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Original article

Sinapic acid alleviates 5-fluorouracil-induced nephrotoxicity in rats via Nrf2/HO-1 signalling

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ABSTRACT

Fluoropyrimidine 5-fluorouracil (5-FU) is a DNA analogue broadly used in chemotherapy, though treatment-associated nephrotoxicity limits its widespread clinical use. Sinapic acid (SA) has potent antioxidant, anti-inflammatory, and anti-apoptotic effects, we investigated its protective effects against 5-FU-induced nephrotoxicity in a rat model. We designated four treatment groups each Group I (control) received five intraperitoneal saline injections (once daily) from days 17 to 21; Group II received five intraperitoneal injections of 5-FU (50 mg/kg/day) from days 17 to 21; Group III received an oral administration of SA (40 mg/kg) for 21 days and five intraperitoneal injections of 5-FU (50 mg/kg/day) from days 17 to 21; and Group IV received an oral administration of SA (40 mg/kg) for 21 days (n-six rats in each group). blood samples were collected on day 22 from each group. Animals were sacrificed and their kidneys removed, and instantly frozen. 5-FU caused oxidative stress, inflammation, and activation of the apoptotic pathway by upregulating Bax and Caspase-3 and downregulating Bcl-2. However, SA exposure reduced serum toxicity indicators, boosted antioxidant defences, and reduced kidney apoptosis, which was confirmed by histopathological analysis. Therefore, prophylactic administration of SA could inhibit 5-FU-induced renal injuries in rats via suppression of renal inflammation and oxidative stress, primarily through regulation of NF-κB and proinflammatory cytokines, inhibition of renal apoptosis, and restoration of tubular epithelial antioxidant activities and cytoprotective defences.

1. Introduction

The fluoropyrimidine 5-fluorouracil (5-FU), first developed by Heidelberg et al. in 1957, is a DNA analogue widely used in chemotherapy for breast (Heidelberger et al., 1957; Mawalizadeh et al., 2021), stomach (Na et al., 2021), head and neck (Dalwadi and Patel, 2018), colorectal(Zhang et al., 2022), and skin cancers (Ruth et al., 2006). 5-FU exerts cellular toxicity by incorporating its metabolites into RNA and DNA, eventually resulting in the inhibition of thymidylate synthase(Chibber et al., 2011). However, hepatotoxicity and nephrotoxicity are caused by the breakdown of 5-FU into dihydrouracil in the liver, which then breaks down into α-fluoro-β-alanine, urea, ammonia, and carbon dioxide (Badawoud et al., 2017; Rashid et al., 2014). 5-FU is a non-targeted cytotoxic agent that causes RNA and DNA damage as well as cell death, resulting in leukopenia, gastrointestinal toxicity, myelotoxicity, mucositis, diarrhoea, vomiting, alopecia, and cardiotoxicity (Lamberti et al., 2012; Tung et al., 2011). A growing number of natural compounds with anti-oxidative and anti-apoptotic properties are being employed to mitigate the toxicity and increase the effectiveness of chemotherapy drugs (Khan et al., 2012), with several studies investigating the potential of natural remedies to side effects of anticancer drugs (Arab et al., 2018; Gelen et al., 2018; Khan et al., 2012; Rashid et al., 2014; Yousef and Aboelwafa, 2017).

Previous studies have demonstrated that 5-FU-induced nephrotoxicity is caused by reactive oxygen species (ROS)/reactive nitrogen species (RNS) production boosting the generation of lipid

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peroxide and decreasing the activity of antioxidant enzymes (Arab et al., 2018; Badawoud et al., 2017; Rashid et al., 2014; Zhang et al., 2022). The production of cytokines, enzymes, and other inflammatory mediators, such as tumour necrosis factor (TNF-α), interleukin-1β (IL-1β), IL-6, and inducible nitric oxide synthase (iNOS), is stimulated by the activation of nuclear factor-κB (NF-κB) in response to ROS/RNS production (Arab et al., 2018). The transcription factor nuclear factor-erythroid-2 p45-related factor-2 (Nrf2) is essential for controlling antioxidation: Keap1 and Nrf2 regulate the transcription of antioxidant genes through the antioxidant response element (ARE) sequence (Itoh et al., 2010; Sies and Masumoto, 1996). As a result, a major goal of antioxidant and cytoprotective drugs is the activation of Nrf2 (Arab et al., 2018; El-Sherbiny et al., 2021; Kang and Hyun, 2017).

Sinapic acid (SA, 3,5-dimethoxy-4-hydroxycinnamic acid) is an orally bioavailable phytochemical with antioxidative, anticancer, antimutagenic, anti-inflammatory, neuroprotective, anti-glycaemic, and antibacterial properties (Chen, 2016; Nićiforović and Abramovič, 2014a). Through a variety of mechanisms, such as activating and safeguarding intracellular antioxidant enzymes or interacting with ROS, SA exerts a chemoprotective effect by inhibiting the pathways of oxidative stress, apoptosis, and NF-κB, and activating the Nrf2/HO-1 pathway(Altindag and Ozdek, n.d.; Ansari et al., 2021; Huang et al., 2018; Nićiforović and Abramovič, 2014b; Raish et al., 2018; Rezaei et al., 2021; Shahid et al., 2022; Verma et al., 2020; Yun and Yang, 2020; Zhao et al., 2021).

