, Macrophage accumulation in human progressive diabetic nephropathy, Nephrology (Carlton, Vic.) 11(3) (2006) 226-31.
[11] R.S. Alruhaimi, A.F. Ahmeda, O.E. Hussein, M.F. Alotaibi, M.O. Germoush, H.A. Elgebaly, E.H.M. Hassanein, A.M. Mahmoud, Galangin attenuates chlorpyrifos-induced kidney injury by

mitigating oxidative stress and inflammation and upregulating Nrf2 and farnesoid-X-receptor in rats, Environmental Toxicology and Pharmacology (2024) 104542.
[12] J. Liu, J. Liu, C. Meng, Q. Gu, C. Huang, F. Liu, C. Xia, NRF2 and FXR dual signaling pathways cooperatively regulate the effects of oleanolic acid on cholestatic liver injury, Phytomedicine 108 (2023) 154529.
[13] A.M. Mahmoud, M.Y. Alexander, Y. Tutar, F.L. Wilkinson, A. Venditti, Oxidative Stress in Metabolic Disorders and Drug-Induced Injury: The Potential Role of Nrf2 and PPARs Activators, Oxid Med Cell Longev 2017 (2017) 2508909.
[14] S. Satta, A.M. Mahmoud, F.L. Wilkinson, M. Yvonne Alexander, S.J. White, The Role of Nrf2 in Cardiovascular Function and Disease, Oxid Med Cell Longev. 2017 (2017) 9237263.
[15] A. Gupta, T. Behl, A. Sehgal, S. Bhatia, D. Jaglan, S. Bungau, Therapeutic potential of Nrf-2 pathway in the treatment of diabetic neuropathy and nephropathy, Mol Biol Rep 48(3) (2021) 2761-2774.
[16] B.M. Forman, E. Goode, J. Chen, A.E. Oro, D.J. Bradley, T. Perlmann, D.J. Noonan, L.T. Burka, T. McMorris, W.W. Lamph, R.M. Evans, C. Weinberger, Identification of a nuclear receptor that is activated by farnesol metabolites, Cell 81(5) (1995) 687-693.
[17] T. Jiang, X.X. Wang, P. Scherzer, P. Wilson, J. Tallman, H. Takahashi, J. Li, M. Iwahashi, E. Sutherland, L. Arend, M. Levi, Farnesoid X receptor modulates renal lipid metabolism, fibrosis, and diabetic nephropathy, Diabetes 56(10) (2007) 2485-93.
[18] S. Cipriani, A. Mencarelli, G. Palladino, S. Fiorucci, FXR activation reverses insulin resistance and lipid abnormalities and protects against liver steatosis in Zucker (fa/fa) obese rats, Journal of Lipid Research 51(4) (2010) 771-784.
[19] X.X. Wang, T. Jiang, Y. Shen, Y. Caldas, S. Miyazaki-Anzai, H. Santamaria, C. Urbanek, N. Solis, P. Scherzer, L. Lewis, F.J. Gonzalez, L. Adorini, M. Pruzanski, J.B. Kopp, J.W. Verlander, M. Levi, Diabetic nephropathy is accelerated by farnesoid X receptor deficiency and inhibited by farnesoid X receptor activation in a type 1 diabetes model, Diabetes 59(11) (2010) 2916-27.
[20] C. Koliaki, J. Doupis, Incretin-based therapy: a powerful and promising weapon in the treatment of type 2 diabetes mellitus, Diabetes Ther 2(2) (2011) 101-21.
[21] S. Umezawa, A. Kubota, H. Maeda, A. Kanamori, K. Matoba, Y. Jin, F. Minagawa, M. Obana, K. Iemitsu, S. Ito, H. Amamiya, M. Kaneshiro, M. Takai, H. Kaneshige, K. Hoshino, M. Ishikawa, N. Minami, T. Takuma, N. Sasai, S. Aoyagi, T. Kawata, A. Mokubo, Y. Miyairi, H. Takeda, S. Honda, H. Machimura, T. Motomiya, M. Waseda, Y. Naka, Y. Tanaka, Y. Terauchi, I. Matsuba, Two-year assessment of the efficacy and safety of sitagliptin in elderly patients with type 2 diabetes: Post hoc analysis of the ASSET-K study, BMC Endocr Disord 15 (2015) 34.
[22] J. Rosenstock, D. Allison, A.L. Birkenfeld, T.M. Blicher, S. Deenadayalan, J.B. Jacobsen, P. Serusclat, R. Violante, H. Watada, M. Davies, Effect of Additional Oral Semaglutide vs Sitagliptin on Glycated Hemoglobin in Adults With Type 2 Diabetes Uncontrolled With Metformin Alone or With Sulfonylurea: The PIONEER 3 Randomized Clinical Trial, Jama 321(15) (2019) 1466-1480.
[23] Y. Li, K.L. Vaughan, Y. Wang, S.J. Yu, E.K. Bae, I.A. Tamargo, K.O. Kopp, D. Tweedie, C.C. Chiang, K.T. Schmidt, D.K. Lahiri, M.A. Tones, M.M. Zaleska, B.J. Hoffer, J.A. Mattison, N.H. Greig, Sitagliptin elevates plasma and CSF incretin levels following oral administration to nonhuman primates: relevance for neurodegenerative disorders, Geroscience 46(5) (2024) 4397-4414.
[24] E.M. Mikhael, S.C. Ong, S.M. Sheikh Ghadzi, Efficacy and Safety of Sitagliptin in the Treatment of COVID-19, J Pharm Pract 36(4) (2023) 980-987.
[25] C.H. Jo, S. Kim, J.S. Park, G.H. Kim, Anti-Inflammatory Action of Sitagliptin and Linagliptin in Doxorubicin Nephropathy, Kidney Blood Press Res 43(3) (2018) 987-999.
[26] A.M. Abd-Eldayem, S.M. Makram, B.A.S. Messiha, H.H. Abd-Elhafeez, M.A. Abdel-Reheim, Cyclosporine-induced kidney damage was halted by sitagliptin and hesperidin via increasing Nrf2 and suppressing TNF-α, NF-κB, and Bax, Scientific Reports 14(1) (2024) 7434.

