

Kidney protective effect of sitagliptin in 5-fluorouracil-challenged rats

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Abstract

The possible protective effect of sitagliptin (SIT) against nephrotoxicity induced by a single dose 5-fluorouracil (5-FU) (150 mg/kg, i.p.) was investigated in rats. SIT treatment (5 and 10 mg/kg/day, p.o.) was given for 7 days, starting 5 days before 5-FU administration. Both SIT doses caused significant reductions of serum creatinine and neutrophil gelatinase-associated lipocalin levels in rats received 5-FU. Both doses of SIT also significantly decreased malondialdehyde, tumor necrosis factor- α , interleukin-1 β , nuclear factor- κ B p65 (NF- κ B p65), Bax/Bcl-2 ratio, and cleaved caspase-3 in kidneys of 5-FU-challenged rats. Additionally, SIT, at both doses, significantly increased renal total antioxidant capacity and nuclear factor erythroid 2-related factor 2 (Nrf2) in rats received 5-FU. Besides, SIT markedly attenuated the 5-FU-induced histopathological kidney tissue injury in rats. It was concluded that SIT, at both doses, provided a significant nephroprotective effect in 5-FU-challenged rats, through its antioxidant, antiinflammatory, and antiapoptotic activities, and by modulating Nrf2 and NF- κ B pathways.

Keywords

sitagliptin, 5-fluorouracil, nephrotoxicity, rats

Introduction

5-Fluorouracil (5-FU) is a pyrimidine anti-metabolite antineoplastic agent used in treatment of many types of malignancies, including colorectal, gastric, pancreatic, cervical, and breast cancers. Similar to many other conventional anticancer chemotherapeutic agents, 5-FU can exert cardiotoxic, hepatotoxic, gonadotoxic, and nephrotoxic effects (Longley et al. 2003). It was found that 5-FU-induced organ toxicity, including nephrotoxicity, was due to its biotransformation into α -fluoro- β -alanine, urea, and ammonia (Badawoud et al. 2017). This leads to disturbance in the oxidant/antioxidant status, increased

generation of reactive oxygen species (ROS), depletion of endogenous total antioxidant capacity (TAC), enhanced membrane lipid peroxidation, and increased production of malondialdehyde (MDA) (Gelen et al. 2021). Additionally, increased production of inflammatory cytokines, as tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL1 β), are implicated in 5-FU-induced kidney injury (Wan et al. 2021). The inflammatory mediators and ROS finally up-regulate the apoptotic pathways and caspase family of proteases, which induce renal cell apoptotic death (Arab et al. 2018; Ahmad Ansari et al. 2023).

Cellular responses to oxidative, inflammatory, and apoptotic insults are greatly controlled by the fine

balance between the anti-inflammatory nuclear factor erythroid 2-related factor 2 (Nrf2) pathway and the pro-inflammatory nuclear factor- κ B (NF- κ B) pathway (Wardyn et al. 2015; Liu et al. 2017). Previous investigations showed that 5-FU-induced nephrotoxicity caused by a marked disruption in the balance between the Nrf2 and NF- κ B pathways, which intensify oxidative stress, inflammation responses, and cell apoptosis (Arab et al. 2018; Ahmad Ansari et al. 2023).

Sitagliptin (SIT), the dipeptidyl peptidase-4 inhibitor, is commonly used for treatment of patients with type 2 diabetes mellitus. SIT also showed significant antioxidant, anti-inflammatory, and antiapoptotic activities. Previous investigations revealed that SIT significantly mitigated nephrotoxicity caused by gentamicin (Abuelezz et al. 2016), cisplatin (Abdelrahman 2017), adenine (Abdelrahman et al. 2019), and methotrexate (Afkhami Fard et al. 2023), and alleviated streptozotcin-induced diabetic nephropathy (Ashraf et al. 2023) in rats. However, to the best of our knowledge, the protective effect of SIT against renal toxicity induced by 5FU was not yet investigated. Therefore, this study was performed to detect the potential protective effect of SIT against 5-FU-induced nephrotoxicity in rats, and to reveal the mechanisms underlying this effect.

Materials and methods

Medications

SIT and 5-FU powders were purchased from Sigma-Aldrich, USA. SIT was prepared in carboxymethylcellulose (CMC), 0.5% solution, and 5-FU was dissolved in physiological saline. The doses of SIT and 5-FU used in the current work were selected from previous studies (Rashid et al. 2014; Abdelrahman et al. 2019).