Numerous studies have suggested that SA provides protection against nephrotoxicity (Ansari, 2017; Ansari et al., 2017, 2016; Singh et al., 2020). In an effort to further investigate SA's potential for use in clinical cancer treatments, we studied its effects against 5-FU-induced nephrotoxicity in rats. We hypothesised that SA would protect against 5-FU-induced nephrotoxicity via the NRF2/HO-1 pathway.

2. Materials and methods

2.1. Chemicals, reagents, and drugs

5-FU and SA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rat TNF-α (RTA00), IL-6 (R6000B), MPO (DY3667) ELISA kits (R&D Systems, Minneapolis, MN, USA). Nuclear and cytoplasmic protein NE-PER assay kits was procured from Thermo Fisher Scientific (Waltham, MA, USA). All antibodies including primary Caspase-3(sc-56053), Bax (sc-20067), Bcl2 (sc-7382), NF-κB (sc-8008), Nrf2 (sc-722), Ho-1(sc-390991) and HRP-conjugated secondary antibody anti-mouse m-IgGκ (sc-516102) and anti-rabbit (sc-2004) were procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Animals

Wister rats (N = 24, 12 weeks old, 185–203 g) were acquired from King Saud University's Central Animal Facility (Riyadh, Saudi Arabia). Rats were kept in cages at 25 ± 2 °C, 50–70% humidity, and a 12 h light/dark cycle. The animals were provided with standard chow and water ad libitum. The animals were acclimatised for one week prior to experimental therapy. The study was authorised by the Research Ethics Committee of the King Saud University Faculty of Pharmacy (KSU-SE-22–80). All experimental procedures were conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals(Guide for the Care and Use of Laboratory Animals, 2011).

2.3. Experimental design and treatment protocol

The rats were randomly assigned to four experimental groups (n = 6 per group): Group I (control) rats received five intraperitoneal saline injections once daily from day 17 to 21; Group II rats received five intraperitoneal injections of 5-FU (50 mg/kg/day) once daily from day 17 to 21; Group III rats received an oral administration of SA (40 mg/kg) for 21 d and five intraperitoneal injections of 5-FU (50 mg/kg/day) once daily from day 17 to 21; and Group IV rats received an oral administration of SA (40 mg/kg) for 21 d. The regimen for 5-FU-induced renal injury was based on previous reports (Arab et al., 2018; El-Sherbiny et al., 2021) and our preliminary results. The SA dosage was consistent with that in previous studies (Ansari, 2017; Ansari et al., 2021, 2017). Ketamine (100 mg/kg) and xylazine (10 mg/kg) were used to anaesthetise the rats on day 22, at which time we collected blood samples from the tail vein and removed the kidneys.

2.4. Assessment of renal dysfunction

As previously described, we measured serum indicators of renal dysfunction, including total albumin, Blood Urea Nitrogen (BUN), lactate dehydrogenase (LDH) and creatinine levels (Scr) (Tikoo et al., 2007a, 2007b). Kidney Injury Molecule-1 (KIM-1; MBS564137) and Neutrophil gelatinase-associated lipocalin (NGAL; MBS564123) were measured by ELISA as per manufacturer's protocol (MyBioSource, Inc, San Diago, USA).

Kidney tissues were collected, thoroughly cleaned in 4 °C PBS, homogenized in 100 mM Tris-HCl buffer (pH 7.4) using a tissue homogeniser, and centrifuged at 14 000 rpm for 20 min at 4 °C. The supernatant was collected in a new tube and the total protein content was examined using the Lowry method (LOWRY et al., 1951).

2.5. Oxidative stress and antioxidant indices

We examined the levels of oxidative stress parameters, such as malondialdehyde (MDA) and nitric oxide (NO); total protein (LOWRY et al., 1951); and the antioxidant enzymes GSH (Jollow et al., 1974), SOD (Peskin and Winterbourn, 2000), and CAT (Aebi, 1974) in the renal homogenate.

2.6. Cytokine and inflammatory markers

We assessed the levels of TNF-α, IL-6 and MPO in renal tissue homogenates using ELISA kits; absorbance was recorded at 450 nm.

2.7. Protein expression

Nuclear protein and cytosol were extracted from renal tissue using the NE-PER Kit (Thermo Fisher Scientific). Immunoblotting was performed as previously described (Towbin et al., 1989). Western blot was performed according to previously published paper (Ansari, 2017) with antibodies against Caspase 3, Bax, Bcl2, NRF2, HO-1, NF-κB (p65), and housekeeping β-actin.

2.8. In vitro cytotoxicity

The human embryonic kidney cell line (HEK293) was grown at 37 °C in CO_2 incubator using DMEM with 1% penicillin–streptomycin, 10% FBS and 1% L-glutamine.

