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Ameliorative Potential of Sitagliptin Against Doxorubicin-induced Nephrotoxicity Via Inhibition of Oxidative Stress and Downregulation of NF-κB/NLRP3 Axis in Rats

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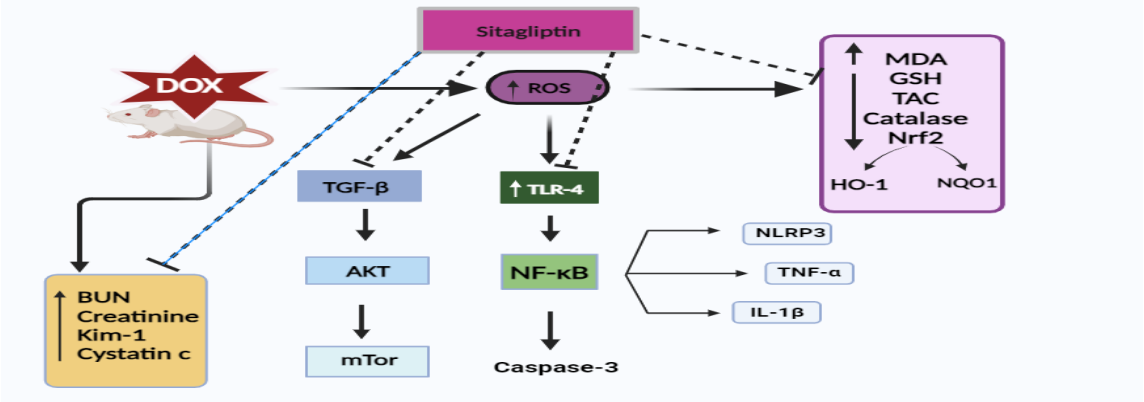
Abstract: Doxorubicin (DOX) is a potent antineoplastic drug. Nevertheless, its invaluable therapeutic application is hindered due to organ toxicities. Nephrotoxicity is one of these toxicities. Sitagliptin is a selective inhibitor of dipeptidyl peptidase-4 enzyme used for the management of diabetes mellitus type II. **Objective:** This study intended to examine the nephroprotective impact of sitagliptin against DOX-induced renal injury and illustrate the potential mechanisms that underlie these protective consequences. **Method:** Rats were subdivided into four groups: Group 1: was administered oral saline for 15 days, with a single i.p injection of saline on the 11th day (negative control), Group 2 (positive control) rats were administered saline for 15 days and treated with a single dose of DOX (15 mg/kg, i.p.) on the 11th day. Group 3: rats received sitagliptin (10 mg/kg) daily for 15 days. Then, a single dose of DOX on the 11th day. Group 4: rats underwent only sitagliptin treatment duration for 15 days. **Results:** DOX administration led to a significant elevation in kidney function biomarkers. Moreover, there was a marked decline in oxidative stress markers, Heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf-2). Additionally, there was a marked increase in inflammatory markers, mammalian target of rapamycin (mTOR), transforming growth factor- β (TGF-β), NOD-like receptor containing pyrin domain 3 (NLRP3), and Toll like receptor 4 (TLR-4). However, sitagliptin demonstrated a significant reduction in DOX-induced injury. **Conclusion:** sitagliptin exhibited a promising nephroprotective effects against DOX-induced renal damage through modulating TLR-4/NF-κB p65/NLRP3 and TGF-β/AKT/mTOR, as well as Nrf-2/HO-1/NQO1 pathways.

Keywords: Nephrotoxicity; Doxorubicin; Sitagliptin.



Graphical Abstract

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1. INTRODUCTION

Doxorubicin (DOX) is an anthracycline chemotherapeutic drug, which is frequently utilized for treating various tumours. DOX nephrotoxicity is amongst the most significant concern during chemotherapy. Previous research has focused on DOX nephrotoxicity; however, an antidote is not available to fully avoid this toxicity. DOX capacity to cause inflammation, oxidative stress, and apoptosis, forms the basis of DOX-induced nephrotoxicity. The accumulation of reactive oxygen species (ROS) can be a key factor contributing to this nephrotoxicity. This effect may stem from the chemical composition of DOX, which promotes ROS generation, thereby inducing oxidative harm to various biological macromolecules and triggering lipid membrane peroxidation[1]. Furthermore, oxidative stress triggered by DOX promotes the release of TNF-α, which subsequently activates multiple signaling cascades, including the NF-κβ-mediated inflammatory pathway. Moreover, DOX exerts a potent pro-apoptotic influence, primarily targeting the mitochondrial pathway [2].

Dipeptidyl peptidase-4 (DPP-4) inhibitors are the preferred oral hypoglycemic agent recommended for management of type II diabetic patients. Sitagliptin is considered as DPP-4 enzyme inhibitor that increase the concentration of glucagon Like peptide-1 (GLP-1)[3]. DPP-4 inhibitors' ability to preserve tissue extends beyond blood glucose regulation. Numerous studies have demonstrated that sitagliptin protects a various organs and tissues; one such study focused on the drug's renoprotective properties against gentamicin-induced nephrotoxicity[4]. Also, sitagliptin inhibited the apoptosis through stimulating the Bcl-2 protein expression and down regulating Bax[5].

The pathogenesis of kidney damage is attributed to an inflammatory response [6]. NLRP3 stands for crucial element in the initiation of inflammation. Adapter protein apoptosis associated speck-like protein (ASC), NLRP3 protein, as well as procaspase-1 are constituents of this inflammasome. Once NF-κB is activated, the NLRP3 protein becomes active, then it interacts with procaspase-1 and ASC, causing the NLRP3 inflammasome to assemble together [7].TGF-β stands for the extremely powerful profibrogenic cytokine that is expressed more frequently in nearly all fibrotic disorders [8]. TGF- β promotes the production of ROS while reducing glutathione [9]. It is widely believed that TGF-β is a significant contributor to the onset of nephropathy caused by diabetes[10]. Interestingly, it was reported that sitagliptin inhibitory impact on diabetic nephropathy is associated with blocking the TGF-β/Smad signal cascade [11]. Unbalanced oxidative stress (OS), inflammation, and apoptosis responses are widely considered as an essential pathogenic factor in advancement of acute kidney injury (AKI) [12].