[27] D. Wang, G. Zhang, X. Chen, T. Wei, C. Liu, C. Chen, Y. Gong, Q. Wei, Sitagliptin ameliorates diabetic nephropathy by blocking TGF-β1/Smad signaling pathway, Int J Mol Med 41(5) (2018) 2784-2792.
[28] R.H. Mohamed, A.A. Sedky, G.G. Hamam, L. Elkhateb, S.A. Kamar, S. Adel, S.S. Tawfik, Sitagliptin's renoprotective effect in a diabetic nephropathy model in rats: The potential role of PI3K/AKT pathway, Fundam Clin Pharmacol 36(2) (2022) 324-337.
[29] N.G. Abraham, J.D. Lutton, R.D. Levere, Heme metabolism and erythropoiesis in abnormal iron states: role of delta-aminolevulinic acid synthase and heme oxygenase, Experimental hematology 13(8) (1985) 838-843.
[30] J. Krawisz, P. Sharon, W.J.G. Stenson, Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity: assessment of inflammation in rat and hamster models, 87(6) (1984) 1344-1350.
[31] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods (San Diego, Calif.) 25(4) (2001) 402-8.
[32] S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx, Methods Mol Biol 1263 (2015) 243-50.
[33] A.C. Wallace, R.A. Laskowski, J.M. Thornton, LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions, Protein Eng 8(2) (1995) 127-34.
[34] T. Szkudelski, The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas, Physiological research 50(6) (2001) 537-546.
[35] S. Zhang, H. Xu, X. Yu, Y. Wu, D. Sui, Metformin ameliorates diabetic nephropathy in a rat model of low-dose streptozotocin-induced diabetes, Experimental and therapeutic medicine 14(1) (2017) 383-390.
[36] M.P. Cohen, R.S. Clements, J.A. Cohen, C.W. Shearman, Prevention of decline in renal function in the diabetic db/db mouse, Diabetologia 39(3) (1996) 270-4.
[37] P. Montilla, M. Barcos, M.C. Muñoz, J.R. Muñoz-Castañeda, I. Bujalance, I. Túnez, Protective effect of Montilla-Moriles appellation red wine on oxidative stress induced by streptozotocin in the rat, The Journal of nutritional biochemistry 15(11) (2004) 688-93.
[38] A. Flyvbjerg, D. Landau, H. Domene, L. Hernandez, H. Grønbaek, D. LeRoith, The role of growth hormone, insulin-like growth factors (IGFs), and IGF-binding proteins in experimental diabetic kidney disease, Metabolism 44(10 Suppl 4) (1995) 67-71.
[39] T. Behl, A. Gupta, A. Sehgal, S. Singh, N. Sharma, M. Garg, S. Bhatia, A. Al-Harrasi, L. Aleya, S. Bungau, Exploring the multifaceted role of TGF-β signaling in diabetic complications, Environ Sci Pollut Res Int 29(24) (2022) 35643-35656.
[40] C.Q.F. Klessens, M. Zandbergen, R. Wolterbeek, J.A. Bruijn, T.J. Rabelink, I.M. Bajema, I.J. DHT, Macrophages in diabetic nephropathy in patients with type 2 diabetes, Nephrol Dial Transplant 32(8) (2017) 1322-1329.
[41] S.E. McKim, E. Gäbele, F. Isayama, J.C. Lambert, L.M. Tucker, M.D. Wheeler, H.D. Connor, R.P. Mason, M.A. Doll, D.W. Hein, G.E. Arteel, Inducible nitric oxide synthase is required in alcohol-induced liver injury: studies with knockout mice, Gastroenterology 125(6) (2003) 1834-44.
[42] Y. Shi, J. Chen, C. Weng, R. Chen, Y. Zheng, Q. Chen, H. Tang, Identification of the protein-protein contact site and interaction mode of human VDAC1 with Bcl-2 family proteins, Biochemical and biophysical research communications 305(4) (2003) 989-96.
[43] M.A. Fuertes, C. Alonso, J.M. Pérez, Biochemical modulation of Cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance, Chemical reviews 103(3) (2003) 645-62.
[44] T. Mohan, K.K.S. Narasimhan, D.B. Ravi, P. Velusamy, N. Chandrasekar, L.N. Chakrapani, A. Srinivasan, P. Karthikeyan, P. Kannan, B. Tamilarasan, T. Johnson, P. Kalaiselvan, K. Periandavan, Role of Nrf2 dysfunction in the pathogenesis of diabetic nephropathy: Therapeutic prospect of epigallocatechin-3-gallate, Free Radical Biology and Medicine 160 (2020) 227-238.

[45] G. Lambert, M.J.A. Amar, G. Guo, H.B. Brewer Jr, F.J. Gonzalez, C.J. Sinal, The farnesoid X-receptor is an essential regulator of cholesterol homeostasis, Journal of Biological Chemistry 278(4) (2003) 2563-2570.
[46] Y. Zhang, F.Y. Lee, G. Barrera, H. Lee, C. Vales, F.J. Gonzalez, T.M. Willson, P.A. Edwards, Activation of the nuclear FXR improves hyperglycemia and hyperlipidemia in diabetic mice, Proceedings of the National Academy of Sciences of the United States of America 103(4) (2006) 1006-1011.
[47] J.D. Wardyn, A.H. Ponsford, C.M. Sanderson, Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways, Biochem Soc Trans. 43(4) (2015) 621-626.
[48] K. Cai, M.B. Sewer, Diacylglycerol kinase θ couples farnesoid X receptor-dependent bile acid signalling to Akt activation and glucose homoeostasis in hepatocytes, Biochemical Journal 454(2) (2013) 267-274.
[49] I.R. Popescu, A. Helleboid-Chapman, A. Lucas, B. Vandewalle, J. Dumont, E. Bouchaert, B. Derudas, J. Kerr-Conte, S. Caron, F. Pattou, B. Staels, The nuclear receptor FXR is expressed in pancreatic β-cells and protects human islets from lipotoxicity, FEBS Letters 584(13) (2010) 2845-2851.
[50] E. Maneschi, L. Vignozzi, A. Morelli, T. Mello, S. Filippi, I. Cellai, P. Comeglio, E. Sarchielli, A. Calcagno, B. Mazzanti, R. Vettor, G.B. Vannelli, L. Adorini, M. Maggi, FXR activation normalizes insulin sensitivity in visceral preadipocytes of a rabbit model of mets, Journal of Endocrinology 218(2) (2013) 215-231.
[51] B. Schittenhelm, R. Wagner, V. Kähny, A. Peter, P. Krippeit-Drews, M. Düfer, G. Drews, Role of FXR in β-cells of lean and obese mice, Endocrinology (United States) 156(4) (2015) 1263-1271.
[52] Z. Gai, L. Chu, Z. Xu, X. Song, D. Sun, G.A. Kullak-Ublick, Farnesoid X receptor activation protects the kidney from ischemia-reperfusion damage, Scientific Reports 7(1) (2017) 9815.

Tables:

Table 1. Binding affinities and interaction of sitagliptin with different protein targets.

Target	Lowest binding energy (kcal/mol)	Polar bonds	Hydrophobic interactions
NF-κB p65	-7.5	His58, Ser45	Lys56, Pro47, Gln220, Glu222, Thr52
iNOS	-8.2	Tyr293, Gln265	Pro291, Ile285, Gln282
Caspase-3	-5.3		Arg164, Glu124, Pro201, Tyr1975, Gly125, Cys264, Tyr195, Val266
Keap-1	-10.2	Leu365, Val465, Cys513, Val418, Arg415	Ala556, Gly462, Leu557, Gly 509, Val604, Gly364, Gly603
HO-1	-9.4	Gly139	Phe214, Gly143, Ser142, Ala28, Asn210, Leu138, His25, Arg136, Phe207, Tyr134
FXR	-9.2	Tyr369, Ser332	Ile352, His447, Phe329, Phe443, Arg331, Met265, Ile335, Gly343, Met328, Val325, Met290, Leu348

NF-κB p65: nuclear factor-kappaB p65; iNOS: inducible nitric oxide synthase**;** Keap-1: Kelch-like ECH-associated protein 1; HO-1: heme oxygenase 1.

