EFFECT OF EMPAGLIFLOZIN ON CISPLATIN-INDUCED NEPHROTOXICITY IN ADULT MALE ALBINO RAT

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Abstract

Background: empagliflozin is currently available for the management of type 2 diabetes mellitus and had a reno-protective effect. Cisplatin (CP) is used in the treatment of different types of cancers but severe complications occur include nephrotoxicity, ototoxicity, neurotoxicity and bone marrow suppression. **Objective:** investigating the effect of Empagliflozin on cisplatin-induced nephrotoxicity in adult male albino rats with detecting possible underlying mechanisms .**Materials and methods:** 32 adult male albino rats were used in this work and divided into 4 equal groups: Control group, cisplatin group, group treated with cisplatin and empagliflozin (10mg/Kg) and group treated with cisplatin and empagliflozin (25mg/Kg) by oral gavage for ten consecutive days and cisplatin (30 mg/kg, i.p.) on the seventh day of treatment. In addition to routine weight assessment, kidney tissue nerve growth factor (NGF-β) and oxidative stress parameters were measured. As part of a biochemical evaluation of serum urea, creatinine, uric acid, albumin, calcium, and glucose. Total antioxidant, superoxide dismutase, TNF-α and IL-6 were measured. **Results:** in our study, Cisplatin significantly reduced antioxidant indices (TAC, Reduced glutathione (RG) and superoxide dismutase (SOD), body weight, NGF-β, and also significantly increased the plasma concentration of inflammatory cytokines (plasma tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), malondialdehyde, urea and creatinine. Histopathologically, CP caused a significant kidney damage compared with control. Prophylactic and therapeutic application of empagliflozin significantly reversed CP-induced changes in all the above renal parameters either by low or high doses ($P \leq 0.05$). **Conclusion:** Empagliflozin significantly ameliorated CP-induced biochemical and histopathological changes in the kidney and may be promising renoprotective effect against cisplatin chemotherapy where it reduced oxidative stress and possess anti-inflammatory, antioxidant and anti-apoptotic action

Keywords: Empagliflozin, cisplatin and nephrotoxicity.

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Introduction

One of the most effective chemotherapy drugs is cisplatin, which is used to treat many different types of cancer, including refractory non-Hodgkin lymphoma, head, and neck cancer, ovarian, testicular, and lung cancer. According to **Dasari and Tchounwou (2014)**, it interacts with DNA, induces oxidative stress, and triggers apoptosis to be effective against cancer. Due to tumor cell resistance and unfavorable side effects such neurotoxicity, ototoxicity, and nephrotoxicity, its usage is restricted despite its efficiency (**Sancho-Martnez et al., 2012**). The most frequent and harmful side effect is nephrotoxicity, which manifests as acute kidney damage. One-third of patients experience it even after just one dose, making it difficult to increase the dosage and reducing therapy efficacy (**Miller et al., 2010**).

It is well established that Cisplatin causes acute tubular necrosis in the kidney, namely in the S3 region of the proximal convoluted tubules. For example, DNA damage, increased oxidative damage, mitochondrial dysfunction, inflammation, intracellular messengers and transducers, and induction of apoptosis and necrosis have all been suggested as possible pathways (**Peres and da Cunha, 2013**).

Numerous methods were attempted on patients to prevent nephrotoxicity, including hydration, diuretics, and the administration of magnesium, which aimed to reduce the concentration of cisplatin in renal cells and increase its excretion. Short hydration and forced diuresis with mannitol, rather than frusemide, may be safer in avoiding Cisplatin nephrotoxicity, according to recent consensus (**Crona et al., 2017**). However, given that mannitol is

only utilized in a few situations and might be hazardous to the kidneys at high dosages, the evidence is still limited. To evaluate hydration across a range of ages and practice contexts, more study is required (**Duffy et al., 2018**).