This instigation seeks to clarify the nephroprotective impact of sitagliptin in rats against DOX-induced kidney damage via evaluating TLR_4/NF-κB/NLRP3 and TGF- β/Akt/mTOR pathways and their potential interplay with other cellular pathways.

2. METHODS

2.1. Animals

Male adult albino Wistar rats of 200-230 g were procured from the National Organization for Drug Control and Research (NODCAR) breeding colony, Giza, Egypt. Animals were maintained within a controlled environment with specific circumstances, including temperature at 23±2°C, humidity at 60±10%, and 12/12-hour light/dark cycle. They had unlimited availability of water ad libitum and standard chow diet. Experimental protocols followed the internationally recognized ethical standards for examinations involving laboratory animals and were verified from the Faculty of Pharmacy's Research Ethical Committee, Al-Azhar University, Cairo, Egypt with permit No. (226/2019).

2.2. Drugs and Chemicals

Doxorubicin (Cat No# D1515) and sitagliptin (CAS No# 486460-32-6) were procured from Sigma-Aldrich, St. Louis, USA. Intraperitoneal (i.p.) administration of DOX was performed using a 23-gauge needle. A saline solution was used to dissolve sitagliptin, which was then given orally utilizing a round-tipped, curved feeding needle. The concentration was adjusted so that 1 ml of the solution contained the required dosage for a 200 gm animal. All additional reagents and chemicals utilized were of high analytical quality and were readily accessible for commercial purchase.

2.3. Experimental Design

Adult male albino Wistar rats (200–230g) were utilized. A random allocation method was employed to divide the rats into 4 groups, eight rats each. The first group represented the control, which was administered saline for 15 days. Additionally, a single i.p injection of saline was administered on the 11th day. The second group animals were given saline for 15 days and then a single DOX dosage (15 mg/kg, i.p.) on the 11th day[13]. The third group of rats

was given sitagliptin for 11 uninterrupted days. Then, they were administered a single DOX (15 mg/kg) i.p. injection on the 11th day, subsequently continued by sitagliptin administration until the 15th day. The fourth group underwent oral sitagliptin administration only (10 mg/kg) for 15 consecutive days [14].

At the end of experiment, rats underwent anesthesia using thiopental (50 mg/kg, i.p). For serum separation, retro-orbital sinuses' blood samples were taken, the animals' weights were measured. After that, rats were euthanized via cervical dislocation. The kidney tissues were then extracted, cleaned with ice-cold saline, dried, and weighed. To create 10% homogenates, kidney tissues were subjected to homogenization within 50 mM phosphate buffer (pH 7.4). To eradicate cell debris, homogenates underwent 5000 rpm centrifuging at 4 °C for 10 min with a refrigerated centrifuge (Hermile Labortechnik, Germany). Biochemical evaluations were conducted using tissue supernatants. A 10% formal saline solution was used to fix other kidney tissues for subsequent histopathological analysis.

2.4. Colorimetric Assay

Renal functions and renal oxidative stress markers were calorimetrically quantified utilizing an ultraviolet (UV)—visible spectrophotometer (Shimadzu UV-1601, 84, Tokyo, Japan). The guidelines provided by the manufacturer were followed while utilizing test kits purchased from Biodiagnostic (Giza, Egypt). BUN measurements depends upon chromogenic reagent, which produces a blue colored complex [15]. Serum creatinine was determined by its reaction with picric acid within alkaline medium, promoting the establishment of a red-colored complex. The red color complex intensity is indicative of the amount of creatinine present [16]. The total antioxidant capacity (TAC), catalase (CAT), Reduced glutathione (GSH), and Malondialdehyde (MDA) contents were evaluated by Biodiagnostic kits (Cairo, Egypt; Cat. # GR 2511; TA 2513; CA 2517; MD 2529, respectively). The evaluation of GSH was conducted following the procedure outlined in Beutler et al.(1963)study. GSH has the ability to reduce the reactivity of Ellman's reagent [5,5-dithiobis (2-nitrobenzoic acid)] (DTNB) to form a stable yellow product (5-mercapto-2-nitrobenzoic acid) that can be quantified using a colorimetric method at 405 nm. Moreover, the assessment of lipid peroxidation was conducted by quantifying the quantity of thiobarbituric acid reactive substances (TBARS) at 534 nm, utilizing a colorimetric method outlined in the study by Ohkawa et al. (1979). TAC was assessed by measuring the sample's antioxidant capacity through its reaction with a specified quantity of externally supplied hydrogen peroxide. The colorimetric determination of residual H_2O_2 is accomplished throughout an enzymatic reaction that converts 3,5 dichloro-2-hydroxy benzensulphonate to a colored product, and this method was originally documented by Koracevic et al. (2001). According to Fossati et al. (1980) CAT was assessed using H_2O_2. The remaining H_2O_2 react with chromogenic agent to form a chromophore whose color intensity having inverse correlation with the CAT level. Renal function markers were evaluated in serum but oxidative stress markers were measured in renal tissue. Data were reported as mg/dl for BUN and serum Creatinine, U/g tissue for CAT, nmol/g tissue for MDA, mg/g tissue for GSH, and mmol/g tissue for TAC.

2.5. Enzyme Linked Immunosorbent Assay (ELISA)

ELISA kits from MyBioSource, San Diego, CA, USA (Cat# 92195-3308) were utilized to measure serum KIM-1 and serum Cystatin C (Cat# 92195-3308), the results were stated as pg/ml. Also, TNF-α and IL-1β tissue levels were quantified utilizing Rat ELISA Kit (Cusabio Biotech, Chins; CSB-E11987r and CSB-E08055r, correspondingly) adhering the manufacturer's instructions, where the readings for TNF-α were reported as ng/mg protein, while the readings for IL-1β were reported as pg/mg protein. Additionally, Caspase-3 and TGF-β concentrations were assessed in renal tissue with ELISA kit (Korain Biotech., Shanghai, China; E0280Ra and E0779Ra, respectively) per the manufacturer's instructions and the findings were reported as ng/mg protein and pg/mg protein for caspase-3 and TGF-β, respectively. Also, protein kinase B (AKT/ PKB) and Rat mTOR were estimated utilizing Rat ELISA Kit (MyBioSource, San Diego, CA, USA; MBS3807836 and MBS744326, correspondingly) in compliance with the guidelines provided by the manufacturer. The values for AKT/PKB and mTOR were reported as ng/mg protein.