2.9. MTT assay

To determine the *in vitro* cytotoxic activity of 5-FU, we assessed cell viability in HEK293 treated with 5-FU, SA, or SA + 5-FU using

the MTT assay (Alshememry et al., 2022). Trypsin was added to the cells in the exponential phase and 5×10^5 cells/mL were separated. The cell suspension was then placed into a microplate with 100 μL medium and allowed to attach. The next day, 100 μL of freshly prepared medium with 5-FU, SA, or SA + 5-FU (5–1000 μg/mL) was added to the old medium. FBS-free DMEM with no treatment was used as a negative control. We tested three duplicates for each dilution. At 24, 48, and 72 h, cell was obtained and washed. Next, 80 μL of the medium and 20 μL of the MTT solution (5 mg/mL in PBS) were added and the mixture was kept at room temperature for 4 h. The culture solution was then spread out to precipitate the formazan crystals. Formazan was solubilised in a 100 μL mixture of sodium lauryl sulphate, DMSO, and acetic acid for 15 min at 37 °C. Reading were monitored at 570 nm.

2.10. Histological analysis

Renal tissues were cut into 4-μm slices, embedded in paraffin blocks, fixed with 10% formalin solution, and dyed with haematoxylin and eosin (H&E), then examined.

2.11. Statistical analysis

All data were presented as the mean standard error of mean (±SEM). Analysis of variance with Dunnett posthoc testing was carried out to determine differences in parameters between controls and treated groups. Significance was set at a P value<0.05. All analyses were performed using Graph Pad Prism version 8 for Windows (San Diego, CA, USA).

3. Results

3.1. Effect of SA on serum kidney function

Substantial nephrotoxicity was caused by 5-FU in comparison to control rats, as seen by elevated levels of serum creatinine (SCr), BUN, LDH, and total albumin at 24 h (Table 1), Kim-1, and NGAL (Fig. 1). The SA pre-treatment considerably reduced the levels of these nephrotoxicity indicators, suggesting a decrease in the renal damage induced by 5-FU. SA reduced the increased levels of Scr (38.59%), BUN (36.77%), LDH (38.73%), total albumin (33.20%), renal Kim-1 (37.07%), and NGAL (38.26%) with respect to 5-FU group,

3.2. SA ameliorates oxidative stress

5-FU induced a significantly elevated lipid peroxide (44.47%), expressed as malondialdehyde (MDA), and NO (32.46%) in comparison to control rats (Fig. 2). SA pre-treatment of 5-FU-treated rats significantly suppressed the elevated levels of MDA (37.05) and NO (20.71%). These variations reduced the oxidative stress response, which helped to lessen the kidney damage driven by 5-FU.

3.3. SA replenishes antioxidant defences

The decreased levels of GPX (52.07%), SOD (43.41%), and CAT (166.43%) in comparison to normal control group rats showed that 5-FU weakened antioxidant defences. Pre-treatment with SA restored the depleted levels of GPX (32.60%), SOD (30.29%), and CAT (106.90%) in 5-FU-treated rats, demonstrating that the SA therapy potently boosted the antioxidant defences. These outcomes suggest that elevating cellular antioxidant levels helps to prevent 5-FU-induced renal toxicity. (Fig. 3).

3.4. SA ameliorates renal inflammation persuaded by 5-FU

5-FU caused a significant increase in renal inflammation as indicated by the increased levels of inflammatory cytokines (TNF-α, 73.87%; IL-6, 62.30%; and myeloperoxidase [MPO], 55.18%) in comparison to normal control groups (Fig. 4). SA pre-treatment of 5-FU-treated rats significantly reduced the elevated levels of TNF-α (68.99%), IL-6 (29.56%), and MPO (28.56%), as a result, the inflammatory response is boosted, and the negative effects of 5-FU on renal function are reversed.

3.5. SA suppresses 5-FU-induced renal apoptosis

The amount of cleaved caspase-3 (3.9 fold) and Bax (1.95 fold), as well as a sharp decrease in the level of the anti-apoptotic Bcl-2, show that 5-FU dramatically improved renal apoptosis. (Fig. 5). SA pre-treatment drastically lowered the increased amounts of cleaved caspase-3 (2.6 times), Bax (0.95 fold), and anti-apoptotic Bcl-2 in rats given 5-FU, indicating that SA has a potent anti-apoptotic impact.

3.6. SA inhibits NF-κB protein expression in 5-FU- caused kidney damage

We looked at how the NF-κB and NRF2/HO-1 signaling pathways responded in order to determine the molecular causes of 5-FU-induced kidney injury. The increased (2.8 fold) protein expression of activated NF-κB p65 indicated that 5-FU activates NF-κB. (Fig. 5). Pre-treatment with SA expressively reduced (1.2 fold; Fig. 5) the expression of NF-κB p65 protein levels in 5-FU-treated rats.

3.7. NRF2/HO-1 activation in 5-FU-induced renal injury

In the 5FU-treated rat kidneys, NRF2 and HO-1 protein expression was markedly decreased. SA pre-treatment of 5-FU-treated rats significantly upregulated NRF2, HO-1 (2.18 fold; Fig. 5), and total protein levels. These results demonstrate that SA restored antioxidant defences through the NRF2/HO-1 pathway.