The anti-diabetic medication empagliflozin inhibits the sodium glucose transporter 2 (SGLT2) in the proximal convoluted tubules. Its anti-diabetic action results from the reduction of 90% of the filtered glucose's absorption (**Neumiller, 2014**). It has recently been discovered that empagliflozin may have a reno-protective activity in diabetic nephropathy through its glycemic control effect, blood pressure lowering effects, and direct renal effects inhibition of inflammatory and fibrotic responses of renal proximal tubular cells to hyperglycemia (**Fioretto et al., 2016**).

Regardless of its anti-diabetic and glucosuria activity, the goal of this study was to determine if Empagliflozin has a reno-protective or therapeutic impact on Cisplatin-induced acute kidney damage, particularly in connection to its influence on apoptosis, oxidative stress, and inflammation.

Materials and Methods

Animals and ethical decision

Our study included 32 adult male albino rats weighting from 120 – 140 g and aged 6 – 8 weeks. Rats were placed in cages, which were made of stainless steel and had mesh floors and hardwood beds. They were housed in a laboratory with a standard light/dark cycle and a constant 25 °C temperature. Throughout the trial, rats had access to food and drink. Before starting the study, the rats were given two weeks to acclimate. The "Guide for the Care and Use of Laboratory Animals" was considered the standard by which all experimental procedures were conducted.

Drugs and experimental design

Drugs and chemicals

Both the Mylan (1 mg/ml) and Boehringer Ingelheim (Jardiance) brands of cisplatin solution and empagliflozin were bought. Sigma Pharmaceutical Company in Quesna, Egypt provided the carboxymethyl cellulose (CMC) that was used in this study. All other reagents were of the analytical quality and were acquired from Sig- ma-Aldrich Co. in St. Louis, Missouri, USA.

Experimental design

Rats were randomly assigned into 4 equal groups (n = 8/group). Regular weight checks were performed.

Group 1: Control—received saline injection intraperitoneally (i.p.), single dose on day 7.

Group 2: Cisplatin—received cisplatin (30 mg/kg, i.p.), single dose on day 7.

Group 3: Cisplatin + Empagliflozin—received Empagliflozin (10 mg/kg), by oral gavage for ten consecutive days and cisplatin (30 mg/kg, i.p.) on the seventh day of the treatment.

Group 4: Cisplatin + Empagliflozin—received Empagliflozin (25 mg/kg), by oral gavage for ten consecutive days and cisplatin (30 mg/kg, i.p.) on the seventh day of treatment. Sample gathering and animal sacrifice.

Rats was slaughtered using isoflurane inhalation anesthesia on day 10 following Cisplatin treatment. Blood samples were collected from the retro-orbital plexus into vacuum tubes, and serum was separated by centrifuging blood at 4,000 rpm for 20 min.

Until further biochemical investigation, serum was stored at -80°C in plastic Eppendorf containers. After that, the rats had been killed via cervical dislocation; their kidneys were harvested, weighed, and cleaned in ice-cold saline.

Kidney samples were fixed in 10% buffered formalin from (AlGomhorya, Cairo, Egypt) for histopathological analysis. The kidneys were weighed and cleaned in a phosphate buffer solution. The right one was immediately kept at -80 °C for subsequent measurement of nerve growth factor and oxidative stress parameters; malondialdehyde and reduced glutathione.

Biochemical investigations

With the help of the diamond diagnostic kit (**Diamond Diagnostics Company, Egypt**), serum was used to measure creatinine using the Jaffe colorimetric kinetic method (**Vasiliades, 1976**) and urea using the Berthelot enzymatic colorimetric method (**Fawcett and Scott, 1960**). Calcium was measured using fully automated chemistry analyzer BS-120, MINDRA (**Shenzhen, China**). According to the PAP (peroxidase-antiperoxidase) technique, uric acid was measured using a uric acid liquicolor kit (**Human Diagnostics Company, Germany**) (**Fossati et al., 1980**). Plasma tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were measured by ELISA kit (**Life Technologies Corp., Frederick, MD, USA**), Using the SPINREACT kit (**SPINREACT, S.A./S.A.U., Spain**), albumin was determined in serum using the bromocresol green colorimetric method (**Rodkey, 1964**), and glucose was determined using the GOD-POD (glucose oxidase and peroxidase) colorimetric method (**Trinder, 1969**).

