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Protective and therapeutic effects of empagliflozin in nephrotoxicity induced by 5-Fluorouracil in rats: Role of caspase-3, inflammation and oxidative stress

Mohamed M. Elbadr[1*], Sabah. M. Mohamed[2], Khaled M. A. Hassanein[3], Hala M. ElBadre[4], Ebtsam S. Abdel-lah[2], Esraa A. Ahmed[1]

[1]*Department of Medical Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt.*
[2]*Department of Pharmacology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.*
[3]*Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.*
[4]*Department of Medical Biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt.*

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*Correspondence:

Corresponding author: Mohamed M. Elbadr
E-mail address: mmelbadr@aun.edu.eg

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ABSTRACT

Empagliflozin (EMPA) is a glucose-lowering agent that is widely used for treatment of diabetes mellitus and diabetic nephropathy. This work aimed to evaluate the therapeutic and prophylactic effects of EMPA and NAC in treating nephrotoxicity caused by 5-Fluorouracil (5-FU) in male albino Wistar rats. 48 rats were divided into 6 groups: The first group used as negative control. The second received 20 mg/kg of 5-FU intraperitoneally for 6 days. The third group received 5-FU plus 10 mg/kg EMPA orally for 10 days. The fourth group received EMPA 10 days before the injection of 5-FU. The fifth group received 5-FU plus N-acetylcysteine (NAC) 40 mg/kg. The sixth group received 5-FU, NAC plus EMPA. Biochemical evaluation for urea, creatinine, uric acid, albumin, TNF-α and IL-1β in serum and GSH and MDA in renal homogenate were done. Histopathological examination of kidneys was done with immunohistochemical analysis of caspase-3. The nephrotoxic effect of 5 FU was characterized by elevation of creatinine, urea, uric acid, MDA, TNF-α and IL-1β with reduction of albumin and GSH. EMPA caused improvement in kidney status especially when used therapeutically or with NAC. The immunohistochemical analysis showed that EMPA caused a reduction in the expression of caspase-3. EMPA is an effective drug in cases of 5-FU induced nephrotoxicity. It is more effective when used as a treatment rather than a prophylactic strategy. The effect of EMPA is enhanced when combined with NAC. EMPA nephroprotective effect is mediated via antioxidant, antiinflammatory effects and by decreasing the expression of renal caspase-3.

Introduction

Nephrotoxicity due to drug use affects more than 1.5 million people every year in USA, with an incidence more than twenty percent in adult persons and may reach to 66% in elderly because of the use of drugs that treat cardiovascular diseases and diabetes. Although renal impairment is often reversible, it cost about 3.5 billion dollars annually to manage it (Davis-Ajami *et al*., 2016).

5-Fluorouracil (5-FU) is an anticancer drug that is active against a range of malignant tumors such as colon, lung and breast cancer and is a member of antimetabolite anticancer drugs. Studies demonstrated that when combined with radiotherapy, certain cancers have better control and survival rates than when treated with radiotherapy alone (Alharthy and Rashid, 2024). 5-Fluorouracil causes damage to many organs, especially the kidneys. It causes nephrotoxic effect by releasing free radicals and reactive oxygen species, depleting the antioxidant system and inducing DNA damage and apoptosis by elevating the oxidative stress (Elsayed and Zaazaa, 2024). According to Yousef and Aboelwafa (2017), 5-FU has a hepatotoxic and nephrotoxic effects. Its nontargeted effect caused DNA and RNA damage as well as cell death in normal cells, with several adverse effects. van Kuilenburg (2004) demonstrated that a deficiency in the catabolic pathway may be responsible for most of the negative effects of the drug. Its nephrotoxic mechanism may be attributed to enhancement of oxidative stress, inflammation and apoptotic pathways in the kidney tissues (Rashid *et al*., 2014).

Sodium-glucose cotransporters (SGLT) are membrane proteins that involved in the passage of glucose, amino acids, ions, and vitamins across the intestinal epithelium and proximal tubules brush border. SGLT-type 2 cotransporter is most widely expressed in the proximal renal tubular apical membrane, where regulation of the sodium and glucose reabsorption from the glomerular filtrate occurs (Scheen, 2015b). Empagliflozin (EMPA), is a member of the gliflozin class that includes also dapagliflozin and canagliflozin. All are inhibitors to the SGLT2. Empagliflozin was approved in August 2014 for management of diabetes mellitus type 2 (Scheen, 2015a). According to Liakos *et al*. (2014), when compared to other SGLT2 inhibitors, EMPA is a highly selective and very powerful SGLT2 inhibitor. The use of SGLT2 inhibitor has been reported to protect the kidneys in clinical trials by causing decrease in the rate of declining in the glomerular filtration rate and delaying the albuminuria progression (Scholtes *et al*., 2021). SGLT2 inhibitors have shown favorable results in patients with kidney disease, especially in diabetic patients. Many clinical trials indicated to the benefits of these drugs in diabetic patients, yet little is known about the benefits in non-diabetic patients (Abdelrahman *et al*., 2024).