2.6. Western Blot Analysis

The renal tissue homogenates were lysed employing an ice-cold lysis buffer comprising glycerol (10%), SDS (2%) within 62 mM Tris-HCl, pH 6.8. This lysis buffer was supplemented with a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). The content of the protein within the combined protein lysates was determined employing the Bradford method, which employed bovine serum albumin as a reference standard [21]. To

analyze the total protein content, a corresponding amount of denatured protein (7.5 µg) was loaded onto sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Afterwards, the proteins have been transferred into nitrocellulose membranes. To prevent non-specific binding, the membranes underwent incubation with a blocking solution consisting of 6% non-fat dry milk within TBS-Tween buffer at 4 °C for 3 hours. The nitrocellulose membranes underwent overnight incubation at 4 °C with the certain primary antibody versus the recognized protein [anti-TLR-4 (1:200; Thermo-Fisher scientific, PA, USA, Cat # 48-2300), anti-NF-κB p65 (1:4000; Thermo-Fisher scientific, PA, USA, Cat # PA5-16545), anti-NLRP3 (1:20000: Abcam, USA, Cat# 214185), anti-Nrf-2 (1:1000; Invitrogen, Thermo-Fisher scientific, PA, USA ,Cat # PA5-27882), anti HO-1 (1:1000; Abcam, USA, Cat #13243), anti-NQO1 (1:1000; Abcam, USA, Cat #2346)]. On the subsequent day, an antibody against β-actin, a commonly used housekeeping gene, was added to the nitrocellulose membranes. The membranes were positioned over a roller shaker and underwent 1-hour incubation at 4 °C. Afterward, the membranes underwent a series of five 5-minute washing via Tris-buffered saline-tween 20 (TBST). After being washed, they were subjected to incubation at ambient temperature for 1 hr with a Horseradish Peroxidase (HRP)-conjugated secondary antibody (1:2000; Novus Biologicals, Centennial, CO, USA; Cat. No. NBP1-75297). Moreover, development and analysis of bands. Following three washes with TBST, equivalent volumes of Solution A and Solution B from the improved chemiluminescence (ECL) fluorescence detection kit (Amersham, UK) were combined in darkness and applied to the membrane. Imaging was performed using the Gel Imaging System (E-Gel® Imager, Beijing Liuyi Biotechnology Co., Ltd., Beijing, China) under chemiluminescent conditions. The gray intensity was analyzed with Quantity One v4.6.2 software (Bio-Rad, Hercules, CA, USA), and the relative expression of the target protein was determined by calculating the ratio of the target gene's gray value to that of beta-actin.

2.7. Histopathological Examination

The kidney tissues from different groups were preserved by immersing them in 10% neutral buffered formalin. Afterward, the samples were subjected to a cleaning process using xylene, followed by immersion in paraffin blocks. The paraffin blocks were left to solidify by incubating them within a hot air oven for 24 hours at 56°C. The tissue samples were used to prepare paraffin beeswax blocks, which were then sliced to a thickness of 4 µm employing a sledge microtome for sectioning. Sections of kidney tissue were mounted onto glass slides, subjected to deparaffinization, and staining with hematoxylin and eosin stain for light microscopy analysis[22].

2.8. Statistical Analysis

The findings were displayed as the Mean ± standard deviation (S.D). The Tukey multiple comparison test was applied following a one-way ANOVA to conduct the statistical analysis. $p < 0.05$ served as the significance criterion. Statistical analysis was conducted employing Version 5 of the GraphPad Prism software (San Diego, CA, USA), and also employed to create graphs. The findings are described as % of change.

3. RESULTS

3.1. Influence of Sitagliptin on Renal Function Markers in DOX-Induced Nephrotoxicity in Rats

Single dose of doxorubicin deteriorated kidney functions which were manifested by elevations in BUN (106%), serum creatinine (385%), cystatin-c (418%), and KIM-1 (195%) in comparison to the controls. In comparison to DOX-treated rats, sitagliptin administration resulted in significant reductions in BUN, serum creatinine, cystatin-c, and KIM-1 levels of (42%, 61%, 55%, and 47%), respectively. Besides, these parameters remain unchanged in the sitagliptin only group as opposed to the control group (table 1).

Table 1. Influence of sitagliptin on renal function markers in DOX-induced nephrotoxicity in rats.

Treatment	BUN (mg/dl)	Serum Creatinine (mg/dl)	KIM-1 (pg/ml)	Cystatin-c (pg/ml)
CONT	24.52 ± 3.25	1.50 ± 0.38	40.95 ± 4.12	16.77 ± 1.32
DOX	50.46 ± 6.93[a]	7.27 ± 0.97[a]	121.20 ± 2.14[a]	86.97 ± 8.19[a]
SITA+DOX	29.30 ± 2.94[b]	2.85 ± 0.38[ab]	64.76 ± 2.24[ab]	39.50 ± 3.42[ab]
SITA	25.19 ± 2.44[b]	1.88 ± 0.11[b]	36.55 ± 2.51[b]	15.25 ± 0.87[b]

Data are represented as Mean ± S.D (n=8). Statistical analysis was carried out employing one-way ANOVA, subsequently using the Tukey multiple comparison test. a: Significantly different from the CONT group at $P < 0.05$. b: Significantly different from the DOX group at $P < 0.05$. Where, CONT; control, DOX; doxorubicin, SITA; sitagliptin, KIM-1; kidney injury molecule-1, BUN; blood urea nitrogen.

