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Study on the Possible Protective Effects of Dapagliflozin Against 5-Fluorouracil-Induced Renaltoxicity in Rats

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ABSTRACT: Background: 5-FU remains a challenging drug in clinical practice because it induces tissue injury, especially in the renal tissue, through activation of oxidative stress, inflammation, and the apoptotic pathway. In contrast, Dapagliflozin (DAPA) is a sodium-glucose inhibitor (SGLT2), an antidiabetic drug used in a large portion of individuals globally because it has antidiabetic, anti- inflammatory, and antioxidant properties. Dapagliflozin demonstrates nephroprotective effects by reducing renal injury through ameliorating oxidative stress, inflammation, and the apoptotic pathway. The aim of this study is to assess the nephroprotective effect of Dapagliflozin in mitigating 5-fluorouracil toxicity by measuring several oxidative stress, inflammatory, and apoptotic biomarkers in rats.

Methodology: In the research, 48 male albino rats were split into six groups (n = 8): G1 (control) received regular saline (1 ml/kg/day). intraperitoneal IP injection; G2 (induction) got a single intraperitoneal dose of 5-FU 100 mg/kg completed with(1ml/kg/day) of normal saline; G3 received 5 mg/kg/day oral dapagliflozin; G4 received the single dose of 5-FU (100mg/kg) intraperitoneal with (5mg/kg) daily of Dapagliflozin orally. G5 received the 10mg/kg daily of Dapagliflozin orally, G6 received the single dose of 5-FU 100mg/kg intraperitoneal with (10mg/kg) daily of Dapagliflozin. At the end of the experiment (after 6 weeks), rats were anesthetized and euthanized. serum sample collected for determination of serum urea, creatinine by using coulometric methods, and IL-1□ & IL-10 by ELISA. Kidney tissue for each rat was harvested; a portion was stored in PBS till homogenized to evaluate GSH and MDA markers by ELISA, while another part was stored at 10% of formalin for histopathology study and IHC to assess Caspase-8 and MYD88 proteins expression.

Results: 5-FU caused renal injury, as evidenced

by elevated serum urea and creatinine levels, MDA and IL-1β biomarkers, and decreased GSH and IL-10. In kidney tissue, histopathological changes after 5-FU induction are associated with upregulation of both caspase-8 and myeloid88 proteins. Meanwhile, rats in groups 4 and 6 were treated with 5-FU plus DAPA, especially at 10mg/kg, which showed amelioration of kidney injury changes and oxidative stress markers, as well as improved histopathological appearance with preserved renal tissue with a reduction of expression of both caspase-8 and myloid88.

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Conclusions: Dapagliflozin has a protective effect to reduce 5-FU therapeutic effects that induced renal injury in rats, through ameliorating oxidative stress, inflammation, apoptotic markers, and histopathological modifications. These findings suggest that Dapagliflozin exerted a strategy to reduce 5-FU toxicity.

INTRODUCTION

A potent anticancer drug called 5-fluorouracil (5-FU) is frequently used to treat a number of cancers, including colorectal, breast, and aerodigestive tract tumors. Due to its structural similarity to nucleobase uracil, 5-FU considerably inhibits the development of tumors by interfering with the production of DNA and RNA (1,2). Incorporating themselves into macromolecules such as DNA and RNA, preventing their normal function, or by blocking biosynthetic processes. Attributed to the suppression enzyme thymidylate synthase (TS) and fluoronucleotides (3). 5-FU has proven to be an anti-cancer agent. Depending on the cancer and the treatment area, used topically or intravenously (4). 5-FU damages DNA, which impacts cell survival and proliferation, triggers apoptosis, and modifies the expression of numerous important cell cycle genes (5). It functions as an antimetabolite to halt cell development by inhibiting thymidylate synthase, which stops the synthesis of thymidine required for DNA synthesis. A major mechanism is metabolized into active intermediates that inhibit thymidylate synthesis (6). Nephrotoxicity is one of the main drawbacks of cancer treatment, and it can lead to mortality and morbidity. Antineoplastic agents cause a range of electrolyte imbalances and renal diseases (7). The kidneys are a major route of elimination of agents via glomerular filtration and tubular secretion (8). Additionally, 5-FU increases reactive oxygen species (ROS), which causes oxidative stress. By increasing intracellular glutathione (GSH) levels and enzymes, cancer cells usually fortify their antioxidant defenses. However, if these defenses are inadequate to counteract the raised ROS levels, the cells may die (9). Through a p53-dependent signaling route, 5-FU increases the formation of ROS in the mitochondria, which causes oxidative damage through cytochrome (10). Its systemic toxicity also affects healthy cells, including intestines and bone marrow with a high concentration of proliferative cells, liver, kidneys, and lungs. The oxidative stress and inflammation caused biochemical changes, inflammatory reactions, and histological disruption (11). Dapagliflozin (DAPA) is a Sodium-glucose inhibitor2 (SGLT2) inhibitor that exhibits its therapeutic effects; its advantages extend beyond glycemic control. It reduces proteinuria and disease progression in chronic kidney disease (CKD)(13). classified as "disease-modifying" medications because of their impacts that surpass the renal proximal tubule. Also, a weight-neutral and glycemic stability(14).SGLT2 is mostly situated in the proximal tubule. This inhibition increases urine glucose excretion, which has been effective in lowering plasma glucose and hemoglobin A1c (HbA1c) (15). SGLT2 inhibitors reduce failure with chronic kidney disease (CKD), and their presence is associated with increased morbidity and death. They also improve non-alcoholic fatty liver disease (16). DAPA demonstrates a decrease in GFR (17). According to research, dapagliflozin-treated individuals with chronic kidney disease, independent of type 2 diabetes, had a decreased risk of dying from cardiovascular or renal causes or developing end-stage kidney disease (18). SGLT2 inhibitors have kidney-protective benefits on type 2 diabetes (19). Because proximal tubular cells cannot effectively reduce glucose transport due to insulin-independent entry (20). Dapagliflozin's beneficial effects, including reducing RAAS activation, oxidative stress, renal inflammation, and fibrosis, all contribute to the development of diabetic renal disease, according to long-term therapy (21). SGLT2 inhibition lowers inflammasome activation and inhibits the production of inflammatory cytokines (22). Inhibitors of SGLT2 stop the proximal tubule from reabsorbing coupled glucose and sodium, resulting in glucosuria and a decrease in plasma glucose concentrations (23). Thus, this study aims to evaluate the nephroprotective effect of DAPA in modifying 5-fluorouracil toxicity by measuring several biomarkers in serum and tissues through oxidative stress, inflammatory cytokines, Histopathological changes, and protein expression of apoptotic pathways.

MATERIAL AND METHODS

Drugs and chemicals

Dapagliflozin and 5-Fu were obtained from Sigma USA. Other chemicals and reagents utilized in this investigation were acquired from reputable vendors.

Animals

There were 48 male Wister albino rats utilized, each weighing between 200 and 250 grams. The animals were kept in appropriate cages in a well-ventilated area within the animal house of the College of Pharmacy, Al-Mustansiriyah University, under ideal

conditions (temperature 24 C, humidity 50%), with free access to food and water, in accordance with the laboratory animals handling code of 66 on 17/3/2026. Before the trial began, the rats were acclimated for a few weeks.

Experimental design

Forty-eight male albino Wistar rats (n=8 for each group) were divided as follows: Group one control(G1) received normal saline IP for six weeks.Group two(G2) received the 5-Flurouracil, received as a single dose of 100 mg/kg IP, completed with(1ml/kg/day)of normal saline IP for six weeks.Group three(G3) received only 5mg of DAPA orally for six weeks. Group four(G4) received 5mg of DAPA and a single dose of 5-Flurouracil(100 mg/kg \IP). Group five(G5) received only 10mg of DAPA orally for six weeks. Group sex (G6) received 10mg of DAPA and a single dose of 5-Fluorouracil 100mg/kg/IP for six weeks.

A cardiac puncture was used to draw five milliliters of blood, which was then centrifuged for fifteen minutes at 3000 rpm after being allowed to cool at room temperature for thirty minutes. Until the interleukin-1 (IL-1) and interleukin-10 (IL-10) markers were evaluated, the serum was kept at -80 °C. After the kidney was dissected, the first part was homogenized for examination of glutathione (GSH) and malondialdehyde (MDA), and the second part was preserved in formalin 10% for histology and IHC for caspase-8 and MYD88.

Analysis of oxidative stress and cytokine markers by ELISA

All biomarkers GSH, MDA, IL-1β, and IL-10 were determined by using a technique called sandwich ELISA by following all instructions from the manufacturing company. Samples and standards were added to the antibody well of the marker plate. Follow that added biotinylated detection HRP, washing with water, and addition of TMB substrate for color changing, and the reaction is stopped by using the stop solution. The wavelength used to measure the absorbance was 450nm. At the same time, the concentration is measured by a standard curve.

Histopathological analysis

Kidney tissue fixed in formalin 10% for one day(24h), adding ethanol gradually. After that, tissues are embedded in paraffin wax, and xylene is cleaned. Using a rotary microtome, kidney tissue was sectioned into thin layers of around 4μ, deparaffinized, and stained with hematoxylin and eosin. The pathologist examines the dried slides of tissue under a microscope.