***NGF-B* (nerve growth factor beta) levels in kidney tissue.**

To assay tissue *NGF-B* with a commercially available rat *NGF-B* ELISA kit from Chongqing Bio-spes Co., Ltd catalogue number BEK1171, kidney was homogenized in 0.1 M sodium phosphate buffer

pH 7.4 at a ratio of 1 g tissue to 4 ml buffer and centrifuged at 4,000 rpm for 20 min at 4 °C. The results were expressed as ng/g tissue.

Analysis of reduced glutathione and malondialdehyde in kidney tissue

Renal catalase, glutathione reductase (GR), total antioxidant capacity (TAC), malondialdehyde and superoxide dismutase (SOD) were measured by a colorimetric assay kit (**BioVision, Milpitas, CA, USA**) as described by **Ohkawa et al. (1979)** and **Beutler et al. (1963)**.

Statistical analysis

Utilizing (Graph Pad Prism version 5 for Windows, 2007, Graph Pad software, Inc.), the results' statistical analysis was carried out. ATN score was reported as median, and data were presented as mean+standard error of the mean (SEM). One-way ANOVA was used to compare several groups, followed by the post hoc Tukey test, the Games Howell test, and the Bartlett test for groups with uneven variances. The threshold for significance was set at the 0.05 level of probability.

Results

Changes in body weight, kidney weight both directly and indirectly

As shown in Table 1 and Figure 1; Rats' initial body weight did not significantly differ across groups. The difference between the starting and final body weights was examined, and it was discovered that the Cisplatin group significantly reduced body weight as compared to the control group ($p \leq 0.05$). However, the CP+EMP (10 mg/kg) group demonstrated a non-significant reduction in comparison to control group, whereas CP+EMP (25 mg/kg) group demonstrated non-significant reduction in comparison to the control group ($p \leq 0.05$).

Table (1): Effect of Cisplatin and Empagliflozin on rat body weight changes.

Parameters/ treatments	Control	CP	CP+ EMP (10 mg/kg)	CP+ EMP (25 mg/kg)
Initial body weight	125.1 ± 0.60	126.5 ± 6.5	127.2 ± 0.7	128.9 ± 0.8
Final body weight	210 ± 0.90 [a]	175 ± 12.90 [b]	190 ± 1.40 [a]	200 ± 1.90 [a]

All data are expressed as mean ± SEM. Different letters are significantly different at $p \leq 0.05$.