3.2. Influence of Sitagliptin on Renal Oxidative Stress Markers in DOX- Caused Nephrotoxicity in Rats

Administering DOX in a single dosage reduced renal content of GSH, CAT activity and TAC levels by (44%, 35% and 62%) and increased MDA level by (142 %), respectively when compared to control rats. Also, immunoblotting of HO-1, Nrf-2, and NQO1 protein levels demonstrated that rats received DOX demonstrated a marked reduction in renal HO-1, Nrf-2, and NQO1 expressions by (72%, 72%, and 69%) respectively relative to the controls. In addition, pre-treatment with sitagliptin enhanced the renal GSH, CAT and TAC levels by (157%, 64%, and 162%), respectively, and induced a substantial drop in the renal MDA by 45% relative to DOX treated rats (table 2). In addition, sitagliptin pretreatment developed a notable elevation in Nrf-2, HO-1 and NQO1 expressions by (189%, 189% and 152%) respectively relative to the DOX-treated rats (Figure 1). In contrast to the controls, sitagliptin alone-treated rats revealed normal levels of all the aforementioned parameters.

Table 2. Influence of sitagliptin on renal oxidative Stress markers in DOX-induced nephrotoxicity in rats

Treatment	GSH (mg/g tissue)	CAT (u/g tissue)	TAC (mmol/g tissue)	MDA (nmol/g tissue)
CONT	20.38 ± 3.79	1.16 ± 0.08	2.22 ± 0.26	14.67 ± 2.50
DOX	11.34 ± 0.41[a]	0.75 ± 0.13[a]	0.85 ± 0.05[a]	35.43 ± 6.36[a]
SITA+DOX	29.15 ± 4.81[ab]	1.23 ± 0.05[b]	2.23 ± 0.15[ab]	19.48 ± 2.61[b]
SITA	25.19 ± 2.44[b]	1.10 ± 0.08[b]	1.87 ± 0.03[b]	12.06 ± 1.12[b]

Data are represented as Mean ± S.D (n= 8). a: Significantly different from the CONT group at P < 0.05. b: Significantly different from the DOX group at P< 0.05. Where, CONT; control, DOX; doxorubicin, SITA; sitagliptin, GSH; reduced glutathione, MDA; malondialdehde, CAT; catalase and TAC; total antioxidant capacity.

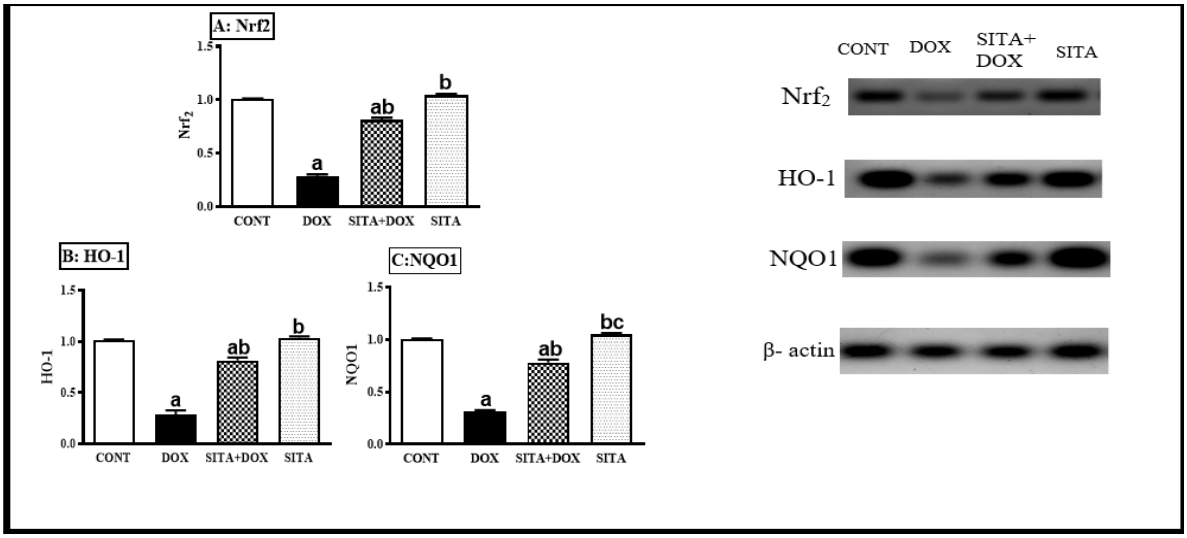

Figure 1. Renoprotective effects of sitagliptin on nuclear factor erythroid 2-related factor 2 (Nrf-2), heme oxygenase-1 (HO-1), NAD(P)H: quinine oxidoreductase 1 (NQO1). Data are represented as Mean ± S.D (n= 8). Statistical analysis was carried out using one-way ANOVA suessed by Tukey multiple comparison test; P< 0.05. a, b Significantly different from the control (CONT) and doxorubicin (DOX) groups, respectively.

3.3. Influence of Sitagliptin on Renal Inflammatory Markers in DOX-Induced Nephrotoxicity in Rats

Following intraperitoneal injection of DOX, kidney contents of IL-1 β and TNF-α increased by (55 % and 177 %), consequently. This marked elevation in TNF-α and IL-1β was decreased by (29 % and 24%) correspondingly in rats pre-treated with sitagliptin relative to the DOX group. Furthermore, rats administered sitagliptin only exhibited no noticeable change in inflammatory markers relative to the controls (table 3).

3.4. Effect Of Sitagliptin on Renal TLR-4, NF-Kb P65 And NLRP3 in DOX-Induced Nephrotoxicity in Rats

Figure 2 demonstrated that DOX promoted a notable elevation in NF-κB p65, TLR 4, and NLRP3 levels by (398%, 397% and 455%), respectively compared to the controls. Alternatively, sitagliptin administration significantly reduced their levels by (66%, 62% and 66%), respectively. Also, non-significant changes in levels of expression were detected for TLR 4, NF-κB p65 and NLRP3 for sitagliptin alone group relative to the controls (Figure 2).

Table 3. Influence of sitagliptin on renal inflammatory markers in DOX-caused nephrotoxicity in rats.

Treatment	TNF- α (ng/mg protein)	IL-1β (pg/mg protein)
CONT	47.20 ± 9.05	124.7 ± 15.41
DOX	130.9 ± 8.43[a]	193.7 ± 17.01[a]
SITA+DOX	92.71 ± 10.48 [ab]	146.1 ± 3.09 [ab]
SITA	79.66 ± 5.21[b]	133.7 ± 9.78 [b]

Data are represented as Mean ± S.D (n= 8). a: Significantly different from the CONT group at P< 0.05. b: Significantly different from the DOX group at P< 0.05. Where, CONT; control, DOX; doxorubicin, SITA; sitagliptin, TNF-α; tumor necrosis factor α, IL-1β; interleukin 1β.