Figures:

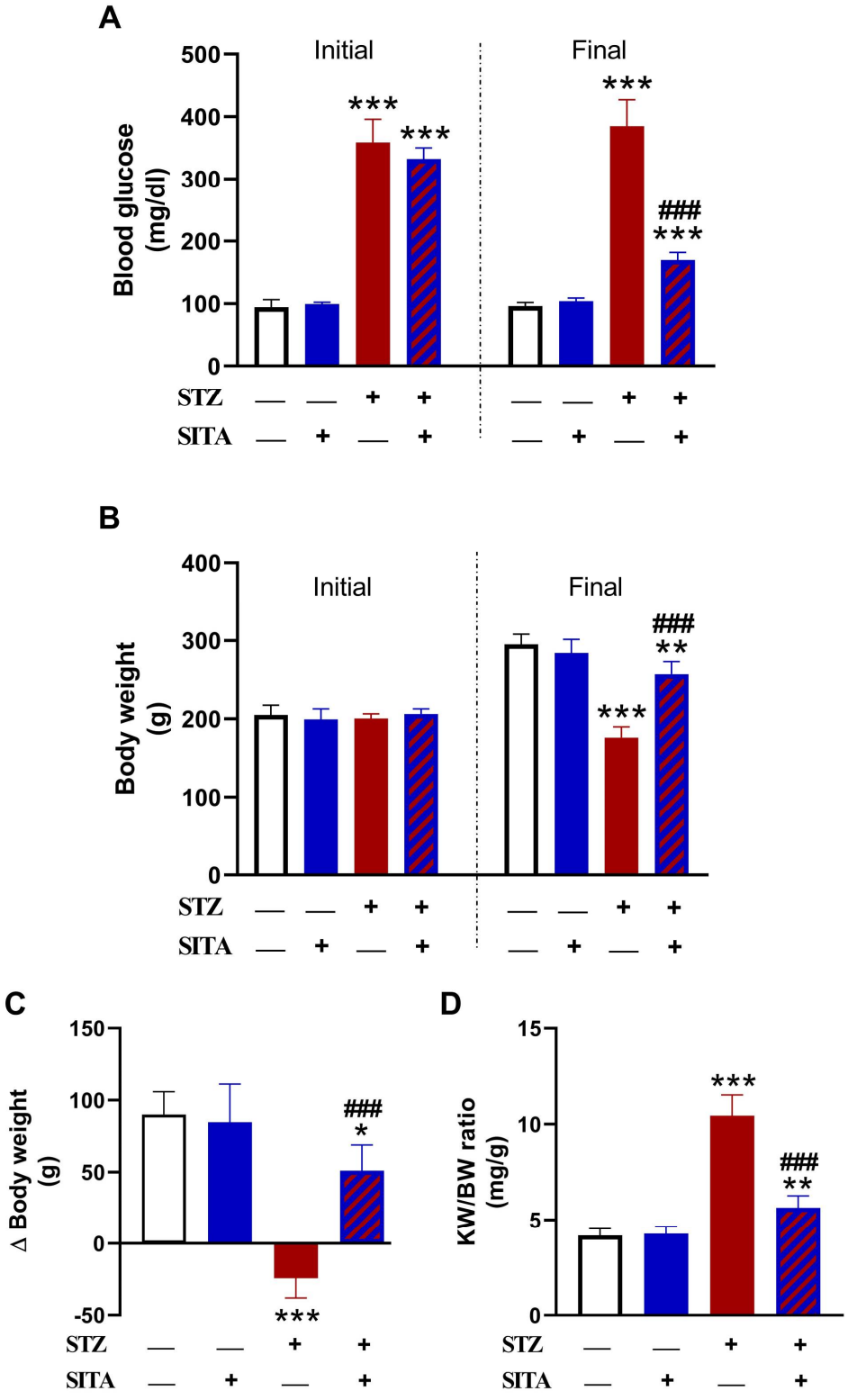

Fig. 1. Sitagliptin ameliorated blood glucose (BG) (A), body weight (BW) (B-C) and kidney weight (KW)/BW ratio (D) in diabetic rats. Data are mean ± SEM, (*n*=8). *P<0.05, **P<0.01 and ***P<0.001 versus Control, and $^{\#\#\#}$P<0.001 versus Diabetic.