N-acetylcysteine (NAC) is one of the most important antioxidant substances and used for GSH synthesis. So, it is used for treatment of many diseases caused by free radicals (Kocamüftüoğlu *et al*., 2024). NAC has been widely used for treatment of toxicity of acetaminophen and in respiratory disorders as a mucolytic agent (Tardiolo *et al*., 2018), or even in symptomatic treatment of coronavirus disease 2019 (Bourgonje *et al*., 2021). Numerous beneficial effects of NAC have been reported, including hepatoprotective, antifibrotic, anticancer, anti-inflammatory, neuroprotective and nephroprotective effects (Heyman *et al*., 2003; Tardiolo *et al*., 2018), by eliminating peroxide, superoxide and other reactive oxygen species (ROS) (Moldeus *et al*., 1985).

The current study was done to evaluate the potential therapeutic, preventive benefits of EMPA and its combination with NAC in rats with 5-FU-induced nephrotoxicity. It also investigated the role of caspase-3, oxidative stress and inflammation in mediating the effects of EMPA on the kidneys.

Materials and methods

Drugs and chemicals

Empagliflozin (EMPA) powder was purchased from Boehringer Ingel-

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heim Pharmaceuticals (USA) and dissolved in 0.5% carboxymethyl cellulose (CMC). 5-Fluorouracil (5-FU) as commercial vial (Utoral 500 mg\10 ml) obtained from Hikma Specialized Pharmaceutical (Egypt). N-acetylcysteine (NAC) powder was obtained from AK Scientific, Inc. (USA) and dissolved in distilled water. Isoflurane 4% obtained from Kahira Pharmaceuticals and Chemical Industries Co. (Egypt). The selection of the doses of NAC and EMPA was dependent on the doses used by Efrati *et al*. (2007) and Elmaaboud *et al*. (2019), respctively). Ellman's reagent, malondialdehyde and reduced glutathione were purchased from Biodiagnostic, Giza, Egypt.

Animals

Forty-eight adult male Albino Wistar rats, weighing between 200 and 250 g, were purchased from Animal House. Assiut, University, Egypt. Animals were kept in conventional, pathogen-free facilities with a temperature of 24.0±2.0°C, and a light/dark cycle of 12 hours per day for 1 week before starting of the experiment. The National Institutes of Health's guide for the care and use of animals (1985) was followed in all animal experiments. The ethics committee at Faculty of Medicine, Assiut University approved this research with an approval number: 17300967.

Nephrotoxicity induction

5-Fluorouracil (5-FU) 20 mg/Kg b.w. was injected intraperitoneally (i.p.) daily for 6 days for the induction of kidney toxicity in all study groups except rats that were used as a control one (Adikwu *et al*., 2019).

Animal grouping

Animals were classified into six groups, 8 rats in each group. The first group was given 0.2 ml/day of 0.5% CMC orally for 10 days and used as a control group. The second group received 20 mg/kg of 5-FU i.p. for 6 days. The third group injected with 5-FU in addition to 10 mg/kg of EMPA orally starting from the first day of the 5-FU for 10 days (considered as therapeutic group). The fourth group received 10 mg/kg of EMPA orally for 10 days prior to 5-FU treatment (considered as prophylactic group). The fifth group injected with 5-FU i.p. in addition to 10 days of i.p. injection of 40 mg/kg of NAC and served as standard group. The sixth group received i.p. injection of 5-FU for 6 days and 10 mg/kg of EMPA orally and 40 mg/kg of NAC i.p. (for 10 days) starting from the first day of the 5-FU injection. When the treatment was completed, animals were decapitated after anesthetizing the animals by using isoflurane 4%. Blood samples were obtained by cardiac puncture. Centrifuging of blood at 3000 rpm for 10 min was done for serum extraction. After that, the serum was kept at -80°C to be used later for the determination of kidney parameters and markers of inflammation. The right kidneys were taken out, weighed and frozen in liquid nitrogen to assess renal oxidative stress. Subsequently, homogenization in ice-cold phosphate buffered saline (PBS) (pH 7.4) was carried out. The homogenization was done for 10 minutes then centrifugation was carried out at 3500 rpm at 4°C. The supernatant was extracted and stored at -80°C for reduced glutathione (GSH) and malondialdehyde (MDA) measurement. The left kidneys were removed and preserved in 10% formalin for staining by hematoxylin and eosin (H&E) and histopathological and immunohistochemistry studies (Elmaaboud *et al*., 2019).