3.8. SA potentiates the anti-tumour activity of 5-FU

We looked at the cytotoxic activity of 5-FU alone and in combination with SA in HEK293 cells to gauge the impact of SA on the anti-tumor capabilities of 5-FU. The treatment of HEK293 cells

Table 1
Effect of kidney function on 5-FU-induced nephrotoxicity in rats.

Parameters	SCr (mg/dl) ± SEM	BUN (mg/dl) ± SEM	LDH ± SEM	Total albumin (mg/d) ± SEM
Normal	0.38 ± 0.14	25.35 ± 0.58	63.58 ± 0.62	6.66 ± 0.14
5-FU	1.44 ± 0.81*	67.95 ± 0.89*	256.80 ± 5.92*	35.83 ± 0.81*
SA 40 mg/kg + 5-FU	0.89 ± 0.06*#	42.96 ± 0.85*#	157.35 ± 5.40*#	23.93 ± 0.96*#
SA 40 mg/kg	0.44 ± 0.10#	21.82 ± 0.43#	58.53 ± 1.76#	5.88 ± 0.10#

Shown as the Mean ± SEM (n = 6 each group) *$p < 0.05$ in comparison to the control group; #$p < 0.05$ in comparison to the 5-FU group.

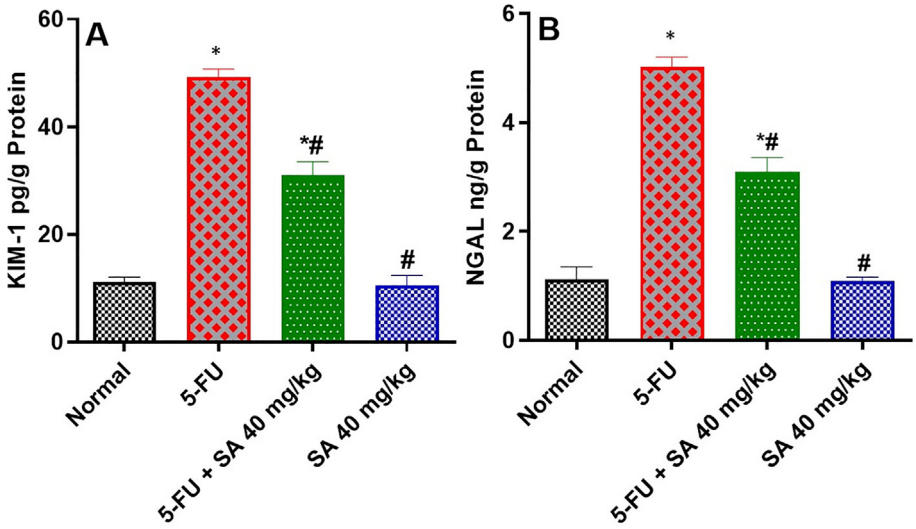

Fig. 1. Levels of Kim-1 (A) and NGAL (B) in 5-FU-treated rats. Shown as the Mean ± SEM., (n = 6 each group). $^{*}p < 0.05$ in comparison to the control group; $^{\#}p < 0.05$ in comparison to the 5-FU group.

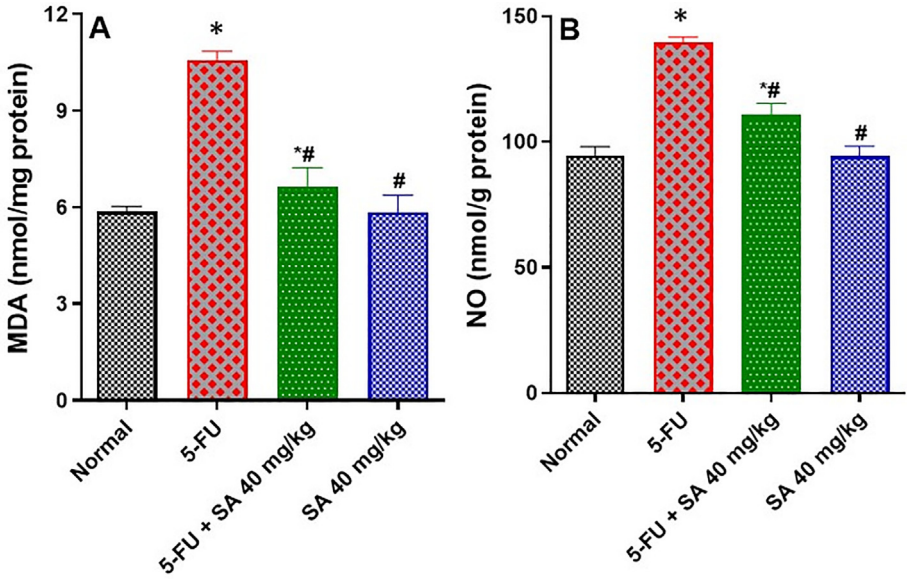

Fig. 2. Lipid peroxide (expressed as MDA) (A) and NO (B) in 5-FU-treated rats. Shown as the Mean ± SEM., (n = 6 each group). $^{*}p < 0.05$ in comparison to the control group; $^{\#}p < 0.05$ in comparison to the 5-FU group.

with either 5-FU or SA resulted in enhanced cytotoxicity. Pre-treatment of HEK293 cells with SA increased their cytotoxic potential by reducing the IC_{50} (Fig. 6).