Laboratory animals and study protocol

Male Sprague-Dawley rats (weight 200 ± 10 g, each) were purchased from National Research Center, Giza, Egypt. Rats were housed in standard facilities, and supplied with ordinary chew and tap water *ad libitum*. They were left to acclimatize for 1 week before experiments. The rats were randomly assigned into 4 equal groups ($n = 8$, each), as follows:

- First group (control) received daily CMC (vehicle of SIT), p.o., for 7 days, and a single i.p. injection of physiological saline (vehicle of 5-FU) on 6th day.
- Group 2 received daily CMC, p.o., for 7 days, and a single dose of 5-FU (150 mg/kg, i.p.) on 6th day.
- Group 3 received SIT (5 mg/kg/day, p.o.) for 7 days, and a single dose of 5-FU (150 mg/kg, i.p.) on 6th day.
- Group 4 received SIT (10 mg/kg/day, p.o.) for 7 days, and a single dose of 5-FU (150 mg/kg, i.p.) on 6th day.

Sampling and biochemical studies

On the 8th day, rats were euthanized by thiopental (70 mg/kg, i.p.), and blood samples were collected via cardiac punctures. They were centrifuged at 5000 rpm for 5 min, after being clotted. Serum creatinine was measured by a colorimetric kit (Biodiagnostic, Egypt), and serum neutrophil gelatinase-associated lipocalin (NGAL) was determined by an ELISA kit (R&D Systems, USA).

The kidneys were dissected out, and their dried weight was measured. The right kidneys were homogenized in cold 0.05 M, KH_2PO_4 buffer (pH 7.4), and centrifuged at 5000 rpm for 10 min. Colorimetric kits were used to determine MDA and TAC (Biodiagnostic, Egypt), and cleaved caspase-3 (R&D Systems, USA) in the resulting supernatant. In addition, ELISA kits were used to measure kidney TNF- α and IL-1 β (R&S Systems, USA), NF- κ B p65 (Novus Biologicals, USA), Bax, Bcl-2, and Nrf2 (LS Bio-Sciences, USA).

Histopathology

The left kidneys were preserved in 10% formalin solution, dehydrated in ethanol, and embedded in paraffin. Sections at 4 μ m were cut, and stained with hematoxylin and eosin (H&E). The slides were examined under light microscope by a pathologist, who was unaware by slide identity. A semi-quantitative score was used to assess renal tubular injury as follows: 0 = absent necrosis; 1 = <10%; 2 = 10–25%; 3 = 25–75%; 4 = >75% (Ramesh and Reeves 2005).

Statistics

Data analysis was performed using one-way ANOVA followed by Tukey test for *post hoc* comparisons using GraphPad Prism Software Program (version 6.01). Results shown as mean \pm S.E.M., and significance was considered at $p < 0.05$ level.

Results

Biochemistry findings

Administration of a single dose of 5-FU (150 mg/kg, i.p.) caused significant increments ($p < 0.05$) of serum creatinine and NGAL in rats as compared to the control values (Fig. 1). SIT treatment (5 and 10 mg/kg/day, p.o.) for 7 days, starting 5 days prior to 5-FU administration significantly reduced ($p < 0.05$) serum creatinine and NGAL (Fig. 1). Additionally, 5-FU caused a significant elevation ($p < 0.05$) of MDA, and a significant decrease ($p < 0.05$) of TAC in rat kidneys (Fig. 2). However, SIT led to a significant decrease ($p < 0.05$) of kidney MDA, and a significant increase ($p < 0.05$) of kidney TAC in rats received 5-FU (Fig. 2).

Regarding the inflammatory and apoptotic biomarkers, significant increases ($p < 0.05$) of TNF- α , IL-1 β , NF- κ B

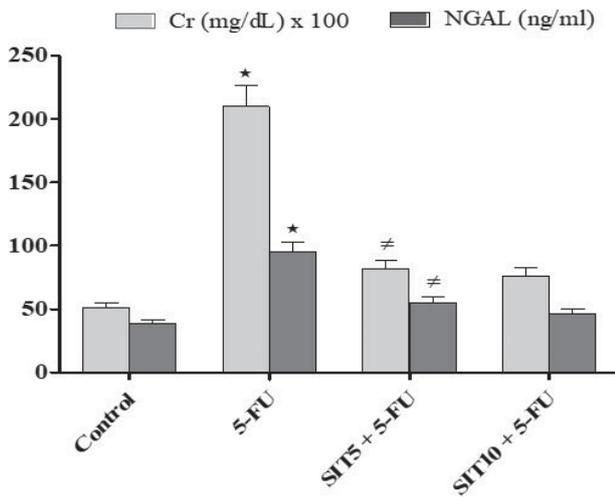


Figure 1. Effects of sitagliptin (SIT) treatment on serum creatinine (Cr) and neutrophil gelatinase-associated lipocalin (NGAL) of 5-fluorouracil (5-FU)-challenged rats. * $p < 0.05$ vs. control, * $p < 0.05$ vs. 5-FU group. Results are mean \pm S.E.M, $n = 8$, in each group.