Determination of renal function parameters

Using the kits which were commercially available and purchased from Schiffgraben, (Germany) measurement of serum creatinine, urea and uric acid was done. Kits obtained from DiaSys Diagnostic System (Germany) were used for measurement of serum albumin. These kidney parameters were used as indicators for kidney function and for assessment of kidney toxicity.

Determination of oxidative stress markers in kidney tissue

Renal reduced glutathione (GSH) assay

Renal GSH levels were estimated using the method indicted by Boyne and Ellman (1972). Renal homogenate was mixed with 10 % trichloroacetic acid. Then cold centrifugation at 3000 revolutions / min for 10 min was done. Disodium hydrogen phosphate buffer (pH 8.4) was mixed with the supernatant with adding of 0.25 ml Ellman's reagent and then incubated for 10 min. At 412 nm, the color absorbance was determined spectrophotometrically.

Renal malondialdehyde (MDA) assay

Renal MDA level was estimated using the method reported by Ohkawa *et al*. (1979). After colorimetric reaction with thiobarbituric acid, MDA was determined spectrophotometrically. Determination of serum inflammatory markers.

Level of serum tumor necrosis factor-α (TNF-α)

TNF-α concentration in the serum of rats was estimated using the rat TNF-α ELISA kit obtained from AssayPro (USA) according to the instructions of the manufacturer (Allan *et al*., 2005).

Level of serum interleukin-1β (IL-1β)

Using the rat IL-1β ELISA kit obtained from Dynatech Microplate Reader Model MR 5000 (Canada), the level of IL-1β in the rat serum was estimated following manufacturer instructions (Gelen *et al*., 2018).

Histopathological examination of renal tissues

Formalin with a 10% neutral buffer was used to fix the kidney tissues. After that, dehydration occurred through increasing alcohol concentrations, xylene clearing, and paraffin embedding. Using the light microscope 4 µm-thick sections stained with H&E were examined (Perry *et al*., 2016).

Immunohistochemical evaluation of renal caspase-3 expression

In summary, 5 µm-sized tissue sections embedded in paraffin were deparaffinized using xylene, then hydrated in a series of graduated alcohols. The samples were then heated in an acidic solution for processing. Sections were allowed to cool for twenty min, then rinsed with distilled water and placed in tris-buffered saline (TBS). The endogenous peroxidase activity was then quenched with a 0.3% H_2O_2 solution for twenty min. The sections were then rinsed in TBS for five min 3 times then incubation with the primary antibody, rabbit polyclonal IgG to rat caspase-3, diluted 1: 100 (Cusabio technology LCC, product code: CSB-PA786000). The sections were then added one by one for one hour at 37°C, followed by a 15-minute incubation period; streptavidin conjugated with horse-radish peroxidase (Streptavidin-HRP) and AEC substrate – HRP reaction dye (AEC substrate) (Elmaaboud *et al*., 2019). Using Image J and the IHC profiler plugin, the proportion of cells exhibiting positive immunostaining was calculated (Varghese *et al*., 2014). Counting of immunopositively cells in five microscopic fields for each slide was done. Calculation of the mean number for every slide was performed. The results were expressed as the mean ± SE for every group.

Statistical analysis

Graph Pad Prism version 9 for Windows, 2007, Graph Pad software, Inc., was used to carry out results statistical analysis. The information was

presented as mean ± standard error of mean (SE). For multiple comparisons, one-way ANOVA was used and when necessary, the Bonferroni test was used afterward. Differences between results were considered significant when P < 0.05.

Results

Effect of Empagliflozin (EMPA) (10 mg/kg) and N-acetylcysteine (NAC) (40 mg/kg) and their combination on biochemical parameters

Rats were given 5-FU for 6 days had significantly higher (P<0.0001) creatinine, urea, and uric acid concentrations in the serum with a remarkable drop in serum albumin concentration (P <0.05) in comparison with -ve control group (Figure 1).

Rat groups received 5-FU+EMPA as prophylaxis or treatment, NAC, and combination of EMPA+NAC exhibited significant (P<0.001) reduction in serum creatinine, urea, uric acid, and significant (P<0.01) increase in serum albumin concentration when compared to 5-FU treated group, with the exception the effect of EMPA prophylaxis on serum uric acid and EMPA treatment on serum albumin where the changes were non-significant differences (Figure 1).