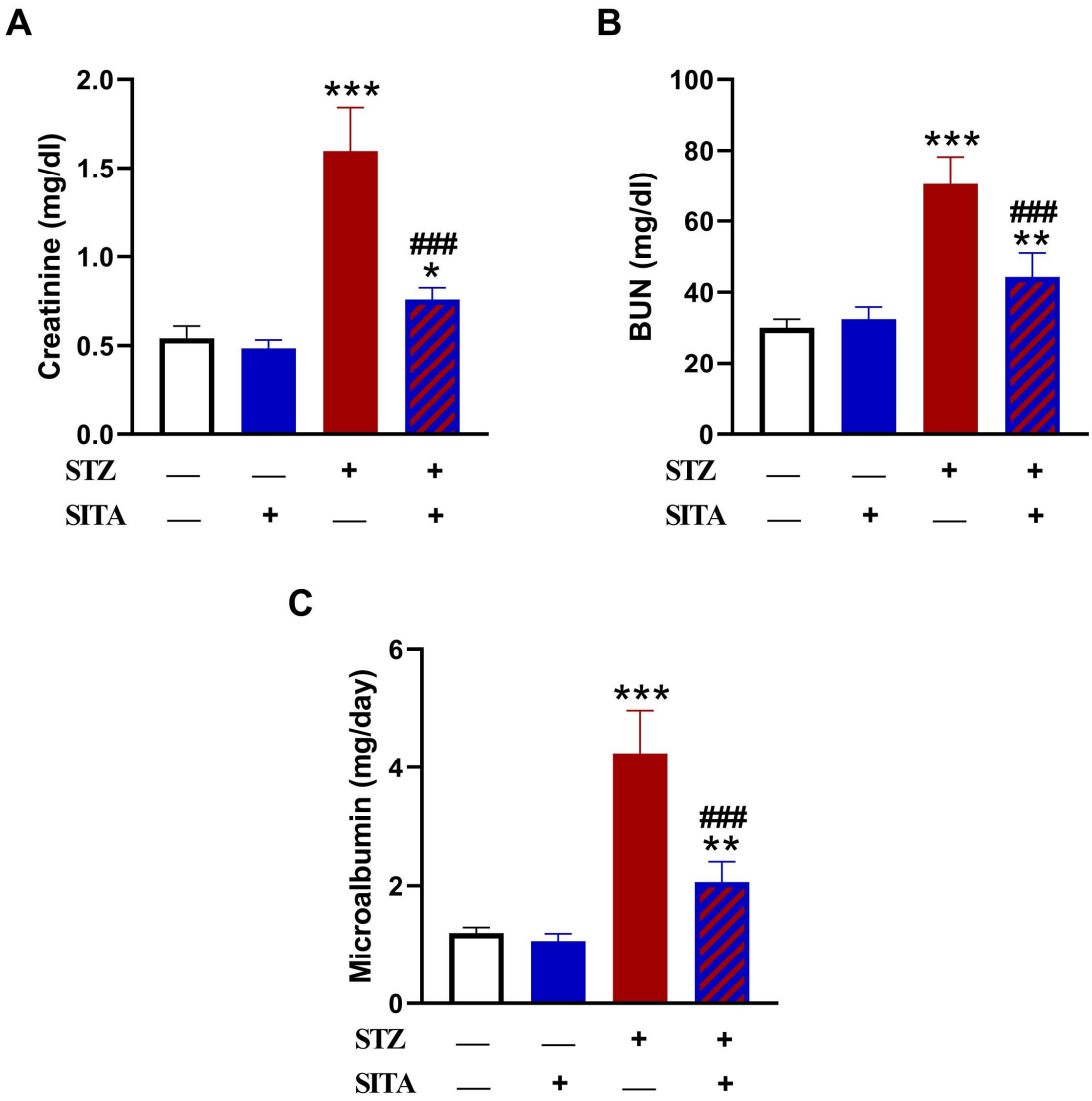

Fig. 2. Sitagliptin alleviated creatinine (A), blood urea nitrogen (BUN) (B) and microalbumin (C) in diabetic rats. Data are mean ± SEM, (*n*=8). *P<0.05, **P<0.01 and ***P<0.001 versus Control, and $^{###}$P<0.001 versus Diabetic.

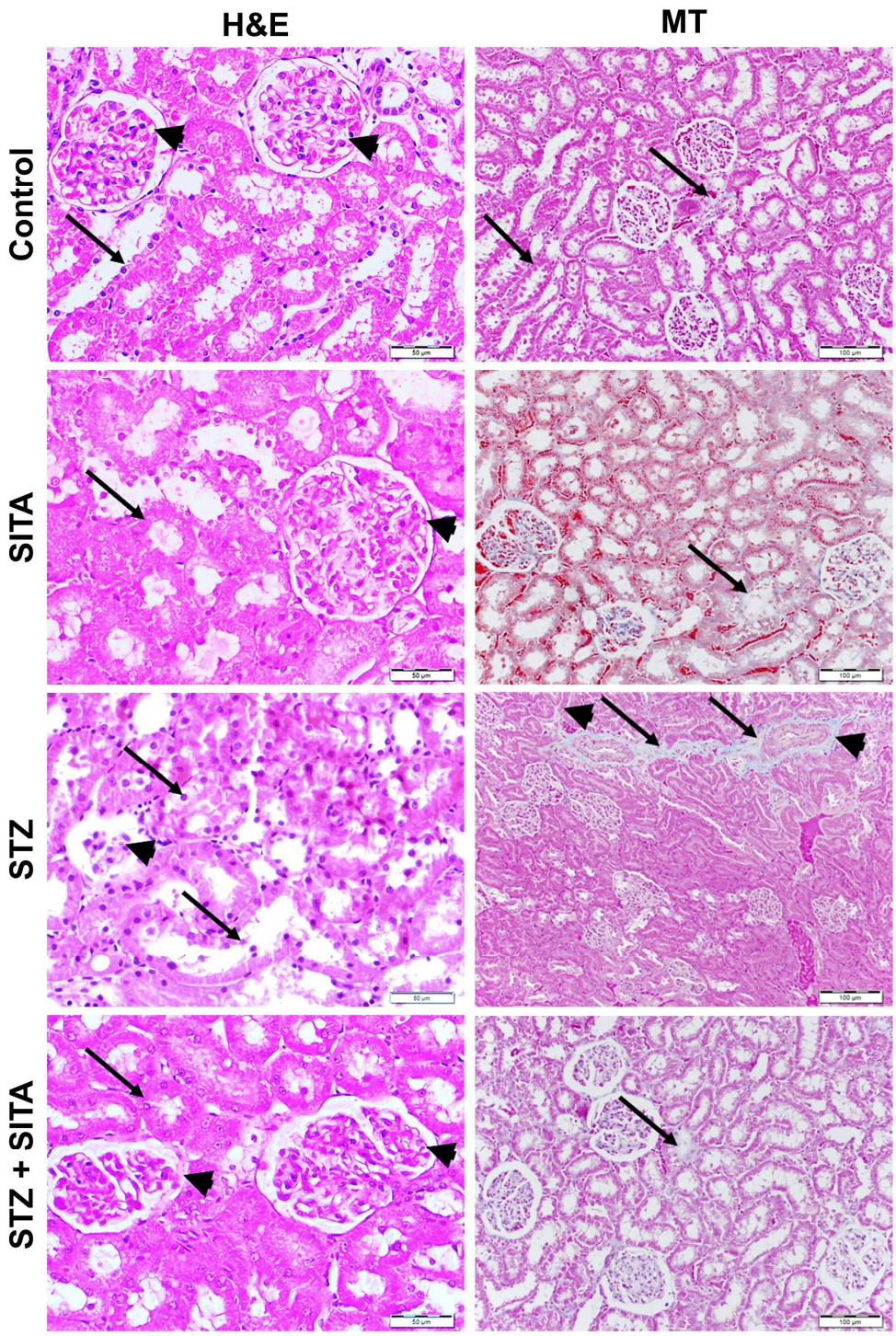

Fig. 3. Sitagliptin attenuated kidney injury in diabetic rats. H&E-stained sections from the control and SITA-supplemented rats showing normal renal corpuscle and glomeruli (arrowhead) and normal different types of tubules (arrow), diabetic rats showing irregular atrophied renal corpuscle (arrowhead), and degenerated tubular epithelium (arrows), and diabetic rats treated with SITA showing remarkable amelioration of the renal corpuscles degeneration (arrowhead) and normal tubules (arrow). (Scale bar = 50 µm). Masson's trichrome-stained sections from the control and SITA-supplemented animals showing normal distribution and amount of interstitial fibers (arrow), diabetic rats showing marked increase in fibrous tissue in the interstitium (arrow), and around blood vessels (arrowhead), and diabetic rats treated with SITA showing marked decrease in fibrotic deposition. (Scale bar = 100 µm).