3.9. Effect of SA on histopathological changes

We investigated whether SA can ameliorate the morphological and structural changes to rat kidneys caused by 5-FU. The renal parenchymal architecture was normal in sections from the control and SA groups (Fig. 7). On the other hand, effective and efficient 5-FU therapy instigated various morphological and structural changes and tissue damage, including localised tubular necrosis, glomerular tuft congestion, tubular epithelial vacuolisation, inflammatory cell infiltration, and intertubular blood capillary congestion (Fig. 7). SA pre-treatment preserved the renal integrity while guarding against these pathological alterations. (Fig. 7).

4. Discussion

5-FU is a potent chemotherapeutic agent of the antimetabolite class; however, its strong toxicity restricts its clinical application (Badawoud et al., 2017). 5-FU-induced nephrotoxicity is caused by oxidative damage and inflammation (El-Sherbiny et al., 2021). In this study, we explained SA's ability to protect the kidneys from 5-FU-induced renal injury. The expression of creatinine, urea, NGAL, and Kim-1 in 5-FU-treated rats showed that SA (40 mg/kg) restored renal function(El-Sherbiny et al., 2021; Paragas et al., 2012; Rashid et al., 2014). The increase in Kim-1 indicates proximal tubular injuries, while the upregulation of NGAL indicates injuries to renal nephrons(Paragas et al., 2012; Rashid et al., 2014). Since Kim-1 decreases apoptosis and promotes tubular re-epithelisation, and NGAL acts as an adhesion molecule that restricts epithelial shedding, their responses to SA treatment could be considered compensatory mechanisms for nephrotoxicity

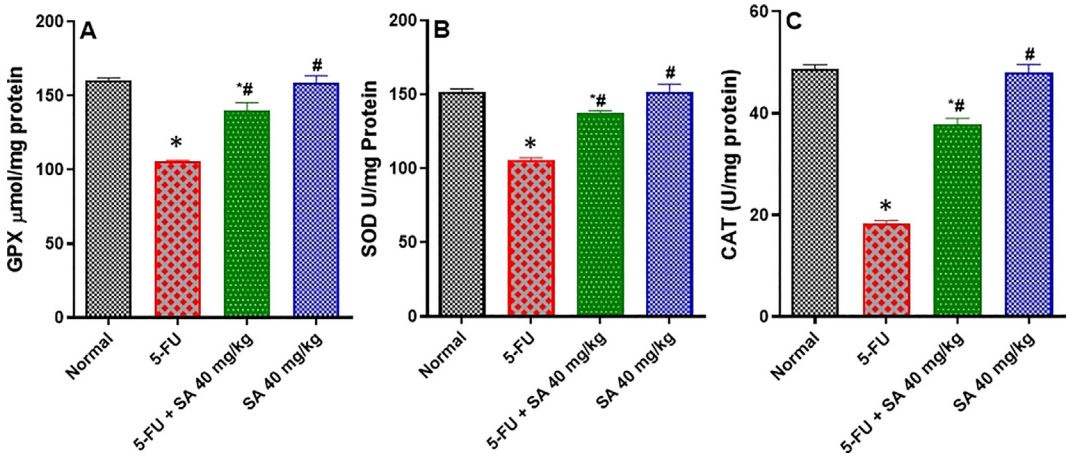

Fig. 3. GPX (A), SOD (B), and CAT (C) in 5-FU-treated rats. Shown as the Mean ± S.E.M., (n = 6 each group). $^{*}p < 0.05$ in comparison to the control group; $^{\#}p < 0.05$ in comparison to the 5-FU group.

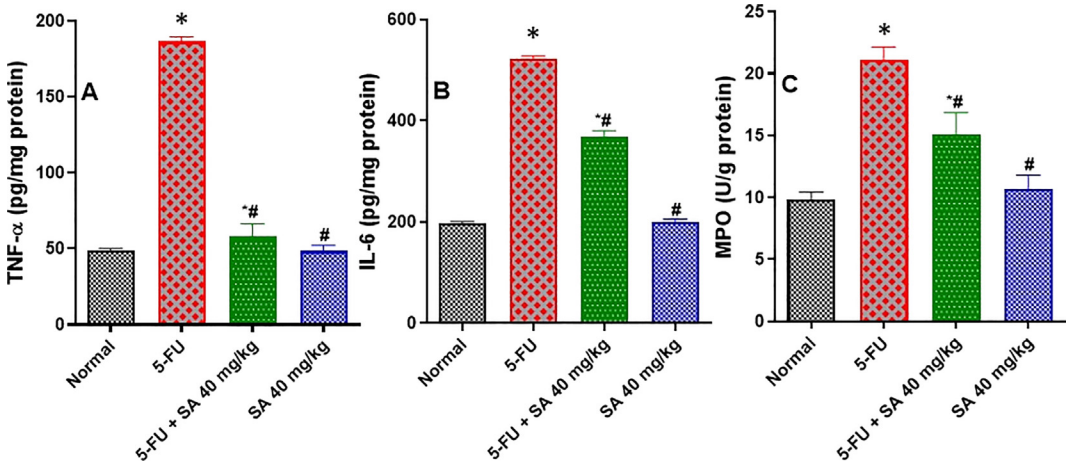

Fig. 4. TNF-α (A), IL-6 (B), and MPO (C) in 5-FU-treated rats. Shown as the Mean ± SEM., (n = 6 each group). $^{*}p < 0.05$ in comparison to the control group; $^{\#}p < 0.05$ in comparison to the 5-FU group.