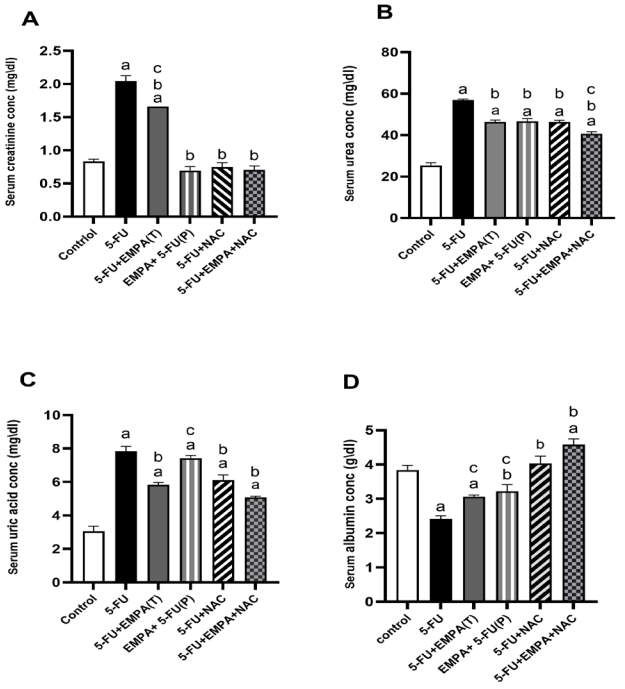

Figure 1. Effect of Empagliflozin (EMPA) (10 mg/kg) and N-acetylcysteine (NAC) (40 mg/kg) and their combination on serum creatinine, urea, uric acid and albumin in cases of nephrotoxicity induced by 5-Fluorouracil (5-FU) (20 mg/kg) in rats.
Data represented mean ± SE of 8 observations. [a]Significant difference at P<0.05 compared with negative control group. [b]Significant difference at P<0.01 compared with positive control (5-FU group). [c]Significant difference at P< 0.01 compared with standard (NAC group).

Effect of the tested drugs on the concentration of markers of oxidative stress (GSH and MDA) in kidney homogenate

GSH concentration in renal homogenate of rats treated with 5-FU was decreased significantly (P<0.0001) in comparison with -ve control group. However, GSH concentration in the animal groups treated by the tested drugs showed significant increase (P<0.01) compared to the 5-FU treated animals. However, prophylactic group did not exhibit any appreciable variations from 5-FU treated rats (Figure 2A).

Rats were injected by 5-FU exhibited a significant increase (P<0.0001) in MDA concentration. Treatment with the tested drugs caused a significant (P<0.0001) reduction in renal MDA in comparison with 5-FU group but this reduction was higher with group that received a combination of EMPA and NAC (Figure 2B).

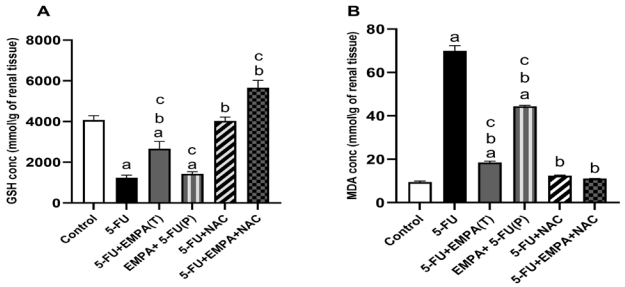

Figure 2. Effect of Empagliflozin (EMPA) (10 mg/kg) and N-acetylcysteine (NAC) (40 mg/kg) and their combination on the level of renal reduced glutathione (GSH) and malondialdhyde (MDA) in in cases of nephrotoxicity induced by 5-Fluorouracil (5-FU) (20 mg/kg) in rats.
Data represented mean ± SE of 8 observations. [a]Significant difference at P<0.05 compared with negative control group. [b]Significant difference at P < 0.01 compared with positive control (5-FU group).[c]Significant difference at P < 0.05 compared with standard (NAC group).

Effect of 5-FU, EMP and NAC on serum TNF-α and IL-1β concentration in rats

Treatment by 5-FU in a dose of 20 mg for 6 days in rats caused an increase in serum TNF-α but this elevation was non-significant in comparison to the negative control rats. The tested drugs caused a reduction in serum TNF-α compared to 5-FU treated group, but this reduction was significant (P<0.01) only with groups received NAC and combination of EMPA and NAC as shown in Figure 3A.

Results demonstrated that 5-FU caused a significant (P<0.0001) elevation in serum IL-1β in comparison to the negative control animals. Treatment with the tested drugs reduced IL-1β level in comparison to 5-FU treated animals; this reduction was significant (P<0.01) except with the rat group which received EMPA as a prophylaxis as the reduction was non-significant (Figure 3B).

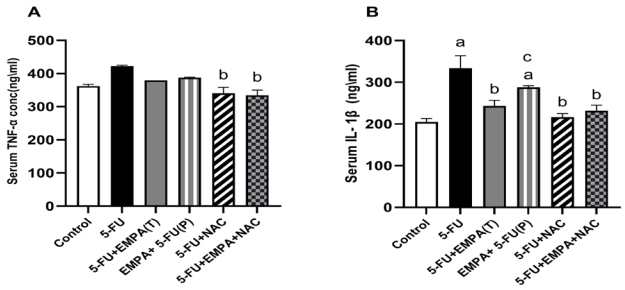

Figure 3. Effect of Empagliflozin (EMPA) (10 mg/kg) and N-acetylcysteine (NAC) (40 mg/kg) and their combination on the level of serum tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in in cases of nephrotoxicity induced by 5-Fluorouracil (5-FU) (20 mg/kg) in rats.
Data represented mean ± SE of 8 observations. [a]Significant difference at P< 0.001 compared with negative control group. [b]Significant difference at P < 0.01 compared with positive control (5-FU group).[c]Significant difference at P < 0.05 compared with standard (NAC group).