Immunohistochemistry analysis

The IHC technique was performed, following the instructions from the manufacturing company(Fine, China). Tissue slices embedded in paraffin were first deparaffinized, rehydrated, and subjected to antigen retrieval before peroxidase activity was blocked. HPR conjugated secondary antibody was then added after primary antibodies containing caspase-8 (1:500) and myd88 (1:100) were incubated. The positive signal was visible as a brownish color after adding the DAB working solution. The slides were analyzed by the pathologist. protein expression was measured depending on allered scoring as follows: 0: None;1: Less than 1/100 cells;2: 1/100 to 0/100 cells;3: 0/100 - 30 /100, i.e., 1/3rd cells; 4: 1/3rd of 100 cells to 2/3rd cells; 5 : > 2/3rd of 100 cells. Intensity of positive stain (Nucleus) score: 0: None; +1: Weak stain; +2: Moderate stain; and +3: Strong stain. Allered score = Percentage of score + Intensity score (range 0, 2 – 8)

Statistical Analysis

To find the impact of different groups on research parameters, the Statistical Packages of Social Sciences (SPSS) (2019) software was utilized. In this study, a significant comparison of means was conducted using the t-test and least significant difference (LSD). A statistically significant value was one that was less than 0.05, while a highly significant value was one that was less than 0.01.

RESULTS

Biochemical analysis

Effect of 5-Fu & Dapagliflozine on urea& creatinine

Results showed significantly increased level of urea in G2 (62.66 ±3.06) compared to G1 control (54.38±2.37), while all groups treated with DAPA alone or combination with 5-Fu (Group3,4,5,6) showed significantly reduction level compared to G2, There

is no difference between these groups(55.32±2.18,57.25 ±2.36, 51.44±1.95,52.96±2.86) regarding to the urea level, as showed in **Table (1)** and **Figure (1).** In addition, the result showed a significantly increased level of creatinine (1.880 ±0.29) in the induction group (G2) versus the control group (G1 (0.4498 ±0.08). All groups treated with DAPA 5 mg and 10 mg alone and in combination with 5-Fu (G3,4,5,6) showed significant reduction, especially in G6 (0.622 ±0.08). There is no difference between G 4,5,6 and control group (0.5682 ±0.13,0.8504 ±0.16,,0.391 ±0.04,0.622 ±0.09) respectively, as illustrated in **Table (1)** and **Figure (2).**

Effect of 5-Fu &Dapagliflozine on the level of GSH&MDA in rat kidney tissue

The level of GSH in rats kidney was significantly higher in G1 (26.81 ±3.85) comparison with G2 (10.68 ±0.44c), Treated groups (G 3,5,6) with Dapa alone and combination showed high level of GSH closely to the control group (25.46 ±1.17, 36.11 ±0.48 and 23.32 ±o.35) respectively, these groups showed significant increase level regarding to the induction group, as seen in **Table (2)** and **Figure (3)**, While The level of MDA in G2 was highly increased versus G1 (724.37 ±23.85 & 205.29 ±16.71) respectively. MDA in groups treated with Dapa +5-Fu (G4,6) showed a significant reduction level compared to G2 (411.06 ±19.06, 309.13 ±22.85), respectively, with P≤ 0.01. Also, G3&5, which were treated with 5&10 mg dAPA alone, showed a close level with the control group, **Table (2**) and **Figure (4).**

Effects of Dapa &5-fu on Interleukins level of IL-1β & IL -10 in rats serum

IL-1 β was increased significantly in G2(304.73 ±17.04) compared to G1(188.45 ±11.74) in rats serum. The combination DAPA 5mg&10 mg with 5-Fu(G4&G6) showed a reduction in IL-1 β level compared to G2, but without a significant difference (283.23±14.68, 297.04 ±16.05), respectively. Regarding to the groups received Dapa, alone (G 3,5), there is no difference with control group (192.67 ±9.76,182.51 ±10.62) respectively, as shown in **Table (3)** and **Figure (5).** IL -10 level showed a significant difference in G2 versus G1 (139.761±8.95&189.1096 ±12.07, respectively, while the level of this cytokine increased in all groups (G4,5,6) that were treated with Dapa alone and in combination with 5-Fu versus G2, as shown in Table 3 and Figure 6**.**

Table 1: Mean level of serum urea and creatinine in rat groups

Groupa	Urea mean ±SE(mg/dl)	Creatinine mean ±SE(mg/dl)
G1 (control)	54.38±2.37 bc	0.449± 0.07 bc
G2 (Induction)	62.66±3.08 a	1.880±0.16 a
G3(DAPA 5mg)	55.32±2.28 bc	0.5682±0.08 bc
G4 (DAPA 5mg+5-FU)	57.25±2.71 ab	0.8504 ± 0.11 b
G5(DAPA 10mg)	51.44±1.95 c	0.139±0.05 c
G6 (SAPA 10mg+5-FU)	52.96±2.46 bc	0.622±0.08 bc
LSD	6.017**	0.4471**
P-value	(0.0296)	0.0074

5-Fu: 5-fluorouracil, DAPA: Dapagliflozin, SE: standard error, LSD: least significant difference.

P values < 0.01 * (significant difference), P values ≤ 0.001 ** (very significant difference), mean ± SE, mean superscript with various lowercase letters (a, b, c, d) that differ statistically, and LSD was used to compare means among groups.

Table 2: Mean level of GSH and MDA in rats' kidney tissue

Groups	**GSH mean ±SE(mg/dl)/**	**MDA mean ±SE(mg/dl)/**
G1(Control)	26.81 ±3.85b	205.29 ±16.71 e
G2(Induction)	10.68 ±0.44c	724.37 ±23.85 a

	25.46 ±1.17b	248.24 ±15.68 d
G4(5mg Dapa+5-FU)	15.23 ±1.38c	411.06 ±19.06 b
G5(10mg Dapa)	36.11 ±0.48a	198.39 ±13.75 e
G6(10mg Dapa+5-FU)	23.32 ±o.35b	309.13 ±22.85 c
LSD	5.147**	38.419 **
P-value	0.0001	0.0001

5-Fu: 5-fluorouracil, DAPA: Dapagliflozin, SE: standard error, LSD: least significant difference.

P values < 0.01 * (significant difference), P values ≤ 0.001 ** (very significant difference), mean ± SE, mean superscript with various lowercase letters (a, b, c, d) that differ statistically, and LSD was used to compare means among groups.

Table 3: Mean level of serum IL-1β and IL-10 in all rat groups

Groups	**IL-1β mean ±SE(mg/dl)**	**IL-10 mean ±SE(mg/dl)**
G1(Control)	188.45 ±11.74 b	189.1096 ±12.07 a
G2(Induction)	304.73 ±17.04 a	139.761 ±8.95 d
G3(5mg Dapa)	192.67 ±9.76 b	146.53 ±9.04 cd
G4(5mg Dapa+5-FU)	283.23 ±14.68 a	168.41 ±11.63 b
G5(10mg Dapa)	182.51 ±10.62 b	167.99 ±9.48 b
G6(10mg Dapa+5-FU	297.04 ±16.05 a	162.67 ±11.04 bc
LSD	31.635 *	20.072 **
P-value	0.0001	0.0074

5-Fu: 5-fluorouracil, DAPA: Dapagliflozin, SE: standard error, LSD: least significant difference.

P values < 0.01 * (significant difference), P values ≤ 0.001 ** (very significant difference), mean ± SE, mean superscript with various lowercase letters (a, b, c, d) that differ statistically, and LSD was used to compare means among groups.