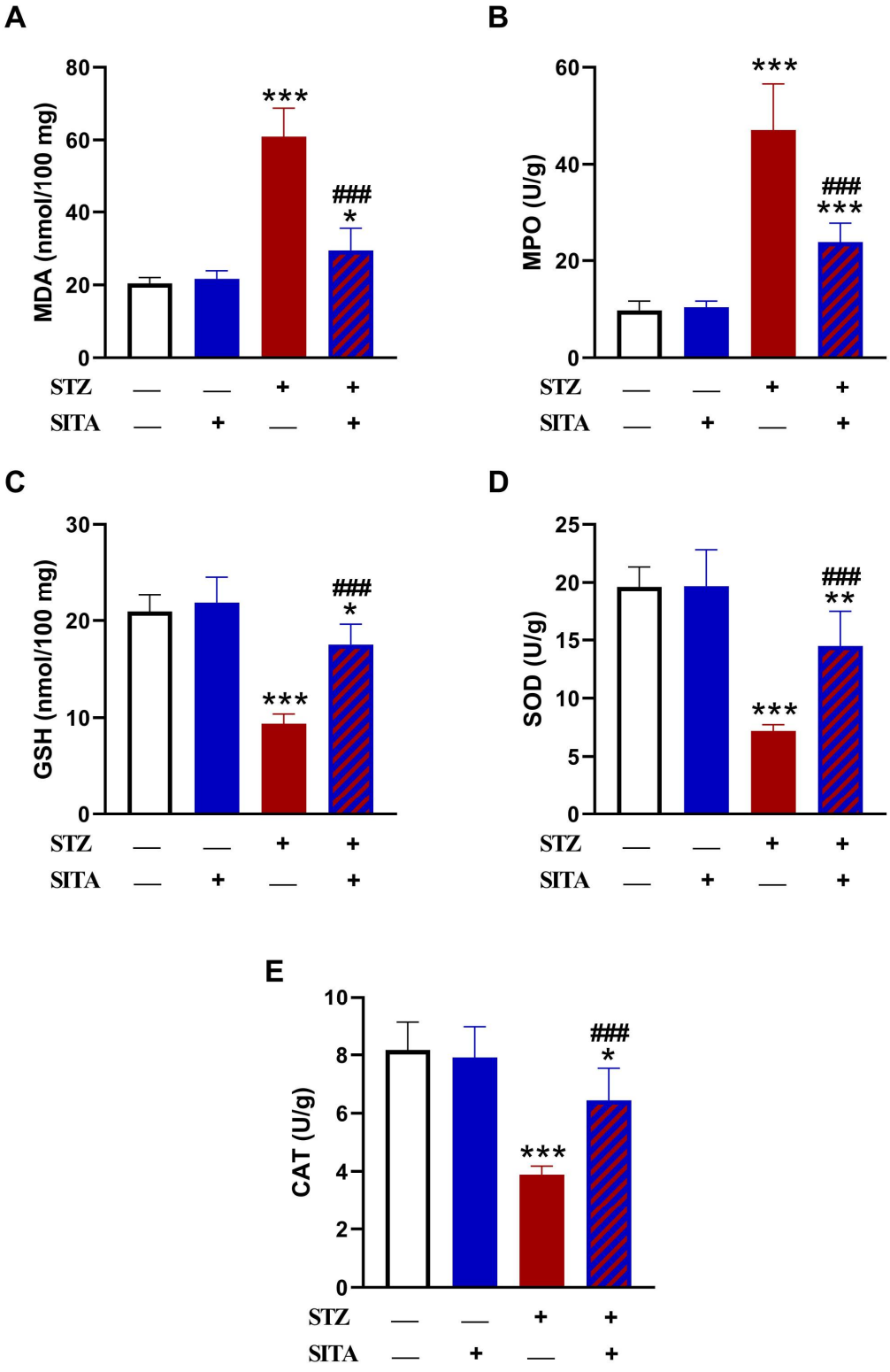

Fig. 4. Sitagliptin prevented oxidative stress in diabetic rats. SITA ameliorated renal malondialdehyde (MDA) (A), and myeloperoxidase (MPO) (B), and increased reduced glutathione (GSH) (C), superoxide dismutase (SOD) (D) and catalase (E) in diabetic rats. Data are mean ± SEM, (n=8). *P<0.05, **P<0.01 and ***P<0.001 versus Control, and $^{###}$P<0.001 versus Diabetic.

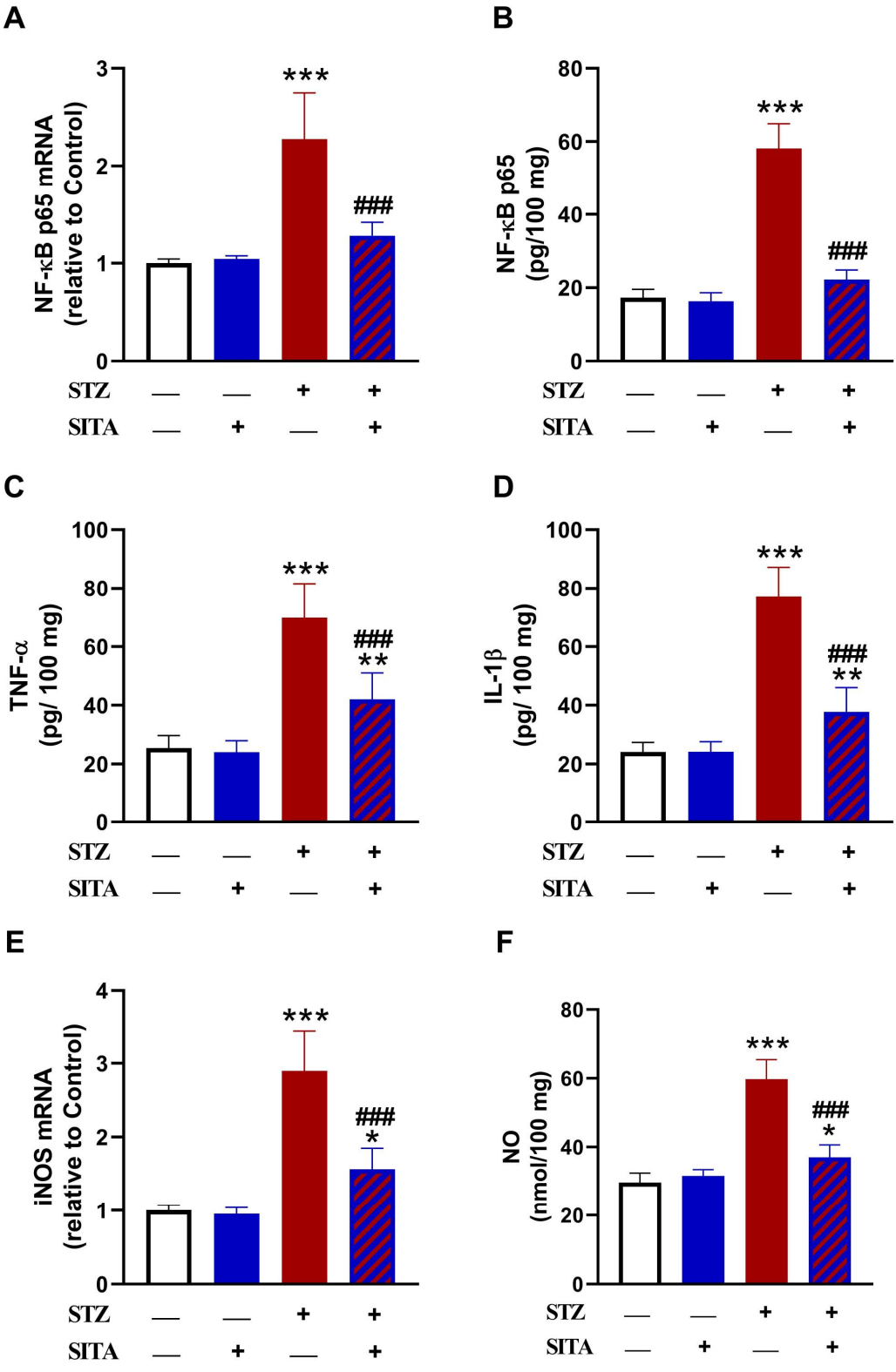

Fig. 5. Sitagliptin downregulated kidney nuclear factor-kappaB (NF-κB) p65 mRNA (A) and protein (B), tumor necrosis factor (TNF)-α (C) and interleukin (IL)-1β (D), inducible nitric oxide synthase (iNOS) mRNA (E), and nitric oxide (NO) levels (F) in diabetic rats. Data are mean ± SEM, (*n*=8). *P<0.05, **P<0.01 and ***P<0.001 versus Control, and $^{###}$P<0.001 versus Diabetic.