Histopathological results

Histopathological examination of the control group revealed normal histology of kidney tubules and glomeruli (Figure 4A). 5-FU treated animals showed severe blood vessels congestion and thickening of the endothelial lining, perivascular mononuclear cell infiltration and perivascular hemorrhage. Kidney tubular epithelium showed vacuolar degeneration with coagulative necrosis in kidney tubules (Figure 4 B-E). Therapeutic group showed congestion and vacuolar degeneration of the renal tubules as shown in Figure 5A, B. Prophylactic group showed congestion and vacuolar degeneration (Figure 5 C, D). Standard (N-acetylcysteine treated) group showed vacuolar degeneration (Figure 5 E). Combination of EMPA and NAC treated group showed normal appearance of renal corpuscles and tubules (Figure 5 F).

Table 1 shows the lesion score for each study group's kidney's histological results. The renal tubular epithelium and glomerulus in the nega

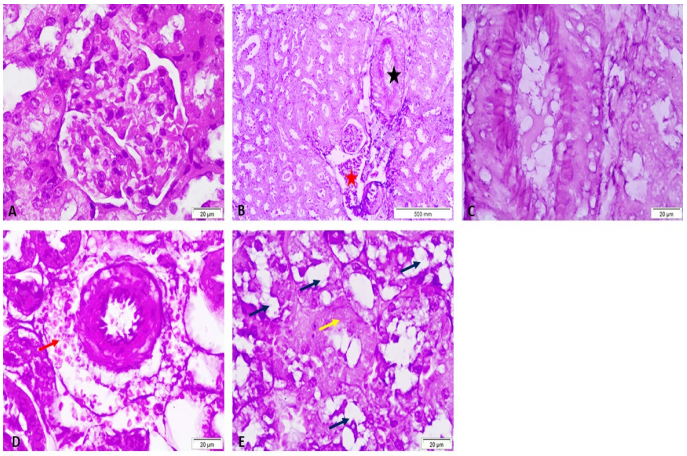

Figure 4. Representative micrograph of the kidneys of the control and induced groups stained with H&E. A) Negative control group showing normal histology of renal tubules and glomeruli B-E) Induced group showing severe congestion of the blood vessels (black star) and thickening of the endothelial lining, perivascular mononuclear cell infiltration (red star), perivascular hemorrhage (red arrow), vacuolar degeneration of the renal tubular epithelium (black arrows), and coagulative necrosis of the renal tubules (yellow arrow).

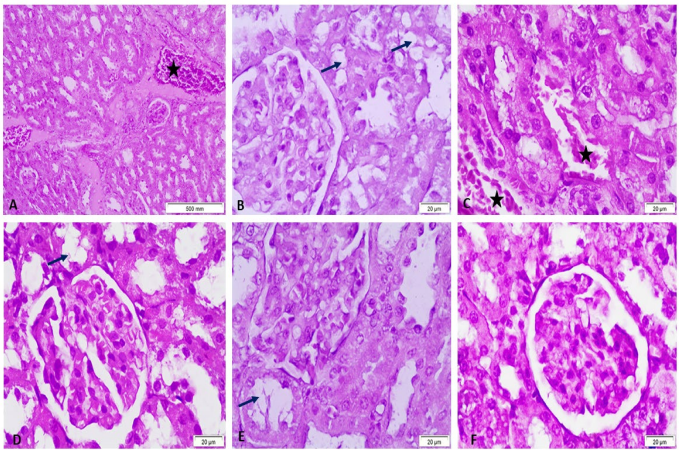

Figure 5. Representative micrograph of the kidney of the treated or prophylactic group of EMPA, N-acetylcysteine and combination treated groups stained by with H&E. A, B) Therapeutic group showing congestion (black star) and vacuolar degeneration of the renal tubules (arrows). C, D) Prophylactic group showing congestion (black stars) and vacuolar degeneration (arrow). E) Standard (N-acetylcysteine treated) group showing vacuolar degeneration (arrow). F) Combination treated group showing normal appearance of the renal tubules and renal corpuscles.

tive control group had normal histology when the kidney sections were examined. Rats in the 5-FU treated group exhibited vascular and parenchymatous alterations, including perivascular mononuclear cell infiltration, hemorrhages, and blood vessel congestion with a thicker endothelium lining. The renal tubular epithelium's vacuolar degeneration and the renal tubules' coagulative necrosis were the parenchymatous alterations, as shown in Figure 4. Upon renal examination, the prophylactic, therapeutic and NAC-treated groups' kidneys showed mild alterations such as renal tubule vacuolar degeneration and congestion. The kidney tissues of the combination treatment group improved, and the renal tubules and renal corpuscles seemed normal, as shown in Figure 5.