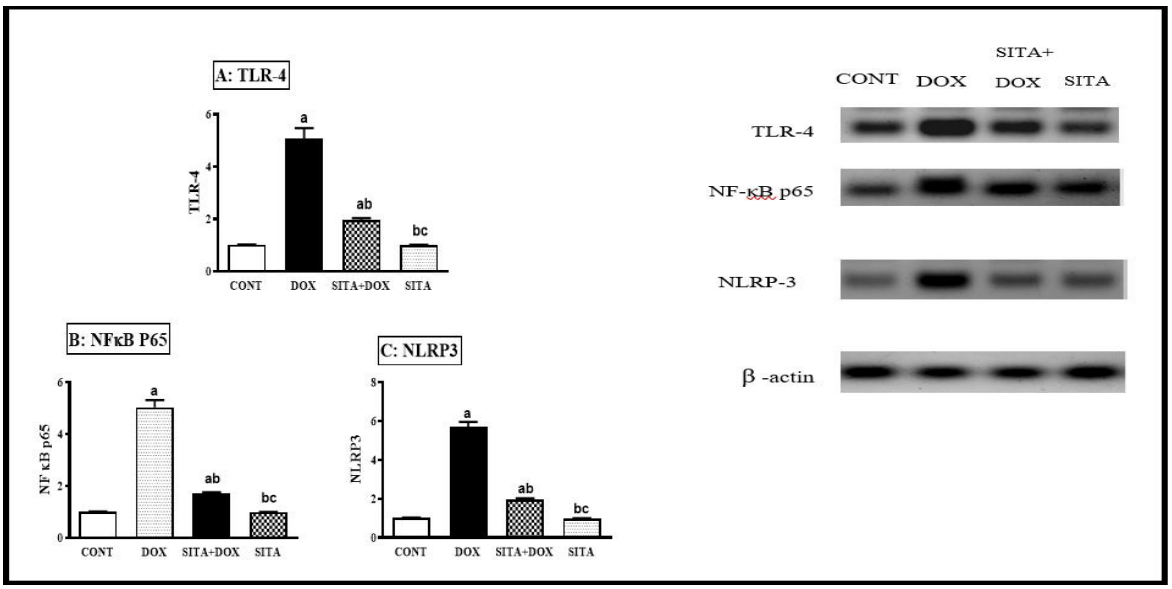

Figure 2. Effect of sitagliptin on toll like receptor 4, nuclear factor κappa B p65 and NOD- like receptor containing pyrin domain 3 in doxorubicin-induced nephrotoxicity in rats. For each group of 8 rats, values are presented as Mean ± S.D. a or b: Significantly different from the control group and doxorubicin (DOX) group correspondingly at P< 0.05 employing one-way ANOVA followed by Tukey multiple comparison test.

3.5. Influence of Sitagliptin on Renal TGF-B, Akt and Mtor in DOX-Induced Nephrotoxicity In Rats

When a single dose of DOX was given, a considerable increase in TGF-β, Akt and mTOR levels by (148%, 160% and 148%) respectively was reported compared to control rats. However, pretreatment with sitagliptin orally for 15 days prior to DOX administration significantly decreased renal TGF-β, mTOR and Akt by (40%, 44% and 42%), respectively, relative to DOX treated rats (table 4).

3.6. Influence Of Sitagliptin on Renal Casp-3 in DOX-Induced Nephrotoxicity in Rats

Administration of DOX induced a marked elevation in the activity of Casp-3 in renal tissue by (76%) relative to the controls. However, sitagliptin pre-treatment evidently reduced the Casp-3 level by (23%) with respect to the DOX-treated group. The sitagliptin alone group revealed no appreciable differences in the Casp-3 expression levels relative to the control group (table 5).

3.7. Influence of Sitagliptin on Histopathological Changes Caused by DOX in Rats

As demonstrated in Figure (3), The pathological inspection of kidney sections from the controls (Figure 3A) and sitagliptin only (Figure 3 D) displayed normal renal histological morphology of glomeruli and tubules, whereas DOX-treated rats exhibited pronounced cortical blood vessel congestion along with degenerative changes in the tubular epithelium (Figure 3 B). They showed significant tubule degeneration along with cortical

blood vessel dilatation and congestion. Additionally, pre-treatment with sitagliptin mitigated the deteriorating impact of DOX on renal glomeruli and tubules (Figure 3 C).

Table 4. Influence of sitagliptin on renal TGF-β, Akt and mTOR in DOX-induced nephrotoxicity in rats.

Treatment	TGF- β (pg/mg protein)	mTOR (ng/mg protein)	AKT/PKB (ng/mg protein)
CONT	12.81 ± 1.71	17.12 ± 3.67	125.90 ± 12.44
DOX	31.73 ± 2.21[a]	42.60 ± 2.27 [a]	327.40 ± 25.84 [a]
SITA+DOX	18.96 ± 1.07 [ab]	23.87 ± 4.51 [ab]	188.70 ± 35.10 [ab]
SITA	11.50 ± 1.50 [b]	15.68 ± 1.95 [b]	112.20 ± 0.93 [b]

Data are represented as Mean ± S.D (n= 8). a Significantly different from the CONT group at P< 0.05.
b Significantly different from the DOX group at P< 0.05. Where, CONT; control, DOX; doxorubicin, mTOR; mammalian target of rapamycin, SITA; sitagliptin, TGF-β; transforming growth factor- β, Akt/PKB; protein kinase B.

Table 5. Influence of sitagliptin on renal Casp-3 in DOX-induced nephrotoxicity in rats.

Treatment	Casp-3 (ng/mg protein)
CONT	2.15 ± 0.25
DOX	3.78 ± 0.15 [a]
SITA+DOX	2.91 ± 0.17 [ab]
SITA	2.26 ± 0.17 [b]

Data are represented as Mean ± S.D (n= 8). a Significantly different from the CONT group at P< 0.05.
b Significantly different from the DOX group at P< 0.05. Where, CONT; control, DOX; doxorubicin, SITA; sitagliptin, Casp-3; caspase-3.