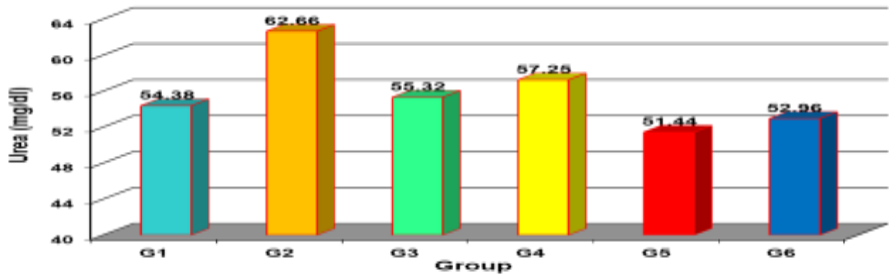


Figure (1): The level of urea in all groups in this study

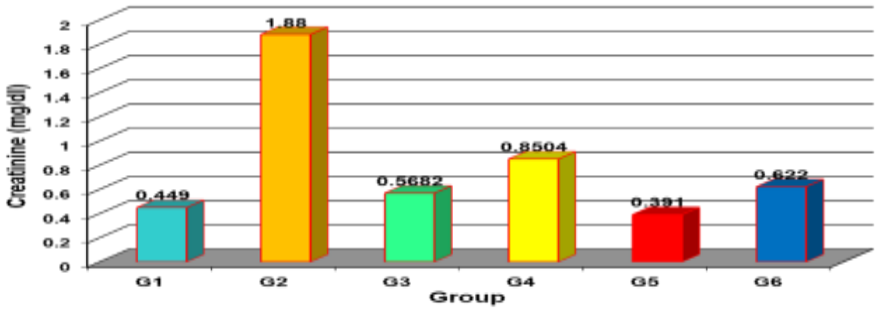


Figure (2): The level of creatinine in all groups in this study

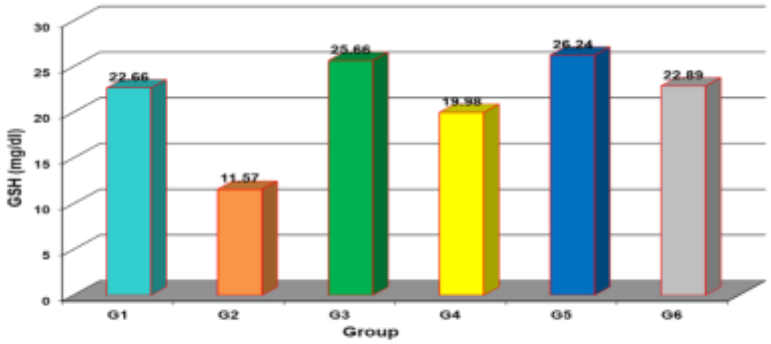


Figure (3): The level of GSH in all groups of rats' kidney tissue

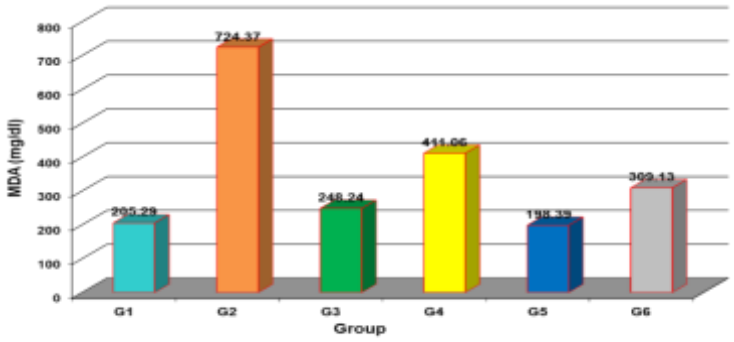


Figure (4): The level of MDA in all groups of rats' kidney tissue

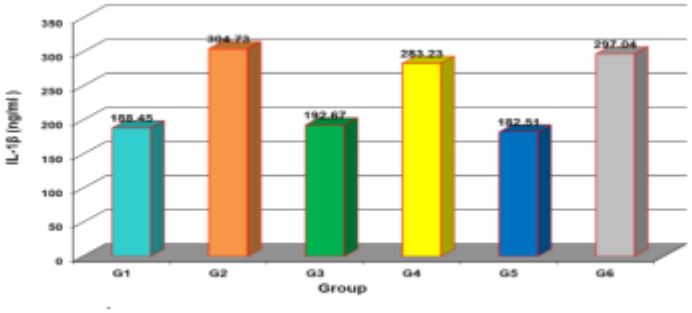


Figure (5): Serum level of IL-1β in all groups of rats

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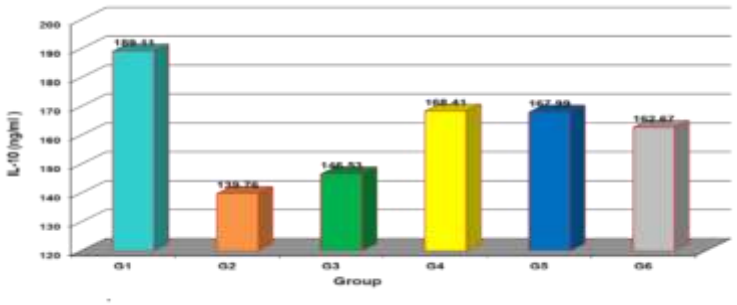


Figure (6): Serum level of IL-10 in all groups of rats

Histopathological evaluation of 5-Fu&Dapa on the rat's kidney

1- Group 1(control): The histopathological figures of the kidney showed a normal renal capsule, normal renal parenchyma comprised of normal lining cells of the renal tubules, and normal glomerular cytoarchitecture **(Figure 7, A &B)**.

2- Group 2 (Induction): Nearly all of the kidney's histological findings had mild nephrosis, which included necrosis of other cells and significant cellular swelling of the renal tubules' lining cells (ballooning) **(Figure 8, C&D)**. A small number of histological pictures showed glomerulonephritis, which was characterized by peri-glomerular leukocyte aggregation, glomerular congestion, significant interstitial hemorrhage with congestion, and mild edema with necrosis of the renal tubule lining cells **(Figure 9, E&F)**.

3- Group 3 (Only 5mgDapagliflozin): All histopathological figures of the kidney were similar to those of the control group **(Figure 10, G&H)**.

4- Group 4 (5mg Dapagliflozin +5-Fu): All histopathological figures of the kidney showed glomerulonephritis with vascular dilation, congestion, interstitial edema, tubular degeneration with necrosis, and deterioration of glomeruli **(Figure 11, I &J)**. Other figures showed mild tubular dilation with atrophy of lining cells; other renal tubules showed degeneration with necrosis **(Figure 12, K&L)**.

5-Group (10mg Only 5mg Dapagliflozin): All histopathological figures of the kidney were similar to those of the control group **(Figure 13.M&N)**.

6- Group 6 (10mg Dapagliflozin +5-Fu): Nearly all kidney histological images had normal interstitium and glomeruli, and little atrophy of the lining cells of collecting tubules. A few images had normal interstitium and glomerular congestion together with normal renal tubule lining cells **(Figure 14, O&P)**.

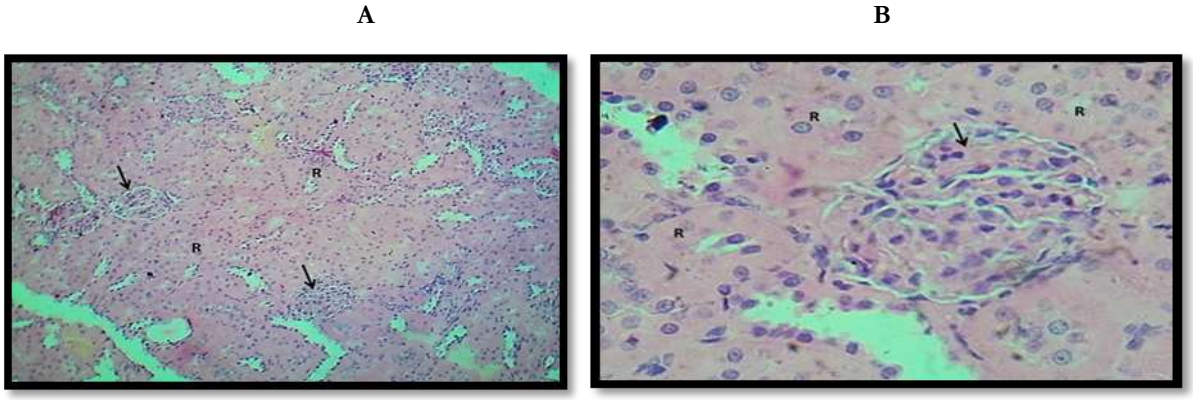


Figure (7): G1 renal tissue sections. A portion of the renal cortex in G1 displays typical renal parenchyma, which is made up of normal glomeruli cytoarchitecture (arrows) and normal renal tubules (R).H&E.100x. B: A section of the renal cortex in G1 displays normal renal parenchyma made up of normal glomerulus cytoarchitecture (arrows) and normal renal tubule lining cells (R). 400x H&E.

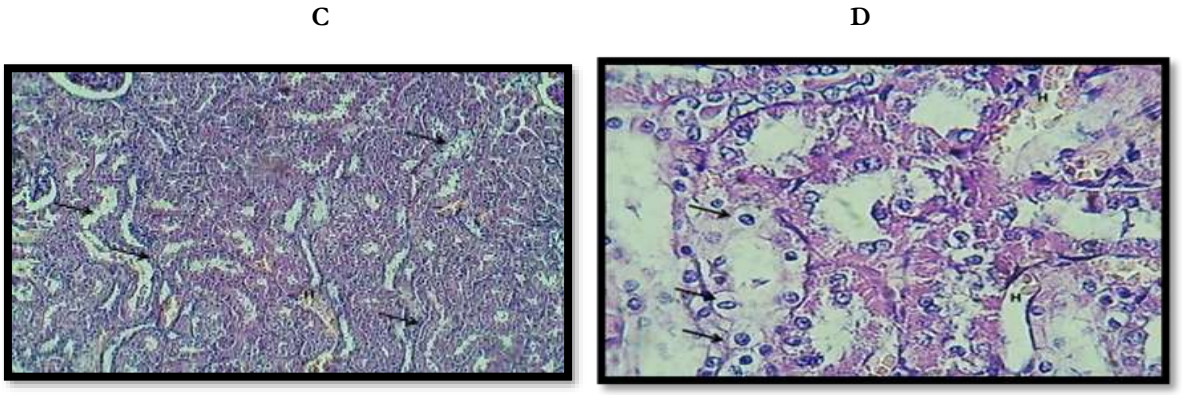


Figure (8): G2 renal tissue sections. C: A portion of the renal cortex in G2 has normal glomeruli, interstitial hemorrhage (H), and considerable cellular edema of the renal tubule lining cells (Arrows). H&E.100x. D: A portion of the renal cortex in G2 exhibits interstitial hemorrhage (H) and considerable cellular edema of the lining cells of the renal tubules (Arrows). 400x H&E.