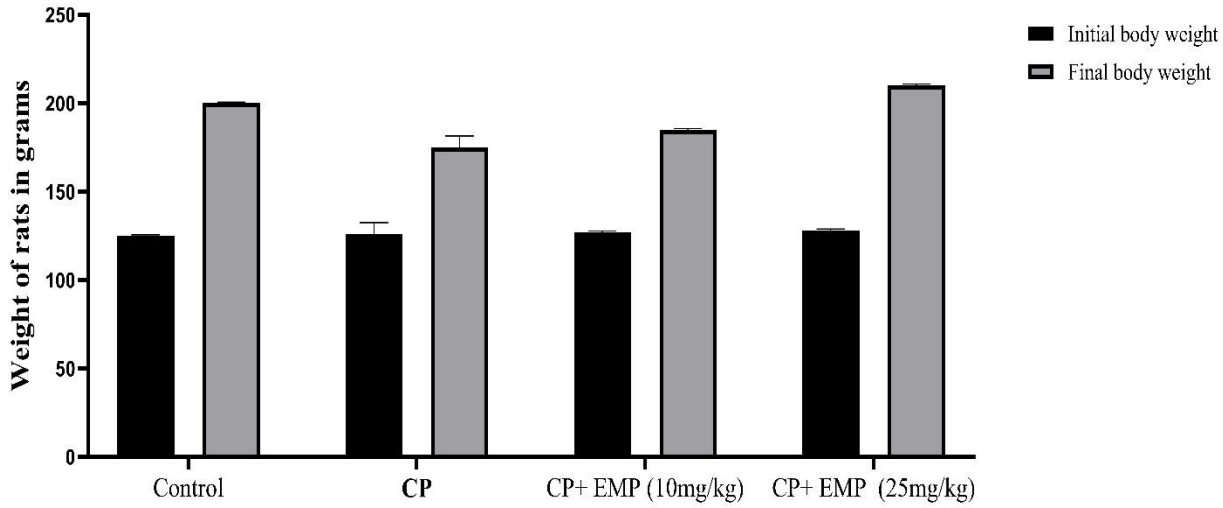

Fig. 1. Effect of Cisplatin and Empagliflozin on rat body weight changes.

Biological effects

As shown in Table 2; Cisplatin administration resulted in a significant increase in serum creatinine, urea, uric acid, and calcium ($p \leq 0.05$) when compared to the control group, while both the CP+EMP (10 mg/kg) and CP+EMP (25 mg/kg) groups showed a significant decrease in serum creatinine, urea, uric acid and calcium ($p \leq 0.05$), when compared to the Cisplatin group. However, there was no significant difference between CP+EMP (10 mg/kg) and CP+EMP (25 mg/kg) groups. Neither Cisplatin nor Empagliflozin significantly altered blood albumin levels. When compared to the control group, the treatment using CP+EMP (10 mg/kg) and CP+EMP (25 mg/kg) groups, both showed a substantial rise in blood glucose levels.

As showed in Table 2 and Figure 2; Results of nerve growth factor-beta (*NGF-B*) in kidney tissue while both the prophylactic and therapeutic groups of empagliflozin did not significantly differ when compared to Cisplatin or when compared to one another, they did show a significant decrease when compared to the control group. However, empagliflozin did not significantly differ when compared to Cisplatin or when compared to the control group.

Table (2): Effect of treatment with Empagliflozin (EMP) on some plasma constituents in rats with cisplatin (CP)-induced nephrotoxicity

Parameters/ treatments	Control	CP	CP+ EMP (10 mg/kg)	CP+ EMP (25 mg/kg)
Urea (mmol/L)	6.1±0.60	41.1±6.50[a]	17.9±2.3[a,b]	7.99±0.8[b]
Creatinine (µmol/L)	14.4±0.90	62.9±12.90[a]	29.9±1.40[b]	20.1±1.9[b]
Uric acid (µmol/L)	80.4±4.70	171.9±10.60[a]	134.5±5.20[a,b]	91.99±4.2[b]
Calcium (mmol/L)	0.81±0.01	1.41±0.02[a]	1.1±0.05[a,b]	1.21±0.02[a,b]
Glucose (mg/dl)	1.2±0.01	33.1±0.71[a]	34.9±0.85[a,b]	41.2±0.94[a,b]
Albumin (g/dl)	3.7±0.079	3.9±0.09	3.9±0.12	3.89±0.13
***NGF-B* (ng/g tissue)**	32.7±2.64	149±10.5[a]	117.8±9.04 [a,b]	97.1±4.6[a,b]

All data are expressed as mean ± SEM. Different letters are significantly different at p≤0.05.