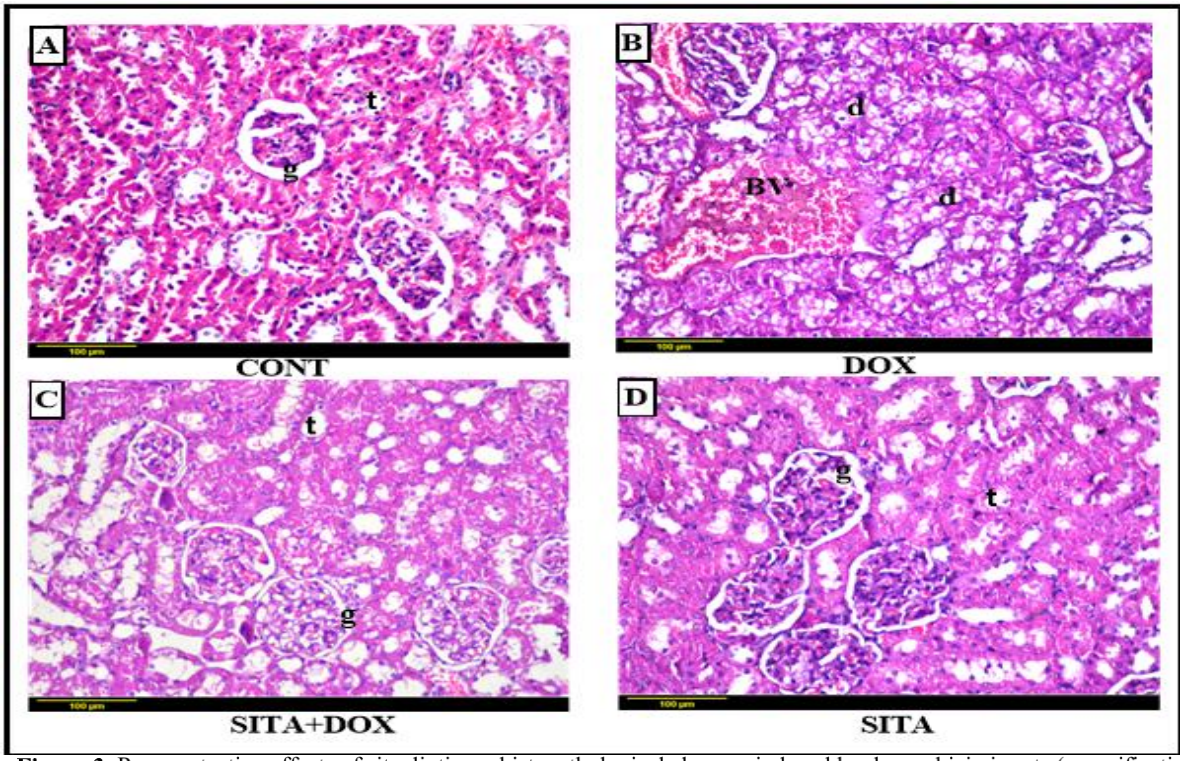

Figure 3. Renoprotective effects of sitagliptin on histopathological changes induced by doxorubicin in rats (magnification ×400). (A–D) H & E staining. Kidney sections from the control and SITA groups exhibit a typical histological arrangement of glomeruli (g) and tubules (t) within the cortex, as illustrated in Figure A and D, correspondingly. Kidney sections from the DOX group exhibited pronounced congestion in cortical blood vessels (BV) alongside degenerative alterations (d) in the tubular epithelial lining, as depicted in Figure B. In contrast, kidney sections from the SITA+DOX group displayed a normal histological architecture of the glomeruli (g) and tubules (t), as illustrated in Figure C.

4. DISCUSSION

Despite the widespread clinical use of doxorubicin (DOX in treating a range of human cancers, the drug's multi-organ toxicity, including nephrotoxicity, places restrictions on how it can be used. Hence, in order to increase the treatment's effectiveness and lessen any negative side effects, adjuvant therapy must be developed for use in conjunction with DOX chemotherapy. DOX capacity to cause inflammation, oxidative stress, and apoptosis is the basis for the mechanism of DOX-induced nephrotoxicity [23]. Tissue damage occurs through a process in which oxidative stress exerts a crucial influence. It arises from a disparity amid the generation of ROS and the body's capability to counteract or mend the subsequent harm [24]. The kidney being a highly metabolic organ with abundant mitochondrial oxidation processes, is particularly vulnerable to oxidative stress (OS) [25]. In our investigation, i.p administration of DOX persuaded intense nephrotoxicity as verified from considerably elevated serum concentrations of urea, creatinine, KIM-1 and cystatin C. These outcomes accorded with those of Soliman et al. (2023) and Abdelrahman et al. (2020). The rise in nephrotoxicity biomarkers is suggested to be a result from the accumulation of DOX toxic metabolites within nephrons, as well as the reduced glomerular filtration rate caused by the direct interaction of DOX with renal DNA. Additionally, the increase in ROS that accumulated after DOX metabolism significantly contributes to DOX-induced nephrotoxicity [28]

The histopathological examination provided further confirmation to the biochemical results. DOX-treated rats displayed significant congestion within the cortical blood vessels, accompanied by degenerative changes within the lining epithelial cells of the renal cortex. These findings agreed with Manawy et al. (2024), who indicated that DOX-treated group exhibited noticeable renal tubular damage.

This research revealed that DOX resulted in significant disturbance of the renal antioxidant/oxidant capacity that indicated by a noticeably increased MDA levels and reduced GSH content, TAC and CAT concentrations along with Nrf-2, HO-1 and NQO1 expressions. These findings agreed with those of Khames et al. (2017); Antar et al. (2023) and Lin et al. (2019). These outcomes could be explained by GSH consumption on by lipid peroxidation induced by DOX, either directly through using the semiquinone structure or indirectly due to the generation of ROS [33]. In addition, the increased MDA level is a reasonable consequence of the lipid peroxidation and intense oxidative stress which caused by DOX [34]. DOX-mediated generation of oxidative free radicals within renal tissues results in significant oxidative stress [33]. This stress cascade drives structural and functional alterations in the kidney, and is widely recognized as the primary mechanism underlying DOX-induced nephrotoxicity [30, 33]. Nrf-2 serves as a vital cellular regulator against oxidative stress. Moreover, HO-1 is a critical protein that actively participates in the body's defense against oxidation [32].