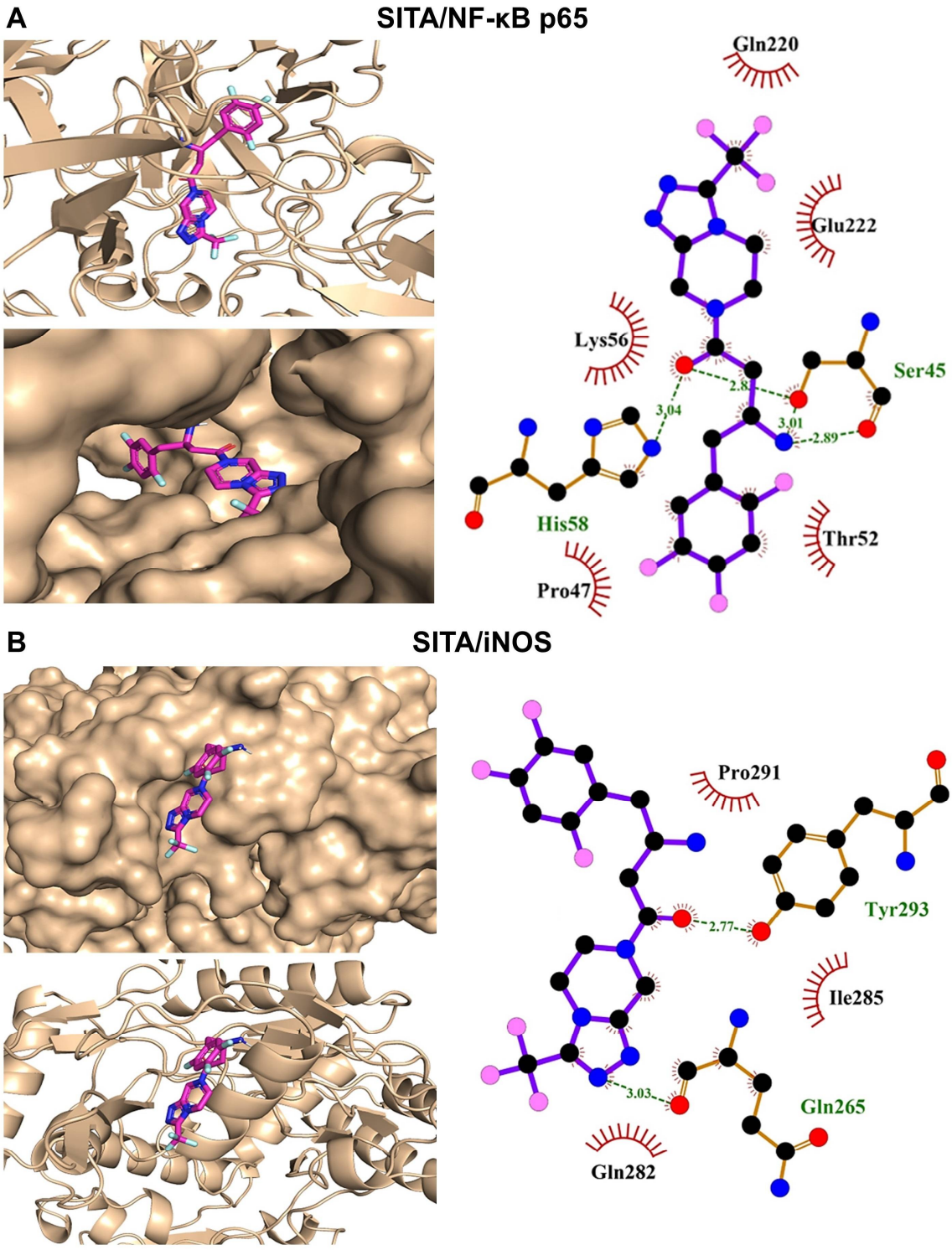

Fig. 6. Molecular docking shows the interaction between sitagliptin and nuclear factor-kappaB (NF-κB) p65 (A) and inducible nitric oxide synthase (iNOS) (B).