Immunohistochemical results of renal caspase-3 examination

Immunohistochemical examination revealed that the control group demonstrated weak expression of caspase-3 as shown in Figure (6A). The 5-FU treated animals reported strong expression of caspase -3 (Figure 6B). Therapeutic, prophylactic group of EMPA and standard treated group showed mild expression of caspase-3 (Figure 6B C, D, E). Combination treated rats demonstrated mild expression of caspase-3 (Figure 6F).

Discussion

According to Dobrek (2023), although the kidney is an important organ for xenobiotic toxicity, it is a compensatory organ that is responsible for maintaining homeostasis. Due to its exposure to chemicals, the kidney is the main target of toxicity induced by drugs because it is the main organ that is responsible for the excretion of drugs and poisons. Drug-induced nephrotoxicity is the main contributing factor (8%-60%) responsible for acute kidney injury in hospitalized patients. Nephrotoxicity is a common adverse effect of anticancer drugs used to treat hematologic and solid malignancies, as mentioned by Kintzel (2001).

Regarding the effect of 5-FU on the kidney, this investigation demonstrated that 5-FU caused a significant nephrotoxicity as indicated by elevation of serum urea, creatinine, uric acid with a drop in albumin level which are closely associated with the progression of kidney injury. The results were consistent with the report of Gelen *et al.* (2018), who indicated a rise in serum urea and creatinine following i.p. injection of a 20 mg/kg dosage of 5-FU in rats for 6 days, and the results of Adikwu *et al.* (2019) who found that serum urea, creatinine, and uric acid levels were significantly elevated, whereas bicarbonate, sodium, chloride, potassium, albumin and total protein concentrations were reduced significantly after i.p. injection of a 20 mg/kg of 5-FU in rats. Also, Elghareeb *et al.* (2021)

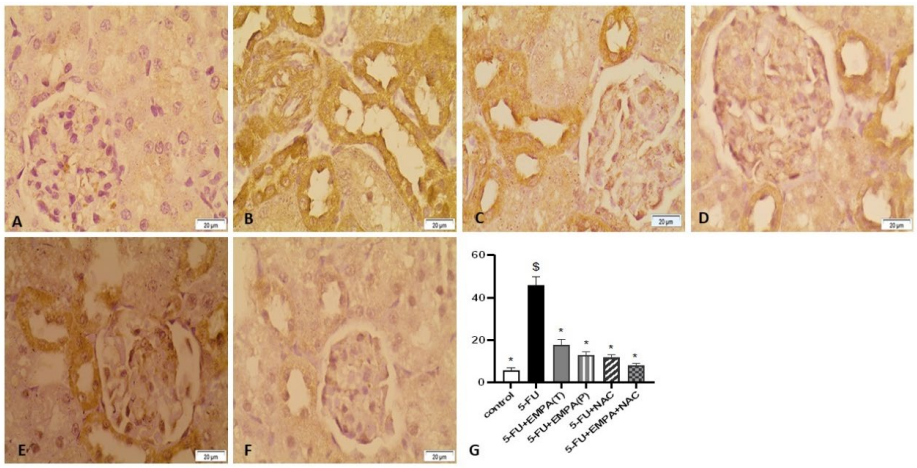

Figure 6. Immunohistochemistry photomicrograph of caspase-3 of the kidney of the studied groups. A) Control group showing weak expression caspase-3. B) 5-FU group showing strong expression of caspase -3. C, D, E) Therapeutic, prophylactic group of EMPA and standard treated group showing mild expression of caspase-3. F) Combination treated group showing mild expression of caspase-3. G) Effect of the combination of empagliflozin and N-acetylcysteine on caspase-3 expression in cases of nephrotoxicity induced by 5-Fluorouracil in rats: Data represented mean ± SE. $ Significant difference at P< 0.0001 compared with negative control group. *Significant difference at P < 0.0001 compared with positive control (5-FU group).

indicated that after 5 days of 5-FU (40 mg/kg b.w) in rats, serum urea, creatinine, and uric acid concentration were significantly elevated.

Nephrotoxicity induced by 5-FU was due to drug metabolism into ammonia, urea and fluoro-β-alanine that influence synthesis of DNA and RNA of normal and malignant cells (Al-Ghamdi *et al*., 2024). This increases the production of ROS that causes disruption in free radicals' scavengers (Gelen *et al*., 2021). In addition to, 5-FU increases formation of cytokines, such as IL-1β and TNF-α which may be implicated in its nephrotoxicity (Wan *et al*., 2021).