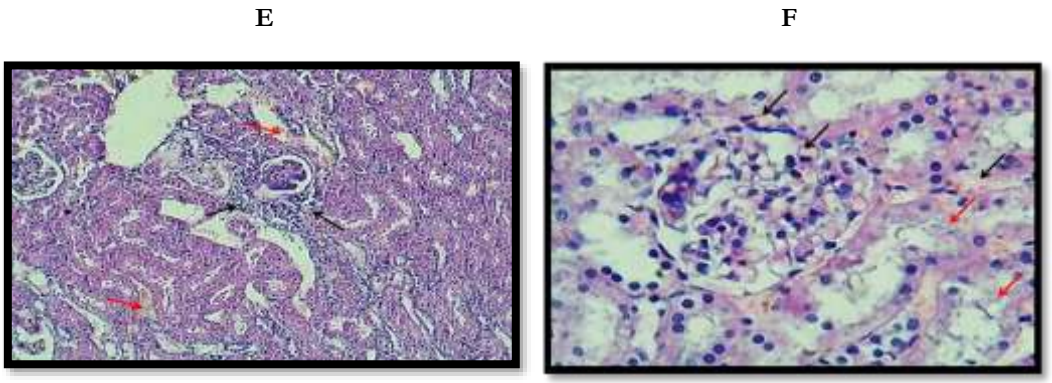


Figure (9): G2 renal tissue sections. E: A section of the renal cortex in G2 displays glomerulonephritis with peri-glomerular leukocyte aggregation (black arrows), interstitial bleeding (red arrows), and necrosis of the renal tubule lining cells.H&E.100x. F: A portion of the renal cortex in G2 displays glomerular and interstitial congestion (black arrows) as well as edema with necrosis of the renal tubule lining cells (red arrows). 400x H&E

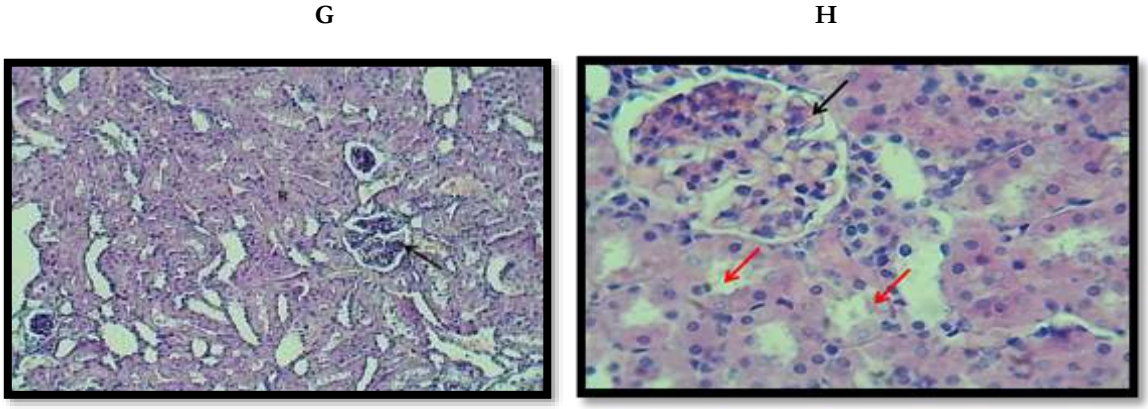


Figure (10): G3 kidney tissue sections. G: A section of the renal cortex in G3 has normal glomeruli (arrows) and renal tubules with normal lining cells (R).H&E.100x. H: A section of the renal cortex in G3 has normal glomeruli (arrows) and renal tubule lining cells (R). 400x H&E.

I J

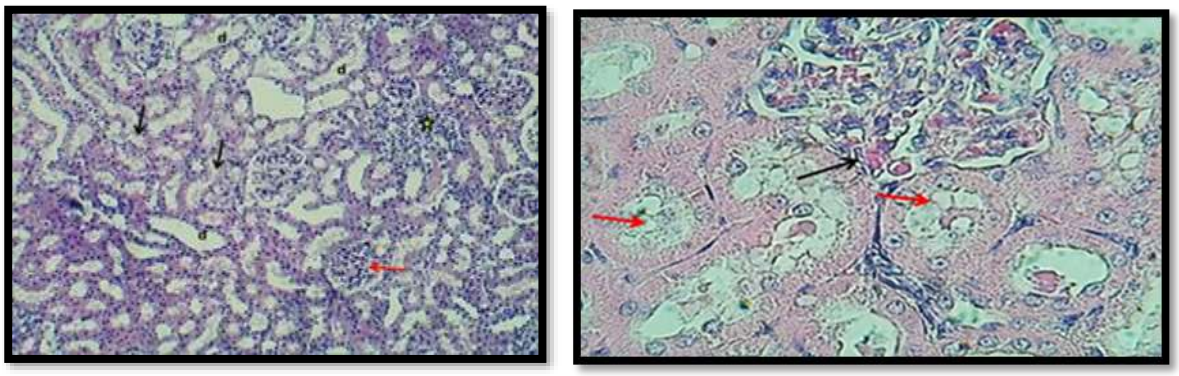


Figure (11): Sections of G4 kidney tissue. I: Section of kidney in G4 shows glomerulonephrosis with vascular dilation with congestion (Black arrows), interstitial edema with interstitial aggregation of leukocytes (Asterisk), glomerular congestion (Red arrow).H&E.100x. J: section of kidney in G4 shows glomerular congestion (Black arrow), interstitial edema (Asterisk), tubular degeneration with necrosis with cast formation (Red arrows).H&E.400x.

K L

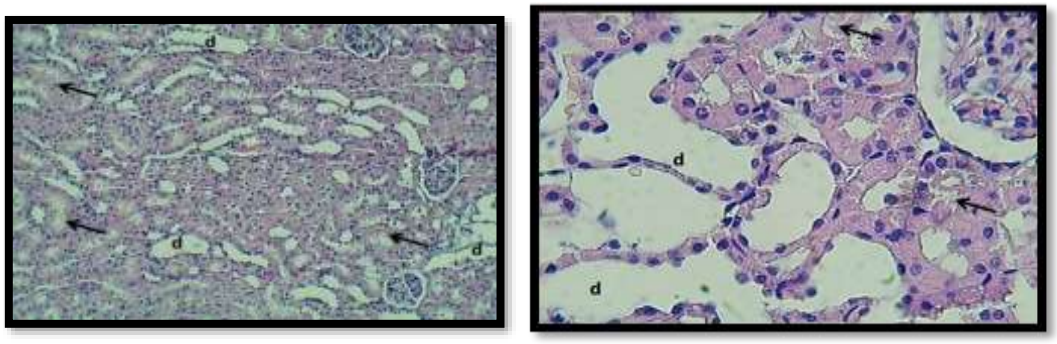


Figure (12): Sections of G4 kidney tissue, K: Section of kidney in G4 shows mild tubular dilation with atrophy of lining cells (d) &other renal tubules showed degeneration with necrosis (Arrows).H&E.100x. L: section of renal cortex in G4 shows tubular dilation with atrophy of lining cells (d), other renal tubules showed degeneration with necrosis (Arrows).H&E.400.

M N

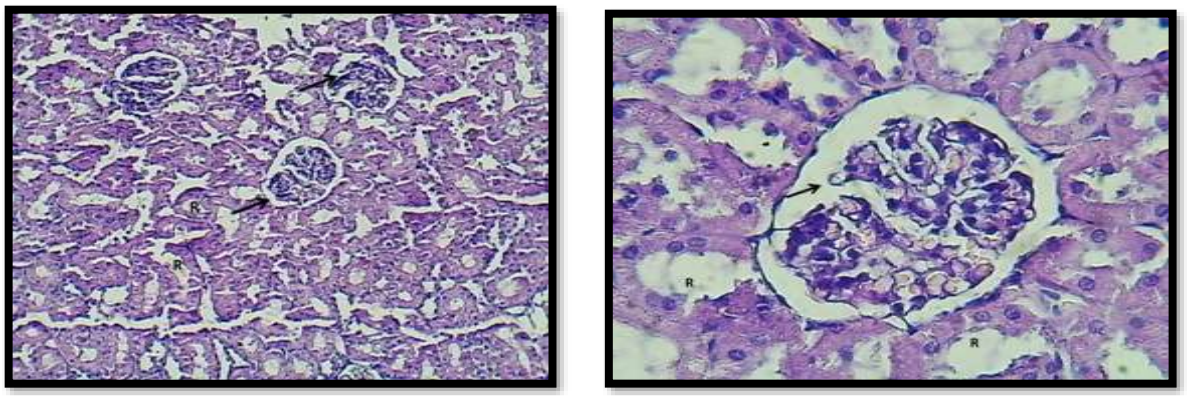


Figure (13): G5 renal tissue sections. M: The renal tubule lining cells (R) and the interstitum and glomerulus (arrows) seem normal in this section of the renal cortex in G5.H&E.100x. N: A section of the renal cortex in G5 displays normal interstitium and glomerulus (arrow), as well as typical renal tubule lining cells (R). 400x H&E.