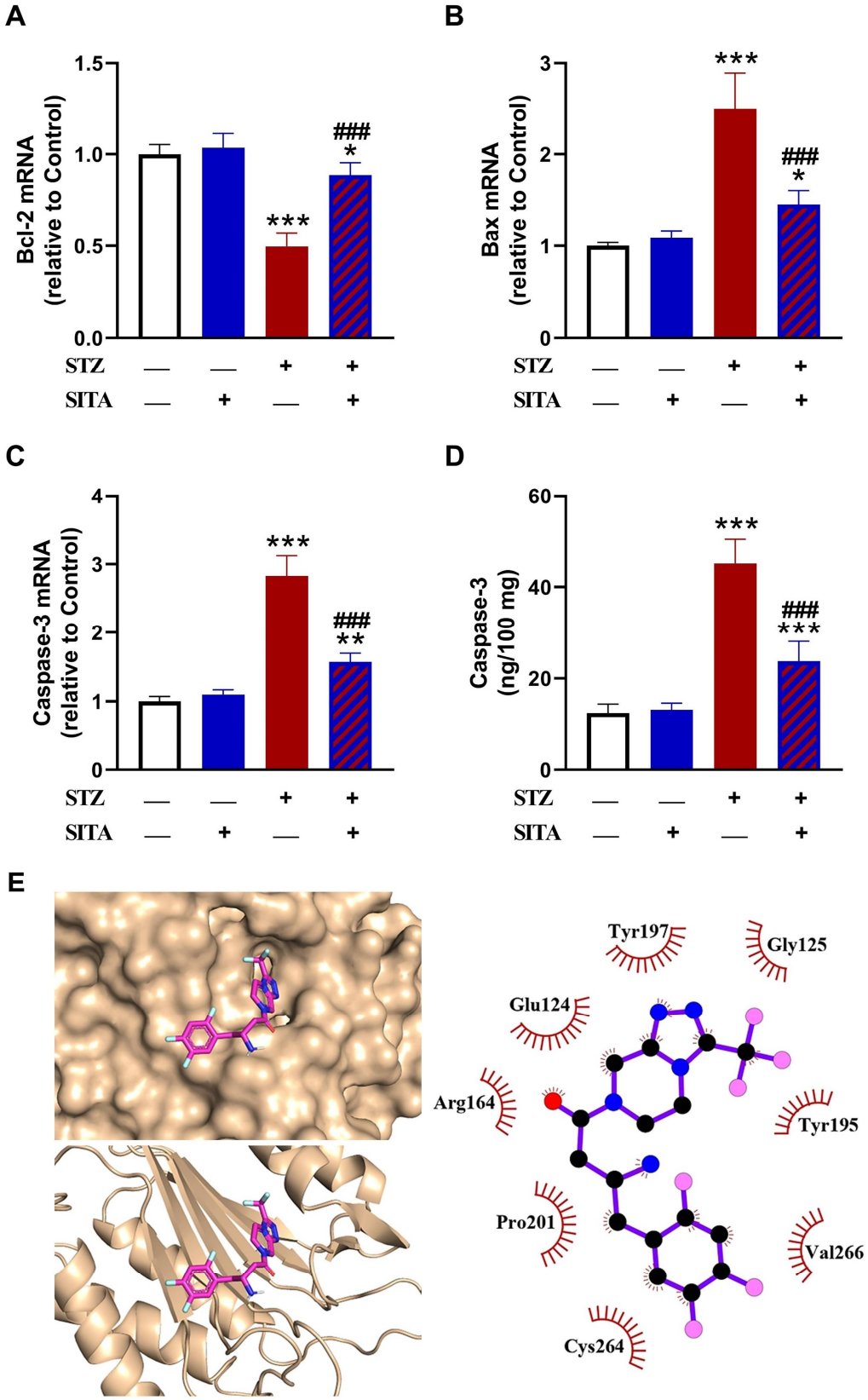

Fig. 7. Sitagliptin upregulated kidney B-cell lymphoma 2 (Bcl-2) (A) and suppressed Bcl-2-associated X protein (Bax) (B) and caspase-3 (C) mRNA, and caspase-3 protein levels (D) in diabetic rats. Data are mean ± SEM, (n=8). *P<0.05, **P<0.01 and ***P<0.001 versus Control, and $^{###}$P<0.001 versus Diabetic. (E) Molecular docking shows the interaction between sitagliptin and caspase-3.

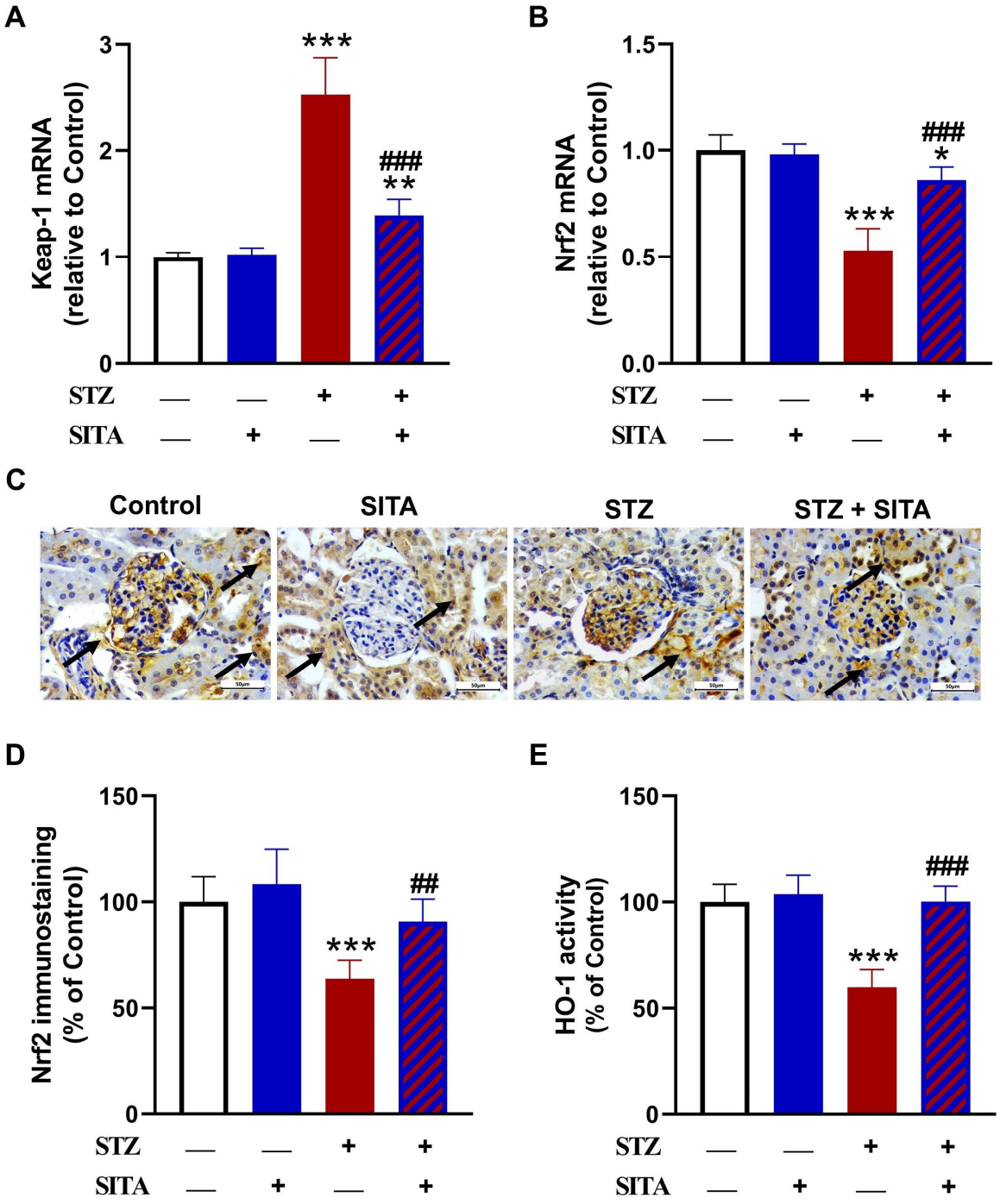

Fig. 8. Sitagliptin enhanced nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase 1 (HO-1) signaling in diabetic rat. Sitagliptin decreased kidney Keap-1 mRNA (A) and upregulated Nrf2 mRNA (B), Nrf2 protein (C-D) and HO-1 activity (E). Data are mean ± SEM, (n=8). *P<0.05, **P<0.01 and ***P<0.001 versus Control. $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 versus Diabetic.