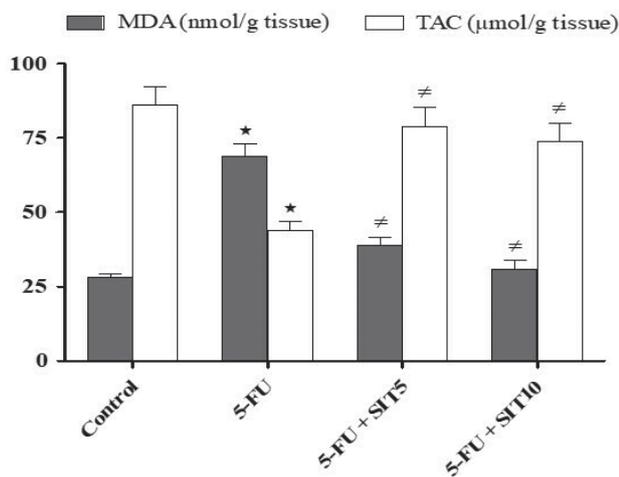
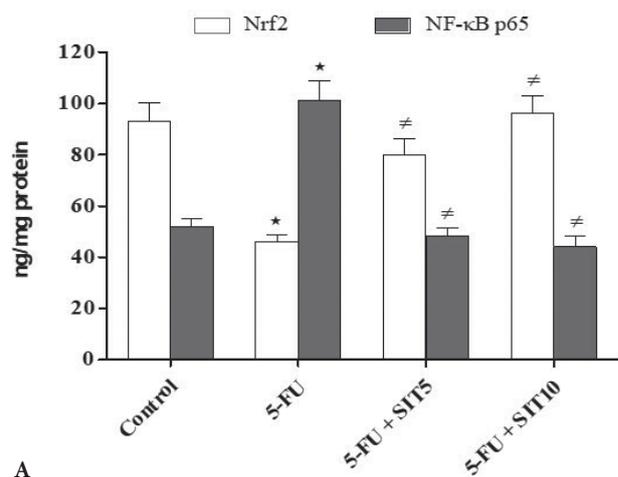


Figure 2. Effects of sitagliptin (SIT) treatment on malondialdehyde (MDA) and total antioxidant capacity (TAC) in kidneys of 5-fluorouracil (5-FU)-challenged rats. * $p < 0.05$ vs. control, * $p < 0.05$ vs. 5-FU group. Results are mean \pm S.E.M, $n = 8$, in each group.

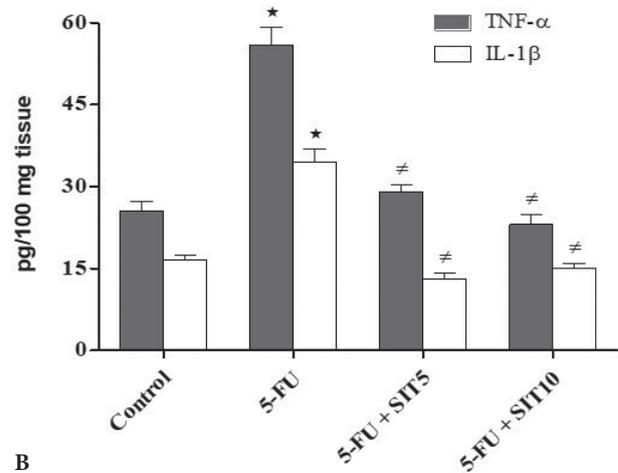
p65, Bax/Bcl-2 ratio, and cleaved caspase-3, and a significant decrease ($p < 0.05$) of Nrf2 were detected in kidneys of 5-FU-challenged rats (Fig. 3A–C). Contrarily, both doses of SIT significantly reduced ($p < 0.05$) renal TNF- α , IL-1 β , NF- κ B p65, Bax/Bcl-2 ratio, and cleaved caspase-3, and significantly increased ($p < 0.05$) renal Nrf2 in rats received 5-FU (Fig. 3A–C).