(Ragab et al., 2014). Our findings are consistent with past studies where SA reduced kidney damage caused by cisplatin, cadmium, and gentamycin (Ansari et al., 2017, 2016; Singh et al., 2020).

Excessive release of free radicals and ROS from oxidative stress contributes to the pathophysiology of 5-FU-induced kidney damage (Raghu Nadhanan et al., 2012; Rashid et al., 2014). 5-FU promoted oxidative stress in terms of lipid peroxidation and nitric oxide levels, which was ameliorated by the administration of SA. Protein expression of iNOS increased in 5-FU-induced renal tissues, which was rescued by the SA treatment. Additionally, the decline in renal NO levels was consistent with information from earlier AKI models and was probably caused by ROS-initiated internalization and deactivation. (Liu et al., 2012a) which is in line with those of earlier research (El-Sherbiny et al., 2021; Rashid et al., 2014; Zhang et al., 2022). As previously reported, 5-FU induces injuries to membranes, lipid peroxidation, and reduces the levels of GSH, CAT, and SOD, while SA pre-treatment reduces lipid peroxidation and NO levels and rescues the levels of GSH, CAT, and SOD (Arab et al., 2018; El-Sherbiny et al., 2021; Gelen et al., 2021; Rashid et al., 2014; Xiong et al., 2016). Moreover, elevated MPO levels cause excess production of hypochlorous acid, a potent oxidant (Eiserich et al., 1998; Salama et al., 2014; Tan and Liu, 2012). Our results demonstrated that SA decreased the oxidative stress caused by the production of ROS and RNS, which resulted in a reduction in MPO levels in pre-treated rats.

We revealed that 5-FU exerted a substantial inflammatory response, which was confirmed by upregulated levels of the proinflammatory cytokines TNF-α, IL-6, and MPO(Ragab et al., 2014). The findings imply that the increase of monocytes is linked to the generation of proinflammatory cytokines, which initiate and maintain the inflammatory response in a number of renal diseases (Liu et al., 2012b). SA significantly downregulated the 5-FU-induced elevated in the inflammatory cytokines and marker TNF-α, IL-6, and MPO, indicating a reduction in the renal inflammatory response, thereby inhibiting cellular damage. NF-κB, an important inducible transcription factor, is activated by ROS and RNS, triggering the production of inflammatory cytokines and an inflammatory response as a consequence of neutrophil infiltration(Liu et al., 2012b). This triggers the inhibitory IκBα's separation from NF-κB, releasing p50 and p65, which travel to the nucleus in order to begin the transcription of inflammatory genes, According to our research, While increasing the expression of Iκ Bα and its downstream cytokines, SA significantly downregulated the expression of NF- κ B, demonstrating its multifaceted anti-inflammatory effects, which is consistent with previous reports (Ansari, 2017; Ansari et al., 2021, 2017; Arab et al., 2018; Raghu Nadhanan