Result of the current investigation revealed that DOX administration notably elevated the contents of the inflammatory mediators in kidneys; TLR4, NF-κB, NLRP3, IL-1β and TNF-α. These findings agreed with those of Kobayashi et al. (2016) and Khames et al. (2020).The elevation of these inflammatory mediators is believed to result from the heightened oxidative stress induced by ROS produced from the semi-quinone form of DOX. This occurs once antioxidant mechanisms become depleted, rendering the tissues more susceptible to inflammation and damage [37]. Transmembrane proteins called toll-like receptors (TLRs) constitute a necessity for innate immunity [38]. Stimuli from immune receptors, such as TLRs, which are increased in response to oxidative stress, activate NF-κB [39]. NF-κB activation facilitates the increased transcription of various components linked to the NLRP3 inflammasome. With the further stimuli, the NLRP3 protein underwent oligomerization, promoting the assembly of procaspase-1, NLRP3 protein, and ASC into the NLRP3 inflammasome complex [7]. The NLRP3 inflammasome effectively initiates the conversion of procaspase-1 into caspase-1, facilitating the production of mature IL-1β and IL-18 from pro IL-1 β and pro IL-18. These mature cytokines are then extracted into the extracellular environment, triggering a cascade of immune or inflammatory responses [7,40]. Also, Ali et al. (2023) observed that, NF-κB activation promotes the transcription of inflammatory cytokines like TNF-α and IL-1β. Data of existing study also revealed that, DOX administration results in marked elevation of apoptotic marker Caspase-3. Such findings agreed with previous researches [41,43]. As the apoptotic pathway activated by oxidative stress and TNF-α, leading to a notable increase in tissue casp-3 level [42].

In this investigation, DOX has been proved to trigger a marked upregulation in TGF-β, AKT and mTOR signaling pathway. Similar outcomes were also documented by Mohamed et al. (2018) and Soltani Hekmat et al. (2021). Elevated ROS concentrations by DOX can subsequently raise the expression of TGF-β and promote the TGF-β releasing from the latent complex, heightening the

bioavailability and activity of this growth factor [45]. Additionally, TGF-β activates various pathways, including the nuclear factor κB (NF-κB), mitogen-activated protein kinases (MAPKs), and phosphoinositide 3-kinase (PI3K) [46]. Upon activation, phosphatidylinositol (3,4)-bisphosphate (PIP2) lipids are converted into phosphatidylinositol (3,4,5)-trisphosphate (PIP3) by the catalytic domain of PI3K. Upon binding of Protein Kinase B (PKB/Akt) to PIP3 at the plasma membrane, PKB/Akt enables 3-phosphoinositide-dependent protein kinase 1 (PDK1) to reach and phosphorylate PKB/Akt, leading to partial activation of PKB/Akt (Akt) [41]. The modification of PKB/Akt in this manner effectively stimulates mammalian target of rapamycin (mTOR) signal by directly phosphorylaing and inactivating tuberous sclerosis protein 2 (TSC2) [47]

Dipeptidyl peptidase-4 (DPP-4) inhibitors as sitagliptin are now employed in treating T2DM to enhance glucose tolerance by prolonging the half-lives of GLP-1 and glucose-dependent insulinotropic peptide [48]. Nevertheless, apart from their ability to lower glucose levels, DPP-4 inhibitors have also been shown to possess renal protective effects [49].

In particular, research has indicated that DPP-4 inhibitors provide kidneys protection against chronic kidney disease, ischemia-reperfusion injury, and diabetic nephropathy [50]. In this study, sitagliptin pre-treatment promoted substantial restoration of kidney function with a decrease in DOX-induced nephrotoxicity markers among rats. This was evident through decreased KIM-1, BUN, serum creatinine, and cystatin c concentrations. These results coincided with Abd-Eldayem et al. (2024) and Sadar et al. (2016) who proposed that the sitagliptin renoprotective effect of sitagliptin is ascribed to its capability to mitigate oxidative stress. Also, sitagliptin has the ability to restore the apparent normal histoarchitecture when compared to DOX group. Similar results were reported by Abuelezz et al. (2016). Also, Abdelrahman (2017) reported that, sitagliptin administration ameliorated cisplatin-triggered changes in kidney function parameters accompanied with an enhancement in both morphological examination of kidney. These outcomes demonstrate that sitagliptin has the potential to function as a nephroprotective agent in countering cisplatin-induced nephrotoxicity due to its anti-apoptotic, anti-inflammatory, and antioxidant attributes [52].

Particularly, co-administration of DOX with sitagliptin renovated the balance between oxidant and anti-oxidant through reducing the MDA level and elevating the TAC, GSH and CAT concentrations. Additionally, the Nrf-2, HO-1, and NQO1 expression was enhanced. These results coincided with earlier researches that have reported the strong anti-oxidant potential of sitagliptin against various animal models [49,53,54]. The current study demonstrated that sitagliptin exhibited nephroprotective consequences against nephrotoxicity stimulated by DOX in experimental animals. These effects were attributed, in part, to its capacity to scavenge free radicals, act as a potent antioxidant [55] and upregulate the Nrf-2/HO-1 pathway [5].