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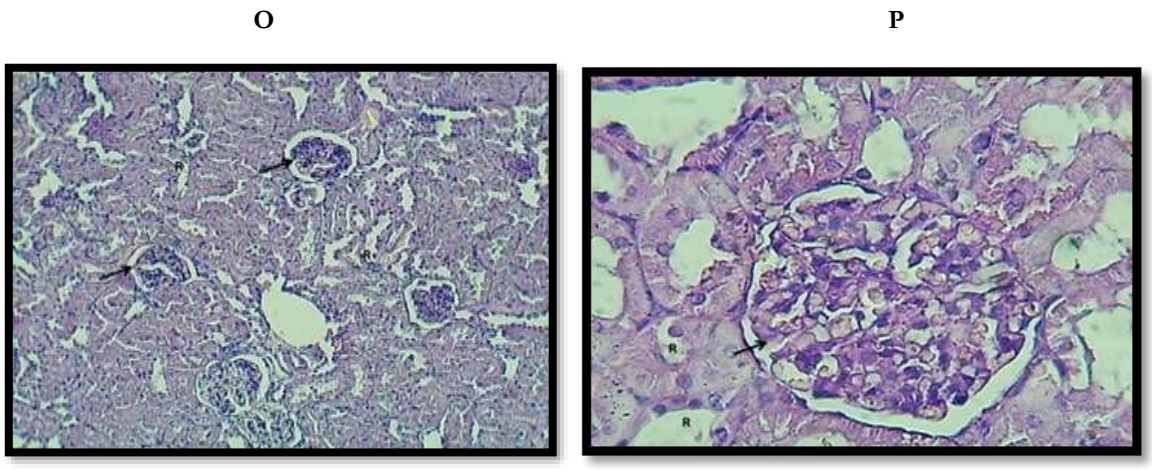


Figure (14): G6 renal tissue sections. O: A portion of the renal cortex in G6 has normal interstitium and glomerulus (arrow) and little tubular atrophy of the lining cells (R).100x H&E. P: A portion of the renal cortex in G6 has normal interstitium and glomerulus (arrow), with little atrophy of the lining cells of collecting tubules (R). 400x H&E

1. Protein expression of caspase-8 in kidney tissue using Immunohistochemistry

Figures (15-20) show different expression scores for caspase-8 in treated groups with dapa alone and in combination with 5-Fu (G3,4,5,6) versus the induction group (G2) in kidney tissue. Caspase-8 shows a significantly high expression score and high intensity in G2, while there is a significant reduction in score in the group that received dapa + 5-Fu (G6). There is no expression for this protein in the control and dapa alone groups (G1,3,5), respectively.

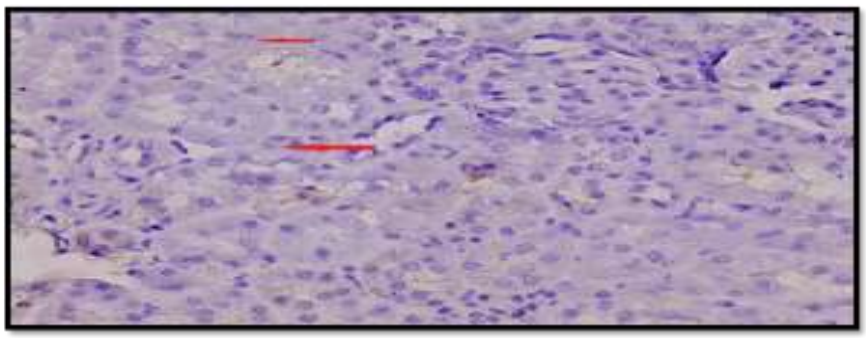


Figure (15): IHC staining for caspase-8 in G1 rat's kidney tissue. Staining showed negative nuclear staining of caspase-8 in epithelial cells of tubules (red arrow) with a score of 0.400xHPF.

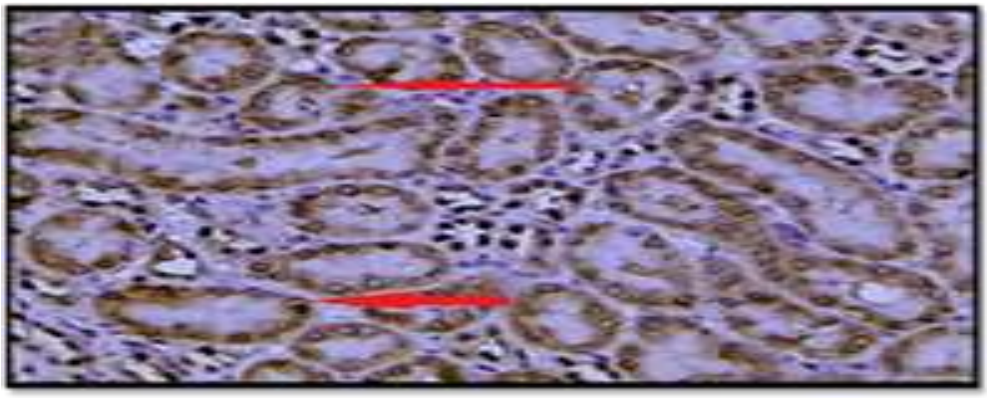


Figure (16): IHC staining for caspase-8 in G2 rat's kidney tissue. Staining showed positive nuclear staining of caspase-8 in epithelial cells of tubules (red arrow) with a score of 8.400x HPF.

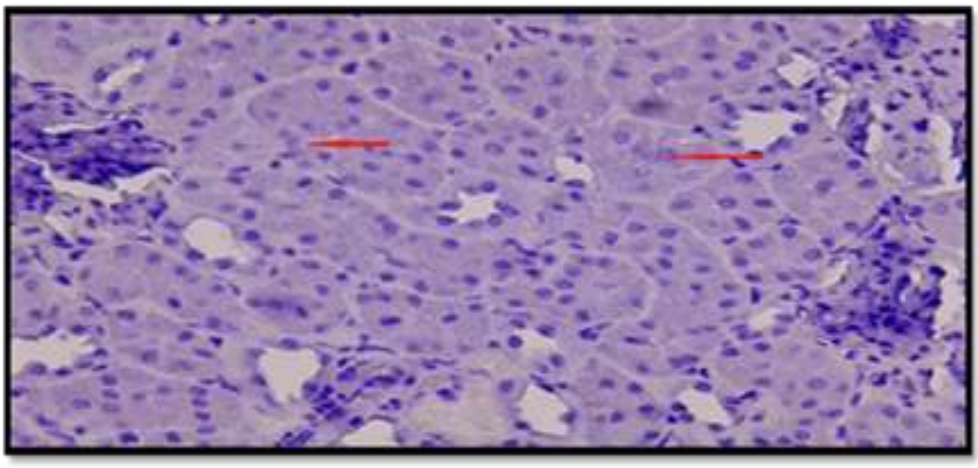


Figure (17): IHC staining for caspase-8 in G3 rat's kidney tissue. Staining showed negative nuclear staining of caspase-8 in epithelial cells of tubules (red arrow) with a score of 0 . 400x HPF.

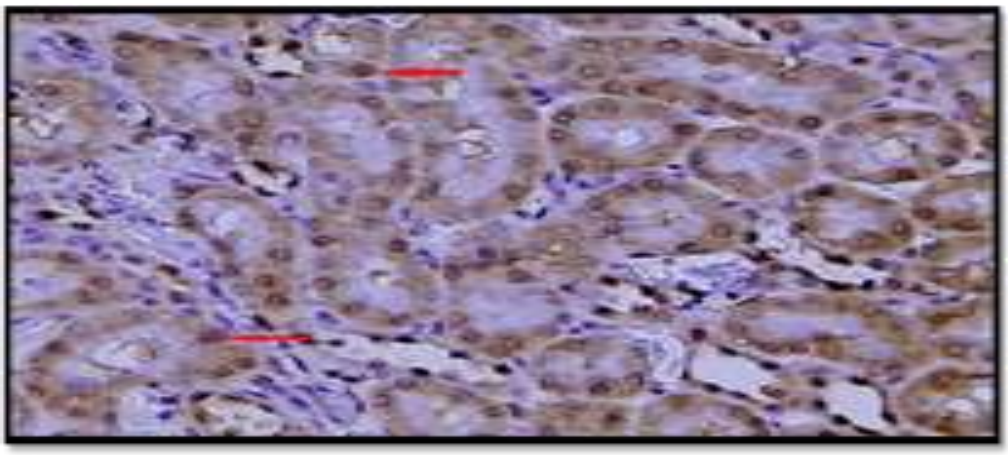


Figure (18): IHC staining for caspase-8 in G4 rat's kidney tissue. Staining showed positive nuclear staining of caspase-8 in epithelial cells of tubules (red arrow) with a score of 7. 400x HPF.

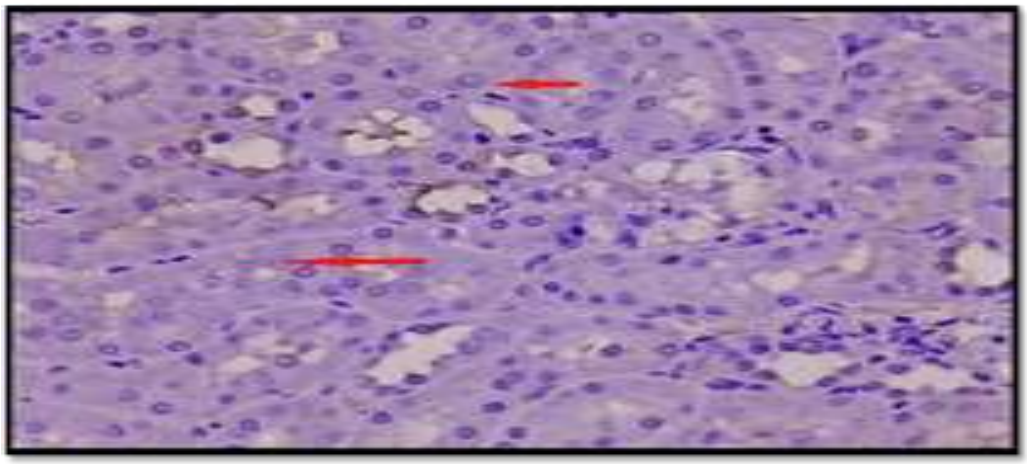


Figure (19): IHC staining for caspase-8 in G5 rat's kidney tissue. Staining showed negative nuclear staining of caspase-8 in epithelial cells of tubules (red arrow) with a score of 0.400x HPF.