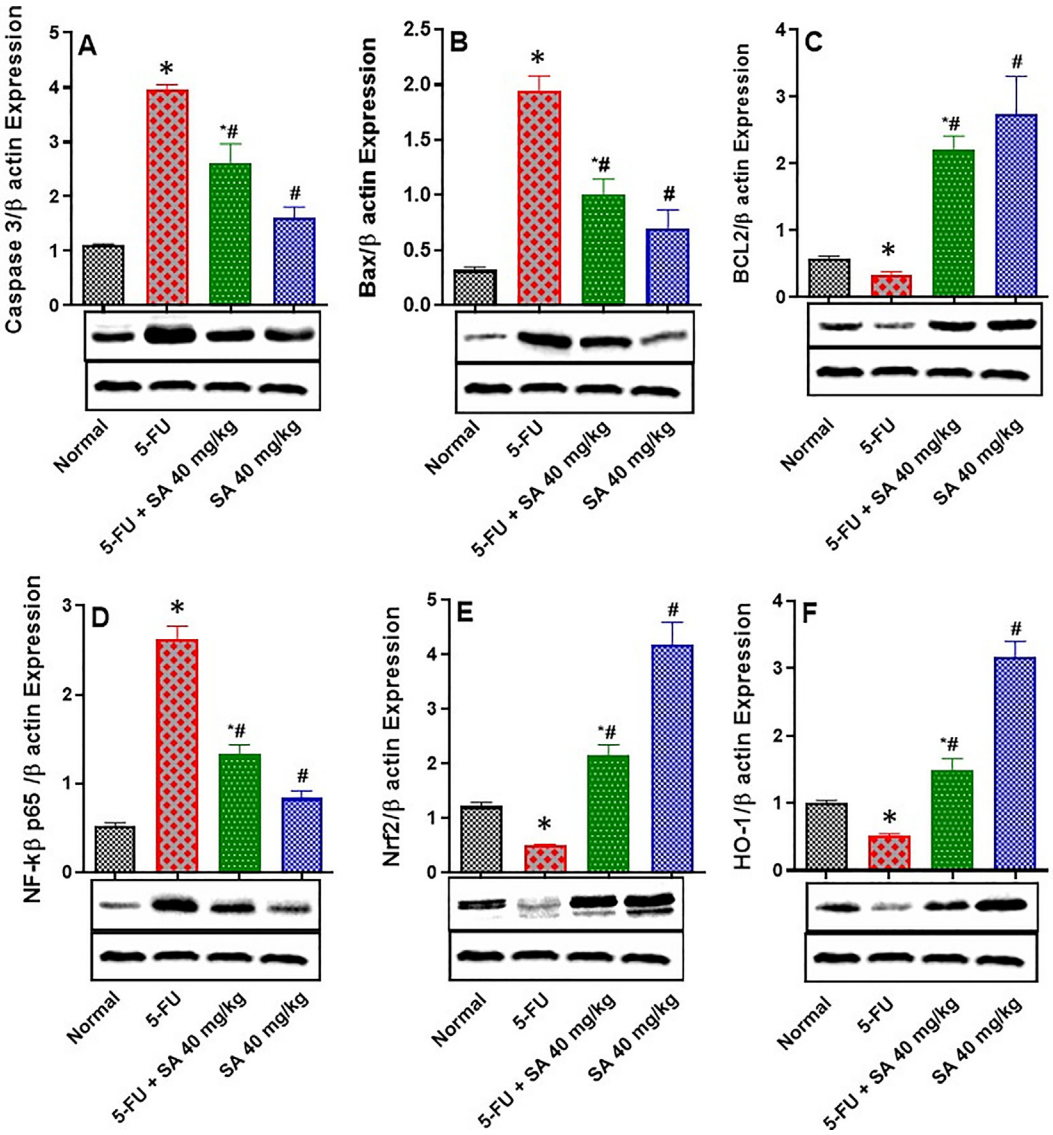

Fig. 5. SA's impact on apoptotic proteins and the recovery of the antioxidant defense. (A) Caspase-3, (B) Bax, (C) BCl_2 (D) NF-κB, (E) NRF2, and (F) HO-1 expression levels in 5-FU-treated rats. Shown as the Mean ± SEM., (n = 6 each group). $^{*}p < 0.05$ in comparison to the control group; $^{\#}p < 0.05$ in comparison to the 5-FU group.

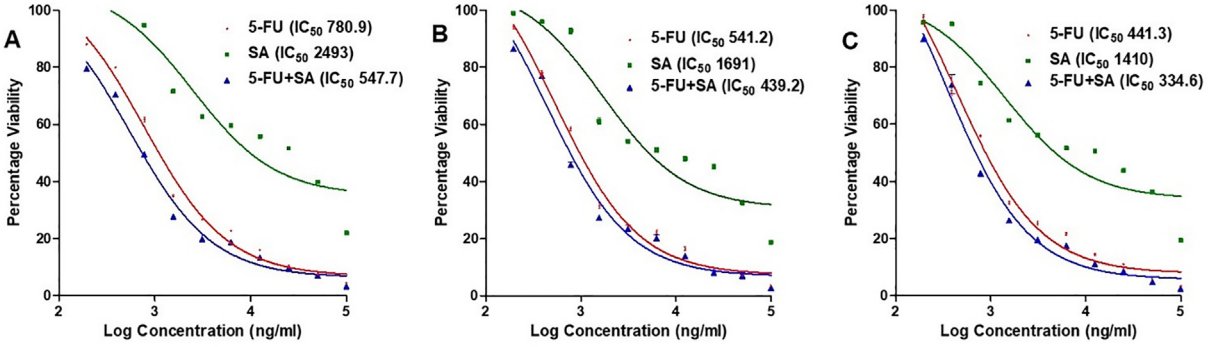

Fig. 6. Cytotoxicity of 5-FU, SA, and 5-FU + SA in HEK293 cells. Results after (A) 24 h, (B) 48 h, and (C) 72 h of incubation. Shown as the Mean ± SEM of triplicate values.

et al., 2012; Raish et al., 2018; Rezaei et al., 2021). We also observed an increase in Nrf2 and HO-1 upstream signals, which prevented ROS augmentation.