Since oxidative stress is a major factor in nephrotoxicity, specifically, the superoxide ($O2^{\cdot-}$) that is the most potent free radical produced by nicotinamide adenine dinucleotide phosphate predominantly found in the kidney [49]. Also, the current study showed that treatment with sitagliptin markedly attenuated DOX-induced expression of TNF-α, TLR4, NLRP3, NF-κB, and IL-1β which agreed with Famurewa et al. (2023); Huang et al. (2022) and Ren et al. (2023). Zhou et al. (2019) indicated that, sitagliptin can act to decrease the ROS levels and improve inflammation in intestinal epithelial cell. Additionally, it inhibit the NFκB pathway by activating Nrf-2 [59]. Also, the trafficking of TLR4 to the plasma membrane is facilitated by ROS, leading to the promotion of TLR4 function [60]. Furthermore, Pahwa and Jialal (2016) postulated that ROS could serve as TLRs activator and in the context of hyperglycemia-induced oxidative stress, TLRs are activated, triggering inflammatory effects in individuals with diabetes. Hajhashemi et al. (2024) have documented that sitagliptin has antioxidant, antiapoptotic, and antifibrotic activities. Sitagliptin treatment reduced Bax, NF-κB, and TNF-α mRNA expression [49]. Furthermore, it was observed that sitagliptin reduced the renal expressions of TNF-α and NF-κB in the context of renal ischemia/reperfusion [49]. Also, sitagliptin exhibited renoprotective effects by mitigating the over expression of Casp-3. This outcome accorded with various investigational studies indicating sitagliptin' antiapoptotic effects [54]. Also, sitagliptin exhibited renoprotective effects by mitigating the overexpression of Casp-3. This outcome accorded with various investigational studies indicating sitagliptin' antiapoptotic effects [54].

According to Arab et al. (2021), sitagliptin was found to suppress the apoptotic events in testes through inhibiting Bax and enhancing Bcl-2 protein expression. The ability of sitagliptin to eliminate ROS contributes to the deactivation of the NFκB pathway and prevents cell apoptosis [5]. The renoprotective effects of sitagliptin that were seen in this investigation may be related to its capacity to reduce TGF-β, which in turn lowers the levels of

AKT and mTOR. These results support those stated by Mohamed et al. (2022) and Abd El-Fattah et al. (2021) who indicated that sitagliptin at different doses reduced AKT levels, which can be linked to the reduced TGF- β levels [63]. Accordingly, Ren et al. (2019) observed the inhibitory effect of sitagliptin on TGF-β. Sitagliptin exerted its effects by reducing oxidative stress. In addition, Abd El-Fattah et al. (2021) suggested that, mTOR activity is inhibited by sitagliptin stimulation of AMPK. This investigation disagreed with Khedr et al. (2018) who found that sitagliptin increase GLP-1 concentration leading to upregulation of GLP-1 receptor and activation of PI3K pathway that modifies the activity of several downstream effectors [3].

While this study highlights the efficacy of sitagliptin in mitigating DOX-induced nephrotoxicity, it is valuable to contextualize its effects against other nephroprotective strategies. Antioxidants such as N-acetylcysteine (NAC) and vitamin E have demonstrated protective effects against DOX-induced oxidative stress by scavenging ROS, yet their clinical utility is often limited to single-mechanism actions. For instance, NAC alleviates oxidative stress but lacks direct anti-inflammatory or anti-apoptotic effects, as shown in cisplatin-induced nephrotoxicity models [67]. Similarly, anti-inflammatory agents like corticosteroids reduce inflammation but may exacerbate oxidative damage or immunosuppression [68]. In contrast, sitagliptin offers a multimodal mechanism simultaneously enhancing antioxidant defenses via Nrf-2/HO-1/NQO1, suppressing inflammatory pathways TLR4/NF-κB/NLRP3, and inhibiting apoptosis via Caspase-3 modulation. This comprehensive action aligns with studies on melatonin and quercetin, which also target multiple pathways but require higher doses or combination therapies for efficacy [69,70]. However, direct comparative studies between sitagliptin and these agents in DOX models are lacking, warranting future research to establish its superiority or synergy in clinical settings.

5. CONCLUSIONS

The mechanistic pathways elucidate how sitagliptin counteracts DOX-induced nephrotoxicity through three interconnected axes:

Nrf-2/HO-1/NQO1 Antioxidant Pathway: Sitagliptin enhances Nrf-2 nuclear translocation (Fig. 4, left panel), upregulating HO-1 and NQO1 to neutralize ROS and restore GSH/CAT levels, directly countering DOX-driven oxidative stress.

TLR4/NF-κB/NLRP3 Inflammasome Pathway: DOX-induced ROS activates TLR4, triggering NF-κB translocation and NLRP3 inflammasome assembly, which amplifies IL-1β and TNF-α production. Sitagliptin disrupts this cascade by scavenging ROS, thereby inhibiting TLR4/NF-κB activation and NLRP3-mediated inflammation, as evidenced by reduced renal IL-1β and TNF-α levels.

TGF-β/AKT/mTOR Fibrotic Pathway: DOX elevates TGF-β, which activates PI3K/AKT/mTOR signaling, promoting renal fibrosis. Sitagliptin suppresses TGF-β expression and inhibits AKT/mTOR phosphorylation, likely via AMPK activation, thereby attenuating fibrotic remodeling

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Conflicts of Interest: There are no conflicts of interest reported by the authors.

Ethical Statement: The Ethics Committee at the Faculty of Pharmacy, Al-Azhar University (permit number: (No. 226/2019) approved all procedures used in this research. The inquiry followed the US National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 2011).

Author Contribution: Hebatalla I. Ahmed, Heba S. Zaky and Amany M. Gad shared developing the research idea, designed the experiments, supervised the experiments performance, executed data analysis, wrote and revised the manuscript. Eman B. Saad performed the experiments, collected the data, carried out the graphical and statistical analysis and wrote the manuscript.

List of Abbreviations: HO-1: Heme Oxygenase, NQO1: NAD(P)H: quinine oxidoreductase 1 , Nrf-2: nuclear factor erythroid 2-related factor 2, MDA: peroxidation malondialdehyde, TLR-4: Toll like receptor 4, NLRP3: NOD-like receptor containing pyrin domain 3, TGF-β: transforming growth factor-β, mTOR: mammalian target of rapamycin, Akt: protein kinase B, Casp-3: caspase-3, H&E: hematoxylin and eosin, IL-1β: interleukin-1β, NF-κB: nuclear transcription factor-κB, ROS: reactive oxygen species, TAC: total anti-oxidant capacity, CAT: Catalase, AKI: Acute Kidney Injury, TBST: Tris-buffered saline with Tween 20.

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