Histopathology findings

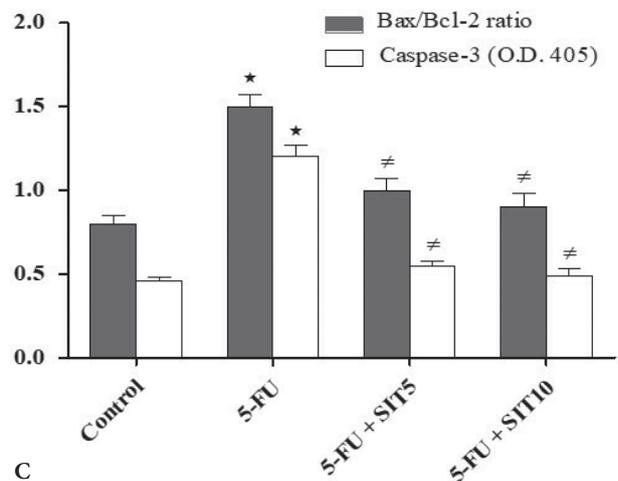
5-FU caused marked distortion of the normal kidney histology in the form of renal tubular dilatation, necrosis and desquamation of epithelial cells, inflammatory cell infiltration, interstitial edema, and multiple areas of coagulative necrosis (Fig. 4). On the other hand, SIT (5 and 10 mg/kg) mitigated the kidney tissue injury,



A



B



C

Figure 3. Effects of sitagliptin (SIT) treatment on: A. Nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor- κ B p65 (NF- κ B p65); B. Tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β); C. Bax/Bcl-2 ratio and cleaved caspases-3 in kidneys of 5-fluorouracil (5FU)-challenged rats. * $p < 0.05$ vs. control, * $p < 0.05$ vs. 5-FU group. Results are mean \pm S.E.M, $n = 8$, in each group.

preserved the renal architecture, and significantly reduced renal tubular injury score in 5-FU-challenged rats (Fig. 4).