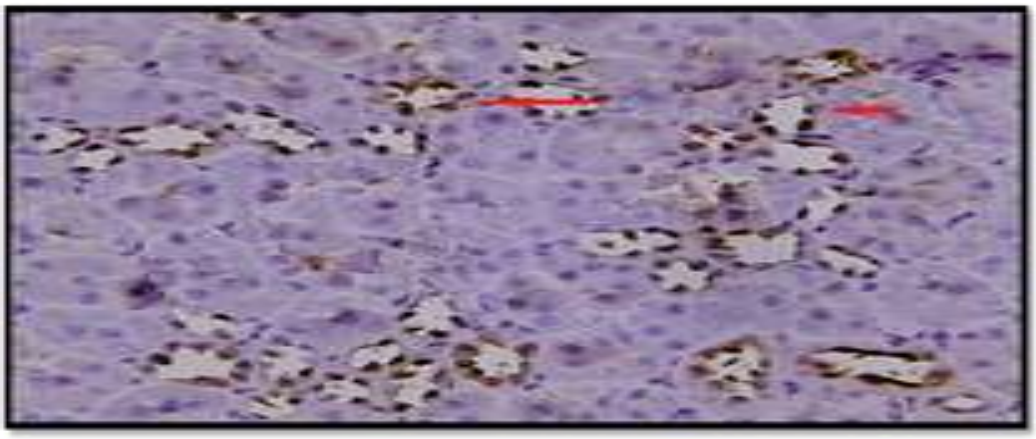


Figure (20): IHC staining for caspase-8 in G6 rat's kidney tissue. Staining showed positive nuclear staining of caspase-8 in epithelial cells of tubules (red arrow) with a score of 4. 400x HPF.

2. Protein expression of myeloid differentiation 88 (MYD88) in kidney tissue using Immunohistochemistry

As shown in **Figure (21-26)**, MYD88 showed high score and high intensity in induction group (G2), while treated groups with dapa plus 5-Fu (G4,6) showed significant reduction in score and intensity of MYD88 protien. There is no expression for this protein in the control and dapa alone groups (G1,3,5), respectively. **Table (4) & Figure (27 &28)** illustrated the mean score for caspase-8, and MYD88 in kidney tissue of all study groups.

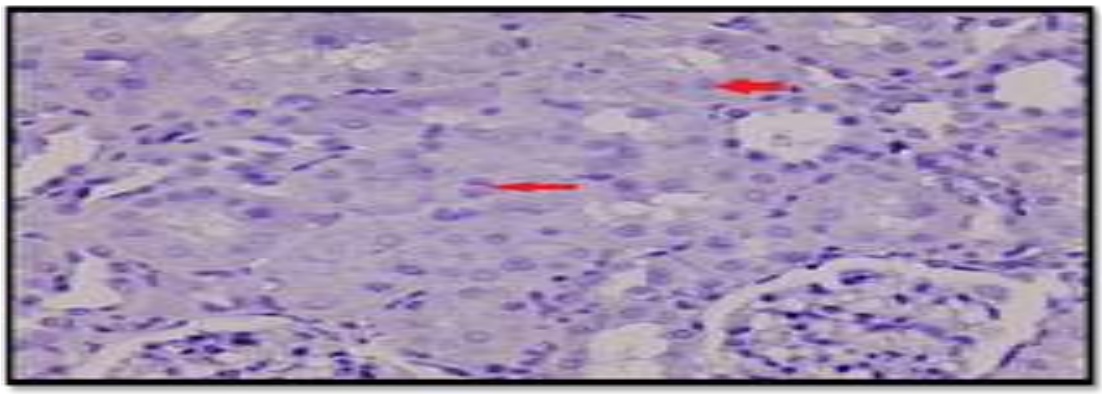


Figure (21): IHC staining for MYD88 in G1 rat's kidney tissue. Staining showed an issue with negative nuclear staining of MYD88 in epithelial cells of tubules (red arrow) with a score of 0. 400x HPF.

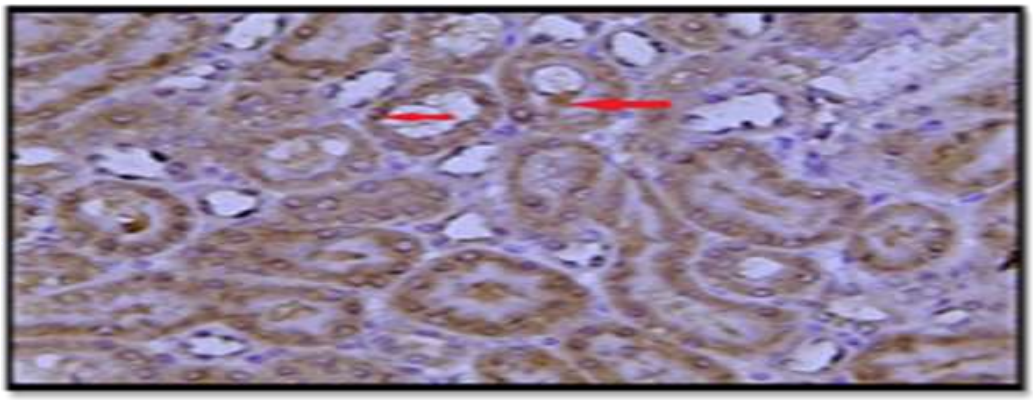


Figure (22): IHC staining for MYD88 in G2 rat's kidney tissue. Staining showed positive nuclear staining of MYD88 in epithelial cells of tubules (red arrow) with a score of 8.400x HPF.

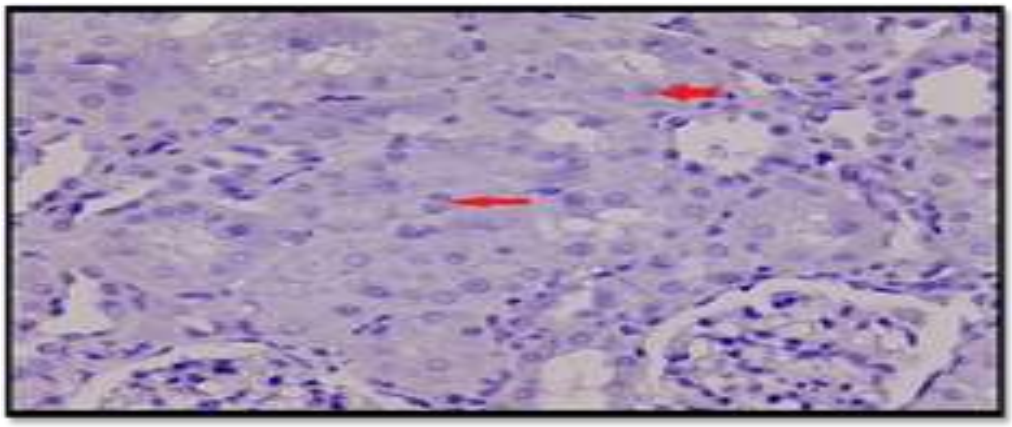


Figure (23): IHC. staining for G3rat's kidney tissue with negative nuclear staining for MYD88 in epithelial cells of tubules (red arrow) with score 0 and 0 intensity.400x HPF.

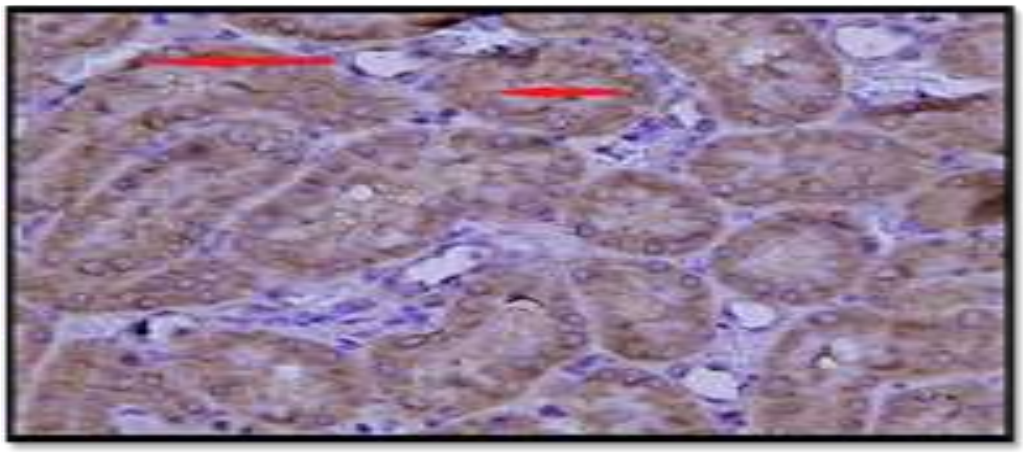


Figure (24): IHC staining for MYD88 in G4 rat's kidney tissue. Staining showed positive nuclear staining for MYD88 in epithelial cells of tubules (red arrow) with a score of 6.400x HPF.