Oxidative stress stimulates the discharge of NRF2 from the NRF2-Keap1 protein composite, it subsequently moves to the nucleus to attach to the antioxidant response element necessary for the transcription of HO-1 and antioxidant enzymes, stimulating the expression of Nrf2, HO-1, and antioxidant enzymes. This clearly demonstrates its potent ability to restore antioxidant defences (Ma, 2013; Rashid et al., 2014). The Augmentation of ROS/RNS

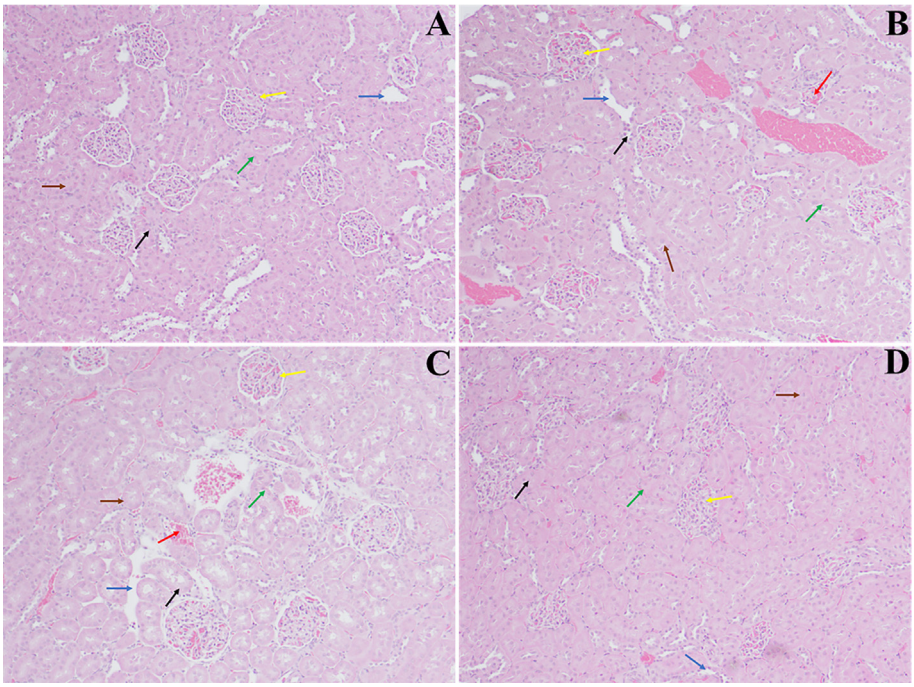

Fig. 7. Effect of SA (40 mg/kg/day) on the histopathological features of 5-FU-treated rat kidneys (H&E staining). (A) Kidney tissue exhibiting normal architecture of distal convoluted tubules (yellow arrow), vacuoles (blue arrow), Bowman's capsule (black arraow), and nephrons (brown arrow). (B) Bowman's capsule-attached glomerular capillaries that were enlarged and irregularly shaped lead to neutrophil infiltration (red arrow) caused by 5-FU. (C) Rats given 5-FU + SA had better histological features in their kidney tissue than rats given 5-FU alone. The tubular epithelium was decreased, but the mesangial cell population was still larger than that in control rats, and the glomerular capillaries maintained their normal size and appearance. (D) Normal histological characteristics may be seen in the kidney tissue of an SA-treated rat.

and superoxide in tubular epithelial cells causes oxidative stress, which induces inflammation, tubular apoptosis, and DNA damage, leading to renal damage and CKD progression (Irazabal and Torres, 2020). In rodent models of kidney damage, apoptotic cell death is closely associated with the degree of renal impairment (Khan et al., 2012), which is closely linked to the influx of ROS and proinflammatory cytokines. ROS causes Bax to undergo conformational changes that eventually cause it to translocate to the mitochondria, releasing CytC into the cytosol and activating Caspase-9 before Caspase-3 (Havasi and Borkan, 2011; Liu et al., 2012a; Salama et al., 2016).

Our immunoblotting analysis clearly demonstrated that 5-FU promoted renal apoptosis, as seen by the induction of pro-apoptotic proteins (Bax and Caspase-3) and the decreased expression of anti-apoptotic proteins (Bcl2), which was reverse by pre-treatment with SA, consistent with previous reports (Eiserich et al., 1998; Khan et al., 2012; Lamberti et al., 2012; Nagata, 1999; Rashid et al., 2014). Our findings were further verified by the histopathological analysis, which showed that 5-FU-treated renal tissues displayed various morphological alterations, such as focal tubular necrosis, glomerular tuft congestion, intertubular blood capillary congestion, and vacuolisation of tubular epithelium; however, SA treatment restored the altered renal architecture and function, which corresponds with its inhibition of apoptotic proteins, consistent with previous reports (Ansari et al., 2017, 2016; Arab et al., 2018; Badawoud et al., 2017; Khan et al., 2012).

5. Conclusion

By diminishing renal inflammation and oxidative stress, we found that prophylactic SA treatment could shield rats' kidneys against 5-FU-induced damage, primarily through regulation of NF-κB and proinflammatory cytokines, inhibition of renal apoptosis, and restoration of tubular epithelial antioxidative activities and cytoprotective defences.

CRediT authorship contribution statement

Mushtaq Ahmad Ansari: Conceptualization, Methodology, Project administration, Writing – review & editing. **Mudassar Shahid:** Formal analysis, Investigation, Resources. **Sheikh F. Ahmad:** Data curation, Validation. **Ajaz Ahmad:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Abdulrazaq Alanazi:** Methodology, Resources. **Abdul Malik:** Data curation, Formal analysis, Investigation. **Yousef A. Bin Jardan:** Data curation, Resources. **Sabry M. Attia:** Software, Validation, Writing – original draft. **Saleh A. Bakheet:** Supervision, Visualization. **Mohammad Raish:** Conceptualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.05.021.

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