According to Gaweł *et al*. (2004), MDA, which is an indicator for peroxidation of lipid, is a dependable marker for assessment of oxidative stress. The prevention of oxidative stress damage is significantly enhanced by the cellular antioxidant mechanism, which includes non-enzymatic and enzymatic defenses (Gulcin and Beydemir, 2013). This was confirmed in this work by the significant decline in antioxidant markers (GSH) and rise in MDA in 5-FU group. 5-FU creates oxidative stress probably through formation of ROS (Fathy *et al*., 2018). The results were in harmony with the results of Adikwu *et al*. (2019) who indicated that 20 mg/kg of 5-FU i.p. in rats for 5 days caused a depletion of renal glutathione, glutathione peroxidase, superoxide dismutase, and catalase levels, whereas MDA level was significantly elevated. As well as, Alharthy and Rashid (2024) who indicated that 5-FU (150 mg/kg) in rats exhausted renal GSH reserves and suppressed the antioxidant enzyme activity with significant elevation in MDA levels.

The study's findings demonstrated a significant increase in serum IL-1β concentration after 5-FU treatment. There was also increase in serum TNF-α, but the rise was insignificant contrasted to the control group. Animal models have demonstrated increase activities of pro-inflammatory mediators such as IL-6, IL-1β and TNF-α in the organs of rats treated by 5-FU which were in keeping with this study results, suggesting that inflammation may be a major factor in nephrotoxicity pathogenesis initiated by 5-FU (Logan *et al*., 2008). According to Elghareeb *et al*. (2021), rats treated with 5-FU exhibited a remarkable rise in serum IL-1β.

In this study, 10 mg/kg of empagliflozin (EMPA) orally to either the prophylactic or therapeutic group led to an improvement of most of renal function parameters. However, in the prophylactic group serum uric acid concentration and in the therapeutic group, albumin concentration improvement was non-significant compared to the 5-FU treated animals. The present findings were in harmony with the records of identical outcomes noted by Abbas *et al*. (2018) who demonstrated that empagliflozin 10 mg/kg/day orally declined urea and creatinine in rat serum with unilateral ureteric obstruction operation. As well as, Bora *et al*. (2021) reported that dapagliflozin 10 mg/kg for 10 days resulted in significant improvement in renal function parameters as serum urea and creatinine in cases of colistin-induced nephrotoxicity. On the other hand, Elmaaboud *et al*. (2019), indicated that EMPA 10 mg\kg orally for 10 days as prophylactic or therapeutic in rats, led to significant reduction in serum creatinine and urea without remarkable variation in albumin and uric acid. The increased excretion of uric acid resulting from glucosuria could be the responsible for the non-significant variation between the treated and control groups.

Clinical studies have exhibited that EMPA preserves renal protective response even when the GFR is reduced (Heerspink *et al*., 2020; Chen *et al*., 2023). In addition to controlling of hyperglycemia, other mechanisms involved in kidney protection caused by SGLT2 inhibitors, include reduction of oxidative stress, inflammatory process, and sympathetic activity. These pleiotropic actions of inhibitors of SGLT2 may be responsible for the clinical efficacy of these drugs in management of non-diabetic renal diseases (Castoldi *et al*., 2024).

The therapeutic and prophylactic use of EMPA indicated a remarkable drop in renal MDA concentration and considerable rise in GSH. This observation in harmony with the study by Bora *et al*. (2021) that found that dapagliflozin had an ameliorating effect on kidney damage caused by colistin, as well as increasing the antioxidant parameters and decreasing peroxidation of lipid. On the other hand, Elmaaboud *et al*. (2019) reported that GSH was significantly elevated in the pretreatment group receiving EMPA as prophylaxis, but that the posttreatment (therapeutic) group of EMPA caused negligible rise in GSH in contrast to cisplatin treated animals.

The current results indicated that use of EMPA either treatment or prophylaxis caused non-remarkable drop in TNF-α. The prophylactic group showed non-significant reduction of IL-1β, but the therapeutic group showed remarkable reduction compared to 5-FU- treated group. The results were in harmony with of the findings of Benetti *et al*. (2016) who showed that, EMPA exhibited antiinflammatory effects and decreased macrophage infiltration in the liver, heart and kidneys in animal models of diabetes mellitus. Maayah *et al*. (2021) also observed that EMPA 10 mg\kg 3 days caused inhibition of inflammation in mice following lipopolysaccharide induced septic renal injury, suggesting that the suppression of inflammation caused by EMPA might be due to a direct effect on T-cells.