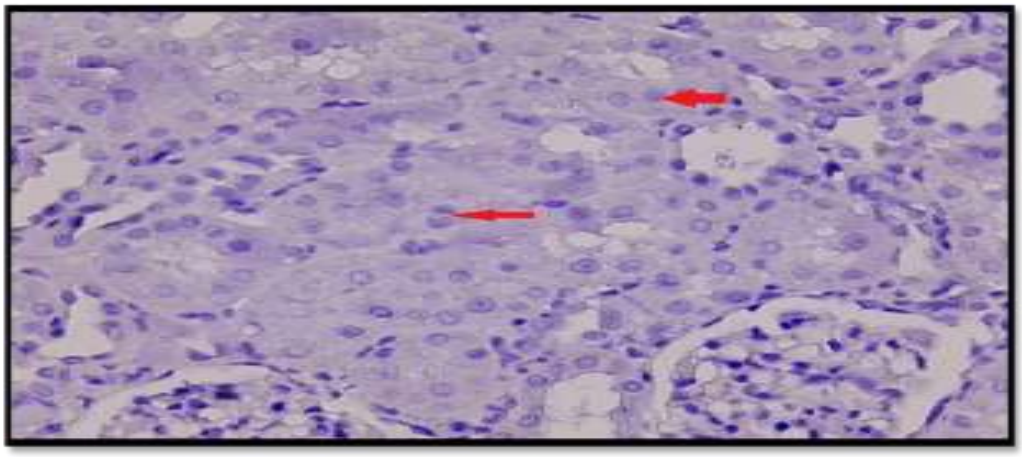


Figure (25): IHC staining for MYD88 in G5 rat's kidney tissue. Staining showed negative nuclear staining with MYD88 in epithelial cells of tubules (red arrow) with a score of 0.400x HPF.

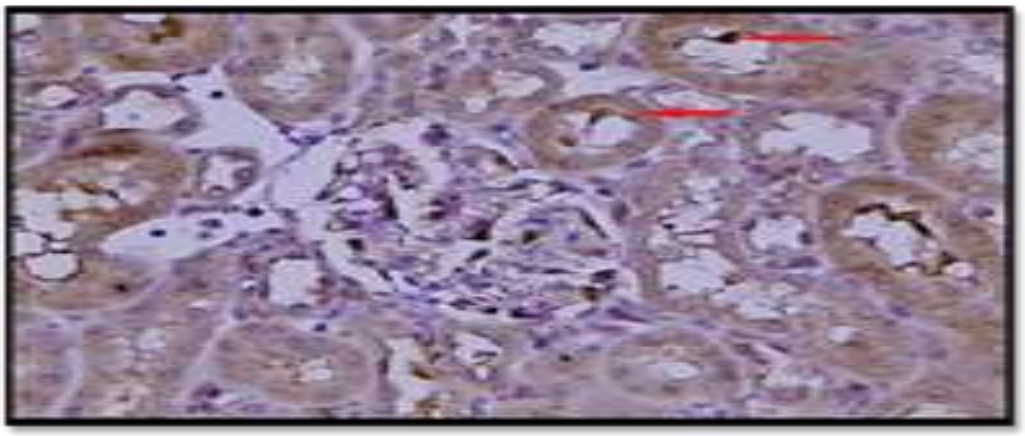


Figure (26): IHC staining for MYD88 in the G6 rat's kidney tissue. Staining showed positive nuclear staining of MYD88 in epithelial cells and endothelial cells of tubules (red arrow) with a score of 4. 400x HPF.

Table (4): Mean score of Caspase-8& MYD88 in rat's kidney tissue

Groups	Mean ±SE of Caspase-8 in kidney	Mean ±SE of MYD88 in kidney
G1(control)	0c	0c
G2(Induction)	8a	8a
G3(5mg DAPA)	0c	0c
G4(5mgDapa+5-Fu)	7a	6ab
G5(10mgDAPA)	0c	0c
G6(10mgDAPA+5-Fu)	4b	4b
LSD	2.905**	2.737**
p-value	-0.0001	-0.0001

The means of the several letters in the same column varied considerably. ** (P≤0.01).

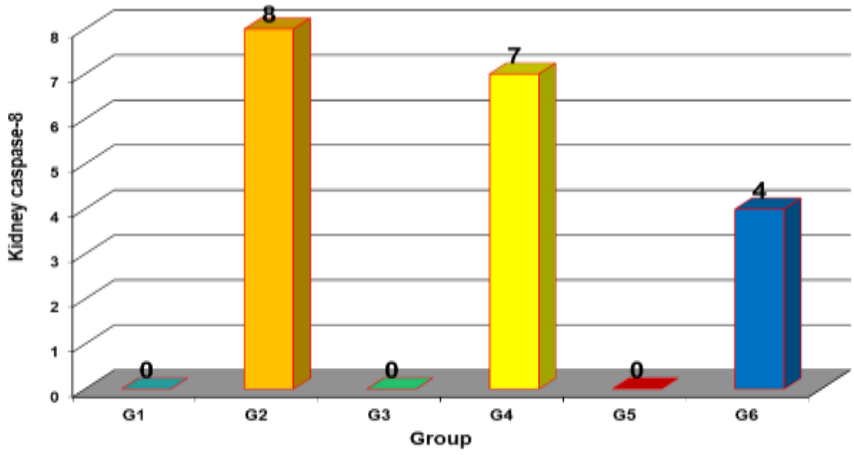


Figure (27): Comparison between the mean score of caspase-8 in all groups of rat's kidney tissue

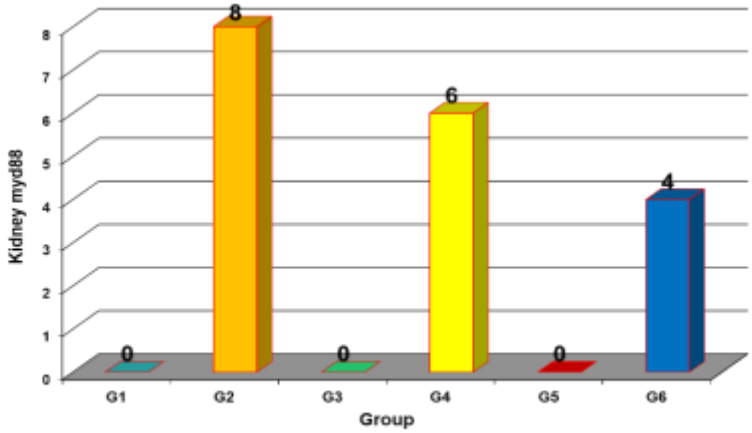


Figure (28): Comparison between the mean score of MYD88 in all groups of rat's kidney tissue

DISCUSSION

5-Fluorouracil (5-FU) remains a cornerstone of systemic chemotherapy for a broad spectrum of solid tumors; however, its clinical utility is markedly limited by a well-documented toxicity profile. Although 5-FU has potent antineoplastic activity, its therapeutic benefit of 5-FU is frequently canceled out by significant organ toxicity, including nephrotoxicity, neurotoxicity, and damage to other vital organs, which limits optimal dosing and compromises patient outcomes. The sodium-glucose inhibitor 2 (SGLT2) inhibitor dapagliflozin (DAPA) has attracted a lot of attention lately because of its pleiotropic Renal protective effects that go beyond glycemic management. DAPA attenuates oxidative stress, suppresses inflammatory responses, preserves mitochondrial function, and reduces apoptosis, ultimately improving renal function and limiting tissue damage (26). The present findings demonstrate elevated urea and creatinine levels in induction(G2) with 5-FU administration compared to control(G1), while treated groups (G4,6) showed reduced levels consistent with several experimental studies. Al-Abbas (2024) reported that 5-FU induced significant renal damage, reduced antioxidant enzyme activity, and upregulation of NF-ϰB/TLR4 inflammatory pathways (24). According to Rashid et al. (2023), 5-FU caused oxidative stress in the kidneys, which resulted in notable changes in renal indicators, such as serum creatinine, urea, and uric acid, as well as decreased activity of superoxide dismutase and catalase (25). The protective role of dapagliflozin observed in the present study is further corroborated by Santos et al. (2025), who demonstrated that dapagliflozin ameliorates oxidative stress, apoptosis, and inflammation in drug-induced nephrotoxicity (26). A major mechanism of 5-FU is oxidative stress, which induces tissue injury. Both GSH and MDA are widely used biomarkers that evaluate the antioxidant mechanism in kidney tissue. The finding that GSH depletion in renal tissue following

5-FU administration is well supported in the literature. Through the activation of oxidative stress, 5-FU injection significantly depleted the renal antioxidant defense machinery, including GSH, glutathione reductase (GR), and glutathione peroxidase (GPx), according to Rashid et al. (2023) (27). The importance of GSH depletion in 5-FU-induced nephrotoxicity was further confirmed by Akbari et al. (2024), who showed that sitagliptin partially restored GSH levels in 5-FU-challenged rats (28). The current results showed a strong activation of inflammatory pathways after 5 FU treatment, with IL-1β levels significantly higher in the induction group (G2) than in the control group (G1). IL 1β is a key pro-inflammatory cytokine implicated in the amplification of tissue injury and perpetuation of oxidative and inflammatory damage. In contrast, treatment with dapagliflozin in combination with 5 FU (G4 and G6) reduced IL-1β concentrations relative to G2, although this decrease did not reach statistical significance. The tubular epithelium, glomeruli, and renal parenchyma of the control group were all intact, indicating normal renal architecture. In contrast, kidney sections from the 5-FU–induced group (G2) displayed severe pathological alterations, including nephritis, tubular cellular swelling (ballooning degeneration), coagulative necrosis, vascular congestion, interstitial edema, and marked glomerular deterioration. These findings are in agreement with a study, which reported that 5-FU administration in rats induced severe degenerative changes along the renal cortex, including tubular necrosis, hemorrhage, atrophy, and increased Bowman's capsular space, attributed to the excessive generation of reactive oxygen species and activation of NF-ϰB–mediated inflammatory cascades (29). The immunohistochemical findings revealed a marked elevation in caspase 8 expression in the renal tissue of the 5-FU–induced rats group (G2), with the highest score and intensity, indicating significant activation of the extrinsic apoptotic pathway following chemotherapeutic insult. The activation of the Fas/FasL or TNF receptor signaling cascades in response to oxidative and inflammatory stressors generated by 5-FU is reflected in the overexpression of caspase 8, a crucial initiator caspase in death receptor–mediated apoptosis. These results are in line with Mitra et al. (2014), who showed that 5-FU causes tubular epithelial cell death by upregulating p53, Bax, and caspase cascade proteins while simultaneously suppressing the anti-apoptotic protein Bcl-2 (30).