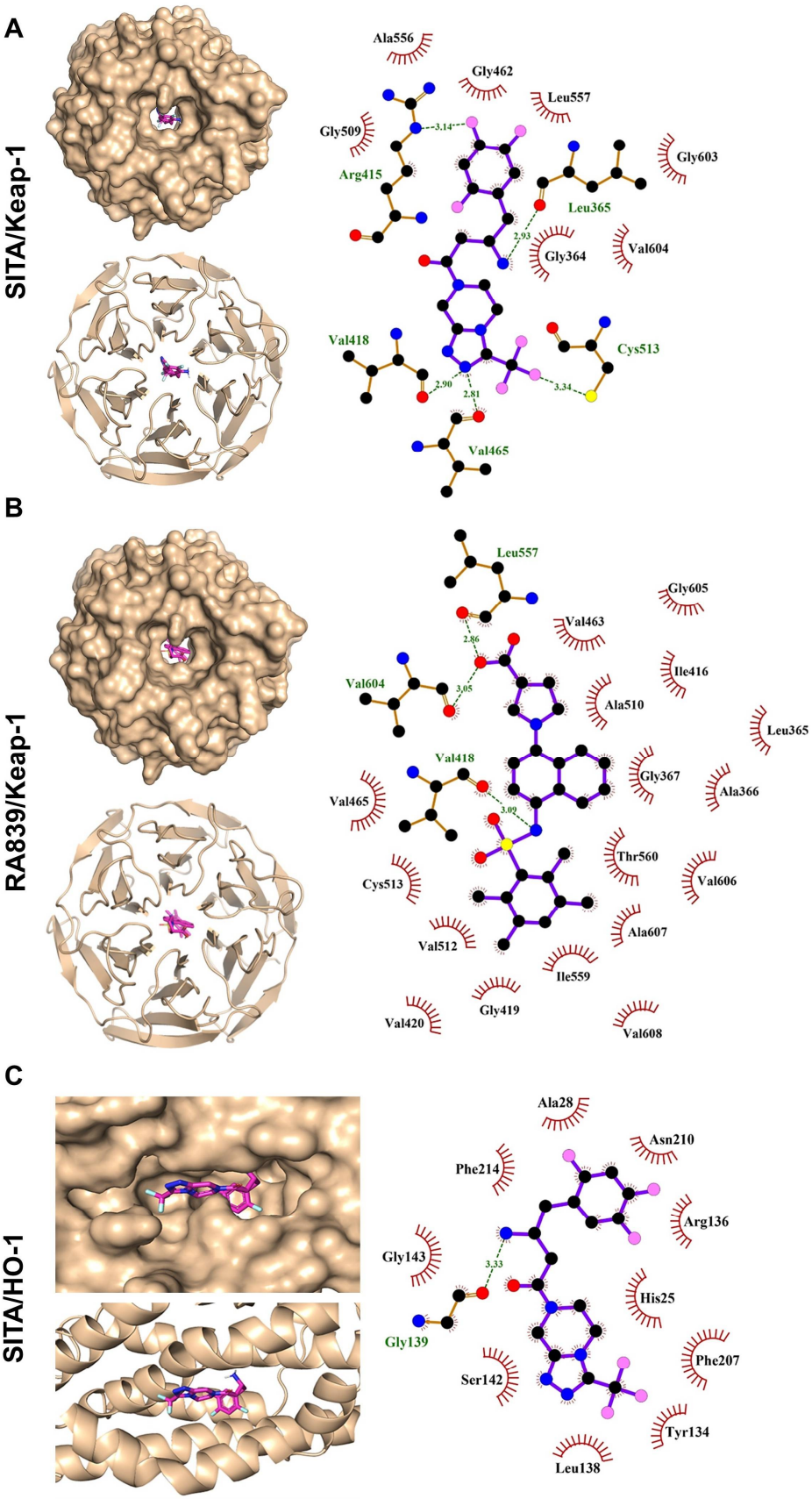

Fig. 9. Molecular docking shows the binding modes of sitagliptin (A) and RA839 (B) with Kelch-like ECH-associated protein 1 (Keap-1) and sitagliptin with heme oxygenase 1 (HO-1) (C).

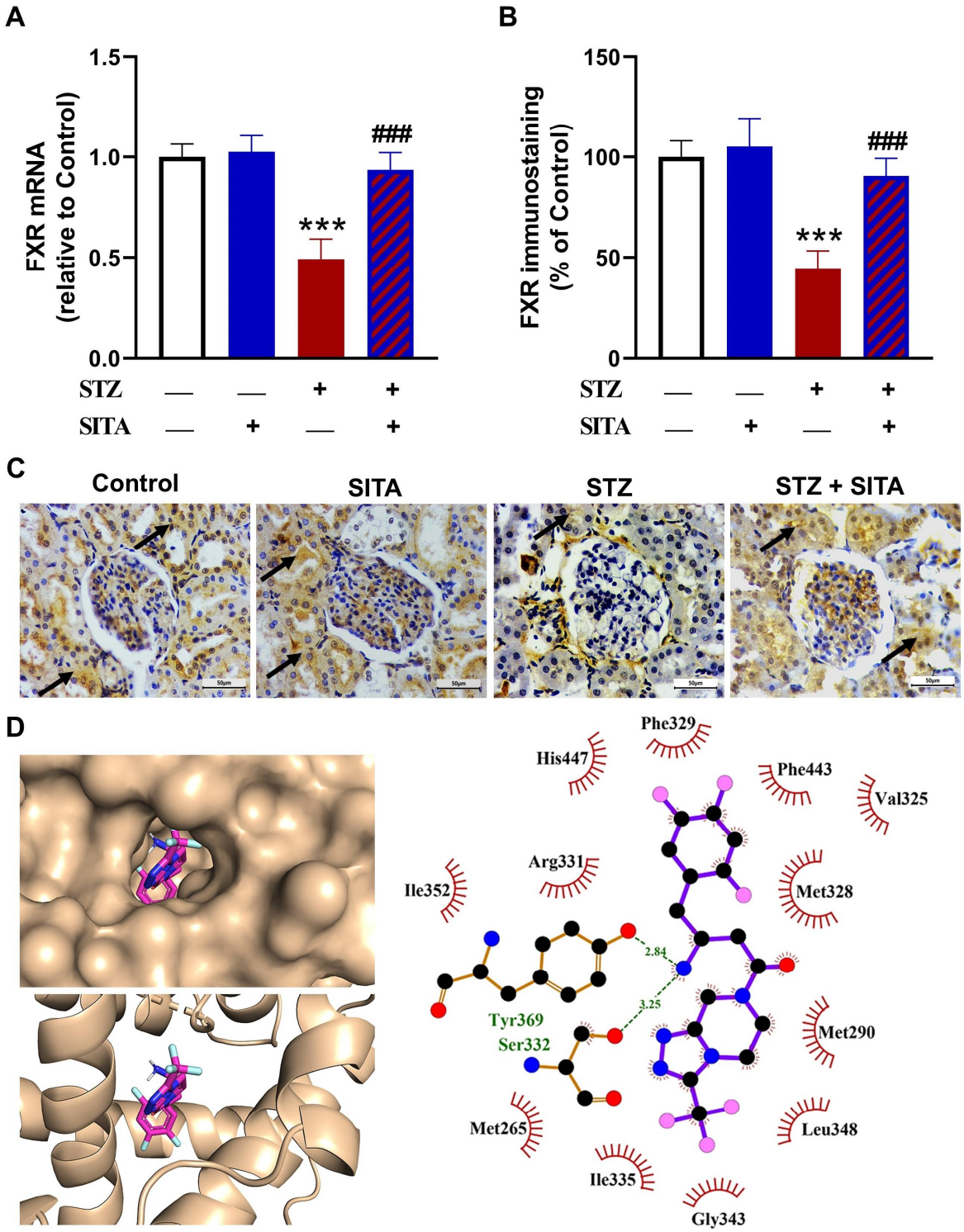

Fig. 10. Sitagliptin upregulated kidney farnesoid X receptor (FXR) in diabetic rat. Sitagliptin increased FXR mRNA (A) and protein (B) in the diabetic kidney. Data are mean ± SEM, (n=8). ***P<0.001 versus Control, and $^{###}$P<0.001 versus Diabetic. (D) Molecular docking showing the binding of sitagliptin with FXR.