The results of the current investigation indicated that the use of N-acetylcysteine caused a noticeable improvement in renal parameters with a significant increase in albumin level compared to 5-FU treated rats as an indicator of its renoprotective effect. Treatment with the NAC (0.6 mg kg-1 i.p.) for 3 days reduced the concentration of urea, creatinine and uric acid and increased albumin level in cases of toxicity by mercuric chloride in rats. This indicates that NAC tended to prevent renal damage by maintaining cell membrane integrity, thereby it inhibits the leakage of enzymes through membranes, exhibiting renal and hepatic protective activity as mentioned by Joshi *et al*. (2014). Also, Alkhattabi *et al*. (2024) revealed that 200 mg of oral NAC daily just before feeding a diet contaminated by ochratoxin A for 42 days caused remarkable reduction in creatinine, urea and uric acid levels.

In this investigation, NAC treatment indicated a drop in MDA with remarkable rise in GSH levels in contrast to 5-FU group. Similarly, the study of Abdel-Wahab *et al*. (2017) who observed that treatment with NAC 50 mg i.p. significantly reduced the elevated lipid peroxide level in nephrotoxicity induced by cisplatin. Also, Alkhattabi *et al*. (2024) revealed a drop in MDA and a rise in GSH concentration as a result of using 200 mg NAC daily by oral route. NAC is a cytosol-specific antioxidant which acts by elevating the levels of intracellular glutathione levels (GSH) and scavenging reactive species. NAC can decrease toxicity in several organs including the kidneys by rising antioxidant capability (Yu *et al*., 2022).

Al-Rasheed *et al*. (2017) demonstrated that 3 doses of NAC 20 mg\kg caused a marked reduction in IL-1β and TNF-α compared to control group with an improvement of the histological image. These findings agreed with the report of the present study which demonstrated that NAC caused a remarkable reduction in TNF-α and IL-1β.

It has been previously indicated that 5-FU-induced apoptosis is a caspase-dependent mechanism that involves activating both effector caspase-3 and activated caspase-9 (Thant *et al*., 2008). Immunohistochemical analysis for caspase-3 in kidneys demonstrated that the use of 5-FU caused a noticeable rise in expression of caspase-3 which was in a line with the study that reported by Gelen *et al*. (2021) that after 3 doses of 5-FU (400 mg/kg) i.p., marked expression of caspase-3 in kidneys was found.

The use of EMPA as a treatment or a prophylaxis and NAC caused a decline in caspase-3 expression indicating that the caspase-3 may have a role in renoprotective effect of EMPA. This results in harmony with Elmaaboud *et al*. (2019), who revealed that the improvement in the pretreatment group with EMPA was indicated by mild positive immunohistochemical staining of caspase-3 in this group. Similarly, the study of Bora *et al*. (2021) demonstrated that there was a minor positive immunohistochemistry staining of caspase-3 in the pretreatment and posttreatment groups of EMPA suggesting that the improvement caused by EMPA may be associated with an antiapoptotic and antioxidant effects in the renal tissues.

The combination of EMPA and NAC caused a marked improvement in renal function parameters, oxidative stress and inflammatory markers,

so it is recommended to combine EMPA with NAC in treatment of nephrotoxicity induced by 5-FU and the similar nephrotoxic drugs. Our result demonstrated that the use EMPA as treatment for 5-FU nephrotoxicity gave better results than as a prophylaxis, so it is recommended to use EMPA as a treatment for nephrotoxicity rather than a prophylaxis strategy.

Histopathological examination of kidney tissues confirmed the biochemical changes caused by the tested drugs. As 5-FU caused blood vessel congestion, thickening of the endothelium lining, perivascular hemorrhage, perivascular mononuclear cell infiltration, vacuolar degeneration of the renal tubular epithelium, and coagulative necrosis of the renal tubules. The study results were in harmony with the findings of Gelen *et al*. (2018). Elghareeb *et al*. (2021) described the histological results in the form of damage to the cardiac cell, distortion of renal corpuscles and tubules, after injection of 5-FU in rats. The results indicated that EMPA and NAC caused an improvement in these histopathological changes caused by 5-FU use as the tested drugs caused less bleeding, tubule degradation, and inflammatory infiltrate and the improvement was better with the combination of EMPA and NAC which confirms the biochemical results.

Conclusion

The trial's outcomes showed that EMPA considerably reduce nephrotoxicity caused by 5-FU. Furthermore, it shows that the antioxidant properties of EMPA, along with its antiinflammatory, antioxidant and modulation of caspase-3 signaling pathway, are the possible mechanisms behind its renoprotective efficiency. These effects exhibit an obvious similarity to those of the standard medication, NAC. It was found that the combination of EMPA and NAC was more effective than either medication alone at modulating the biochemical and histological markers caused by 5-FU. Future research may be needed to look into a number of additional signaling pathways to know if they could play a role in the therapeutic and protective effects of EMPA.

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Conflict of interest

The authors have no conflict of interest to declare.

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