CONCLUSIONS:

Dapagliflozin has a protective effect to reduce 5-FU therapeutic effect in induced renal injury in rats, through improved and ameliorated oxidative stress, inflammatory, and apoptotic markers, and histopathological modifications. These findings suggest that Dapagliflozin exerted a strategy to reduce 5-FU toxicity.

Limitation of the study

Despite the promising nature of these findings, the transition of preclinical results into clinical applications is challenging. Critical factors such as the dose response relationship and overall efficacy of 5-Fu in humans require comprehensive evaluation through well –designed clinical trials. Furthermore, variability in physiological responses to treatment may contribute to differences in therapeutic efficacy among patients, and there were differences in renal health status among patients. Therefore, further research is required in the future to optimize dosage regimens, clarify the underlying mechanisms of action in human physiology, and validate the therapeutic significance of dapagliflozin in reducing 5-Fu toxicity.

Ethical Approval

On March 17, 2026, the Mustansiriyah University College of Pharmacy Ethics Committee granted ethical permission for the animal study.

REFERENCES

[1] Marok FZ, Wojtyniak JG, Selzer D, Dallmann R, Swen JJ, Guchelaar HJ, et al. Personalized Chronomodulated 5-Fluorouracil Treatment: A Physiologically-Based Pharmacokinetic Precision Dosing Approach for Optimizing Cancer Therapy. Clin Pharmacol Ther. 2024;115(6):1282–92

[2] Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: Mechanisms of action and clinical strategies. Nat Rev Cancer. 2003;3(5):330–8.

[3] Noordhuis P, Holwerda U, Van der Wilt CL, Van Groeningen CJ, Smid K, Meijer S, Pinedo HM, Peters GJ. 5-Fluorouracil incorporation into RNA and DNA in relation to thymidylate synthase inhibition of human colorectal cancers. Ann Oncol. 2004 Jul;15(7):1025-32. doi: 10.1093/annonc/mdh264. PMID: 15205195.

[4] Filipa V, Martins S. Benefits and drawbacks for the clinical use of 5-fluorouracil. Master's Thesis, Univ Lisboa. 2023;

[5] De Angelis PM, Svendsrud DH, Kravik KL, Stokke T. Cellular response to 5-fluorouracil (5-FU) in 5-FU-resistant colon cancer cell lines during treatment and recovery. Mol Cancer. 2006;5:1–25.
[6] Costa J. Neurocognitive effects Fog: Cancer chemotherapy Vol. 678, Advances in Experimental Medicine and Biology. 2010.26–32p.Availablefrom:http://www.scopus.com/inward/record.url?eid=2-s2.0-77956636560&partnerID=tZOtx3y1..
[7] Małyszko J, Kozłowska K, Kozłowski L, Małyszko J. Nephrotoxicity of anticancer treatment. Nephrol Dial Transplant. 2017;32(6):924–36.
[8] Harutyunyan K, Nersesova L, Ayvazyan V, Avagyan E, Melkumyan M, Sargsyan M, et al. In silico and in vitro evaluation of drug-like properties and anticancer potential of novel 5-fluorouracil derivatives. Sci Rep. 2025;15(1):1–15
[9] Zhang Y, Liu Z, He Q, Wu F, Xiao Y, Chen W, et al. Construction of Mode of Action for Cadmium-Induced Renal Tubular Dysfunction Based on a Toxicity Pathway-Oriented Approach. Front Genet. 2021;12(July):696892
[10] da Silva MC, Fabiano LC, da Costa Salomão KC, de Freitas PLZ, Neves CQ, Borges SC, et al. A Rodent Model of Human-Dose-Equivalent 5-Fluorouracil: Toxicity in the Liver, Kidneys, and Lungs. Antioxidants. 2023;12(5):1005
[11] Talebi M, Mohammadi Vadoud SA, Haratian A, Talebi M, Farkhondeh T, Pourbagher-Shahri AM, et al. The interplay between oxidative stress and autophagy: focus on the development of neurological diseases. Behav Brain Funct [Internet]. 2022;18(1):1–22. Available from: https://doi.org/10.1186/s12993-022-00187-3,
[12] Afzal S, Biyabani U, Patil N, Babladi VP, Salimath P V. REVIEW ARTICLE A Systematic Review of Pharmacodynamics and Cardio- Renal Outcomes of SGLT-2 and DPP-4 Inhibition in Type 2 Diabetes Mellitus. 2026;4(1):013-022.
[13] Kato S, Horita N, Utsunomiya D. Sodium-glucose cotransporter 2 inhibitors in heart failure with chronic kidney disease. ESC Hear Fail. 2022;9(5):3661–2.
[14] Suades R, Cosentino F, Badimon L. Glucose-lowering treatment in cardiovascular and peripheral artery disease. Current Opinion in Pharmacology [Internet]. 2018 Apr 1;39:86–98.Available from:https://www.sciencedirect.com/topics/ https://www.sciencedirect.com/topics/neuroscience/sodium-glucose-cotransporter-2
[15] Merovci A, Solis-Herrera C, Daniele G, Eldor R, Vanessa Fiorentino T, Tripathy D, et al. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest. 2014;124(2):509–14.
[16] Scheen AJ, Delanaye P. Understanding the protective effects of SGLT2 inhibitors in type 2 diabetes patients with chronic kidney disease. Expert Rev Endocrinol Metab. 2022;17(1):35–46. Available from: https://doi.org/10.1080/17446651.2022.2014322
[17] Fekete A, Committee FE, Members D, Committee FE. NOVEL THERAPEUTIC OPTIONS IN DIABETIC KIDNEY DISEASE [PhD thesis]. D ó ra Bianka Balogh. Budapest (Hungary): Semmelweis University; 2020.128 p.
[18] Trevella P, Ekinci EI, MacIsaac RJ. Potential kidney protective effects of glucagon-like peptide-1 receptor agonists. Nephrology. 2024;29(8):457–69.
[19] Shin SJ, Chung S, Kim SJ, Lee EM, Yoo YH, Kim JW, et al. Effect of sodium-glucose co-transporter 2 inhibitor, dapagliflozin, on renal renin-angiotensin system in an animal model of type 2 diabetes. PLoS One. 2016;11(11):1–18.
[20] Kuriyama S. A Potential Mechanism of Cardio-Renal Protection with Sodium-Glucose Cotransporter 2 Inhibitors: Amelioration of Renal Congestion. Kidney Blood Press Res. 2019;44(4):449–56.
[21] Dong Y, Shi S, Liu L, Zhou X, Lv J, Zhang H. Effect of SGLT2 inhibitors on the proteinuria reduction in patients with IgA nephropathy. Front Med. 2023;10(September):1–7.
[22] Montomoli M, Gonzalez M, Gorriz JL, Puchades MJ, Nuñez-Marín G, Lecueder MS. Addressing Renal Sodium Avidity in Chronic Heart Failure: There Is Always More than One Way to Skin a Cat. CardioRenal Med. 2025;15(1):399–412.
[23] Saber S, Abd El-Fattah EE, Sharaf MH, El-Ahwany E, Amin NA, Alsharidah M, et al. Dapagliflozin protects against doxorubicin-induced nephrotoxicity associated with the nitric oxide pathway: a translational study. Free Radic Biol Med. 2023;208:213–24.
[24] Dawwam GE, Abd El-Ghany RM, Ahmed EK, Bayomy MF. Renoprotective effects of dapagliflozin versus pentoxifylline in chronic kidney disease: antioxidative stress and anti-inflammatory action via inhibition of HMGB1. J Ren Inj Prev. 2023;12:e32245.
[25] Cha RH, Lee JE, Park HJ, Kim HW, An WS, Chin HJ, et al. Dapagliflozin does not protect against Adriamycin-induced kidney injury in mice. Kidney Blood Press Res. 2024;49(1):81–92.
[26] Soltan MA, Abdel-Daim MM, Elbadawy M, Zedan H. Dapagliflozin exerts renoprotective effect by activating the Nrf2/HO-1 pathway in glycerol-induced acute kidney injury. Egypt J Hosp Med. 2025;99:453–62.

[27] Gad EM, Abdel-Rahman HG, Abd-El-Fattah ME, Kamal MM, Eltahan AS, Dessouki AA. Renoprotective impact of dapagliflozin and mulberry extracts on FR STZ-induced diabetic nephropathy in rats: biochemical and molecular aspects. Adv Anim Vet Sci. 2024;12(S1):112 26.
[28] Ergin B, Uzun H, Doğan Z, et al. Protective effect of dapagliflozin on colistin-induced renal toxicity in rats. Signa Vitae. 2021;17(4):92–7.
[29] Zhao X, Wang L, Chen H, et al. Inhibition of caspase-3/GSDME pathway-mediated pyroptosis of renal tubular epithelial cells by dapagliflozin. Diabetes Metab Syndr Obes. 2023;16:515–34.