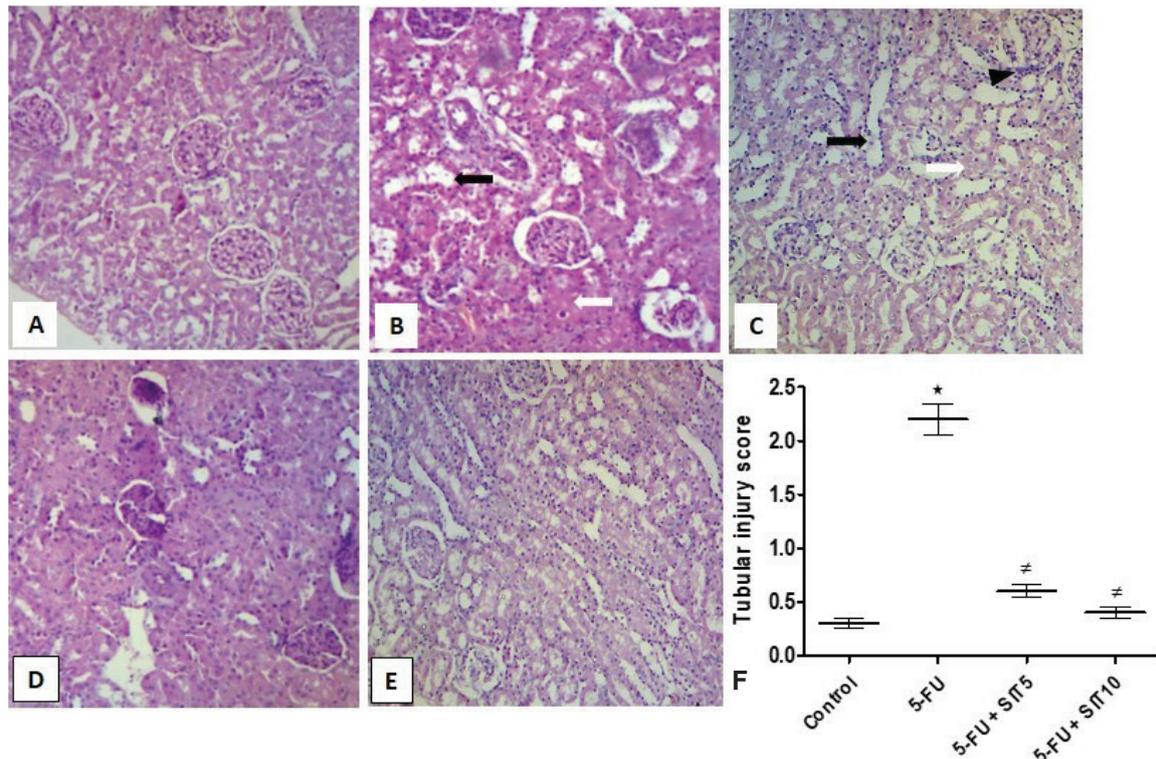


Figure 4. H&E (200 \times) of rat kidneys from: **A.** control showing normal renal histology; **B, C.** 5-fluorouracil (5-FU) group demonstrating marked distortion of kidney architecture, renal tubular necrosis and dilatation, desquamation of lining epithelium (black arrows), interstitial edema, areas of coagulative necrosis (white arrow), and inflammatory cell infiltration (black head); **D, E.** sitagliptin (SIT 5 mg/kg) + 5-FU and (SIT 10 mg/kg) + 5-FU, respectively, showing preservation of the normal kidney architecture; **F.** tubular injury score. * $p < 0.05$ vs. control, # $p < 0.05$ vs. 5FU. Results are mean \pm S.E.M., $n = 8$, in each group.

Discussion

The current study, in agreement with the previous ones, showed that 5-FU-induced renal toxicity is due to oxidative stress, exhaustion of endogenous antioxidant defences, and increased production of MDA, the end product of peroxidation of lipid biomembranes (Gelen et al. 2021; Ahmad Ansari et al. 2023). Similarly, activation of inflammatory cascades and increased generation of inflammatory cytokines were proved to participate in kidney injury induced by 5FU (Arab et al. 2018; Ahmad Ansari et al. 2023). Prior investigations, in accordance with the present one, also revealed that inflammatory cytokines, particularly TNF- α , up-regulated the NF κ B pathway leading to further tissue injury. Phosphorylation of the cytoplasmic inhibitor of nuclear factor- κ B (I κ B) results in release of the active NF- κ B p65 unit, which translocate to the nucleus and activate gene transcription of inflammatory cytokines, and augment the inflammatory responses (Arab et al. 2018; Wan et al. 2021). The current study showed that SIT provided significant antioxidant and anti-inflammatory effects, as evidenced by reduction of MDA, preserved TAC, and suppression of TNF- α and IL-1 β production in rat kidneys. This can be related in part to the ability of SIT to suppress NADPH oxidase, the main source of ROS generation during oxidative stress (Jo et al. 2018).

The intrinsic mitochondrial-dependent cell apoptotic pathway is controlled by the balance between Bcl-2

(the main anti-apoptotic protein), and Bax (the main pro-apoptotic protein).

Increased Bax/Bcl-2 ratio causes disruption of mitochondrial membrane permeability, and release of apoptogenic factors into the cytosol, which eventually activate caspase-3-dependent execution phase apoptosis (Parson and Green 2010). Similar to the current study, previous investigations revealed that activation of mitochondrial-dependent apoptotic pathway with subsequent events were implicated in 5-FU-induced renal toxicity (Liu et al. 2018; Ahmad Ansari et al. 2023). Consistent with the current results, it was also reported that SIT provided significant antiapoptotic effect by inhibiting the intrinsic apoptotic pathway, with subsequent reduction of Bax/Bcl-2 ratio and caspase-3 activity (Famurewa et al. 2023).

The fine balance between the anti-inflammatory Nrf2 pathway and the pro-inflammatory NF- κ B pathway is the main factor regulating the homeostasis of cellular responses to oxidative stress, inflammation, and apoptosis. Nrf2 up-regulates cellular defenses against oxidative and inflammatory, and apoptotic insults (Wardyn et al. 2015). Contrarily, NF- κ B is responsible for activation of inflammatory cascades (Liu et al. 2017). The previous studies, in agreement with the present one, demonstrated that 5FU administration caused a significant increase of NF- κ B p65 and a significant decrease of Nrf2 in rat kidneys (Arab et al. 2018; Ahmad Ansari et al. 2023). The current study also showed that SIT significantly restored the balance between

the two pathways, as evidenced by the significant increment of Nrf2, and the significant decrement of NF- κ B p65 in kidneys of rats challenged with 5-FU. Similarly, prior investigations showed that SIT provided antioxidant, anti-inflammatory, and antiapoptotic effects through up-regulation of Nrf2 pathway and down-regulation of NF- κ B pathway (Abo-Haded et al. 2017; Famurewa et al. 2023).

Conclusions

SIT significantly mitigated renal injury and dysfunction caused by 5-FU in rats through its antioxidant.

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Ethical approval

The study protocol was approved by the Research Ethics Committee, Faculty of Medicine, Minia University, Egypt (approval number: 245-57152). The international guidelines for care and use of laboratory animals were applied.