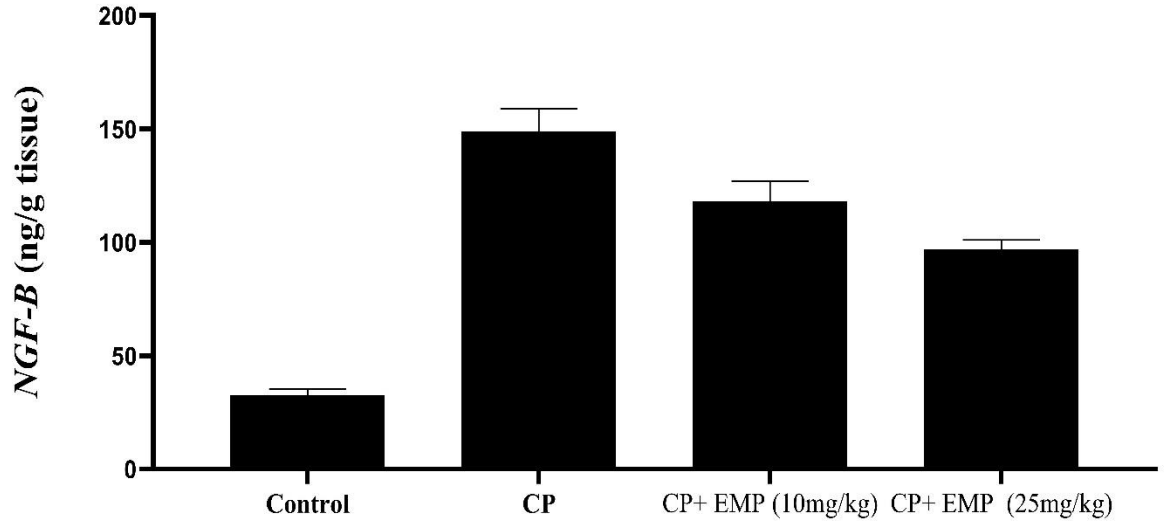

Fig. 2. Effect of Cisplatin and Empagliflozin on kidney tissue nerve growth factor-*β*.

Reduced glutathione and malondialdehyde in the kidney tissue

CP+EMP (10 mg/kg) and CP+EMP (25 mg/kg) treated groups led to a significant elevation of reduced glutathione (p≤0.05), Treatment with Cisplatin caused a significant reduction in reduced glutathione (Fig 3) with an increase in malondialdehyde (Fig 4) (p≤0.05). Malondialdehyde levels in CP+EMP (10 mg/kg) and CP+EMP (25 mg/kg) treated groups did not significantly differ from the Cisplatin group and control groups, displaying a mild inflammatory infiltrate with minimal hemorrhage, areas of tubular necrosis, and areas of tubular degeneration that did not significantly differ from the Cisplatin group. Figure 5 showed that CP increased plasma TNF-α and IL-6 concentrations, respectively. CP+EMP (10 mg/kg) and CP+EMP (25 mg/kg) treated groups reduced CP-induced changes in all the above plasma parameters. Figure 6 and 7 showed that CP reduced TAC, and SOD levels or activities, respectively. CP+EMP (10 mg/kg) and CP+EMP (25 mg/kg) treated groups reversed CP-induced changes in all the above renal parameters.

Table (3): The plasma concentration of tumor necrosis factor (TNF-α), interleukin (IL-6), Total antioxidant capacity, superoxide dismutase, reduced glutathione and malondialdehyde in control and treated rats

Parameters	Control	CP	CP+ EMP (10 mg/kg)	CP+ EMP (25 mg/kg)
TNF (pg/ml)	38.1±0.60	90.1±6.50[a]	80.2±2.3[a,b]	70.1±0.8[b]
IL-6 (pg/ml)	37.2±0.90	95.2±12.90[a]	83.2±1.40[b]	80.1±1.9[b]
TAC (nmol/ µL)	80.4±4.70	171.9±10.60[a]	134.5±5.20[a,b]	91.9±4.2[b]
SOD ((% relative to control)	0.81±0.01	1.41±0.02[a]	1.1±0.05[a,b]	1.21±0.02[a,b]
Reduced glutathione (mmol/g tissue)	4.1±0.01	3.1±0.71[a]	3.7±0.85[a,b]	4.2±0.94[a,b]
Malondialdehyde (mmol/g tissue)	100±0.079	155±0.09	120±0.12	110±0.13

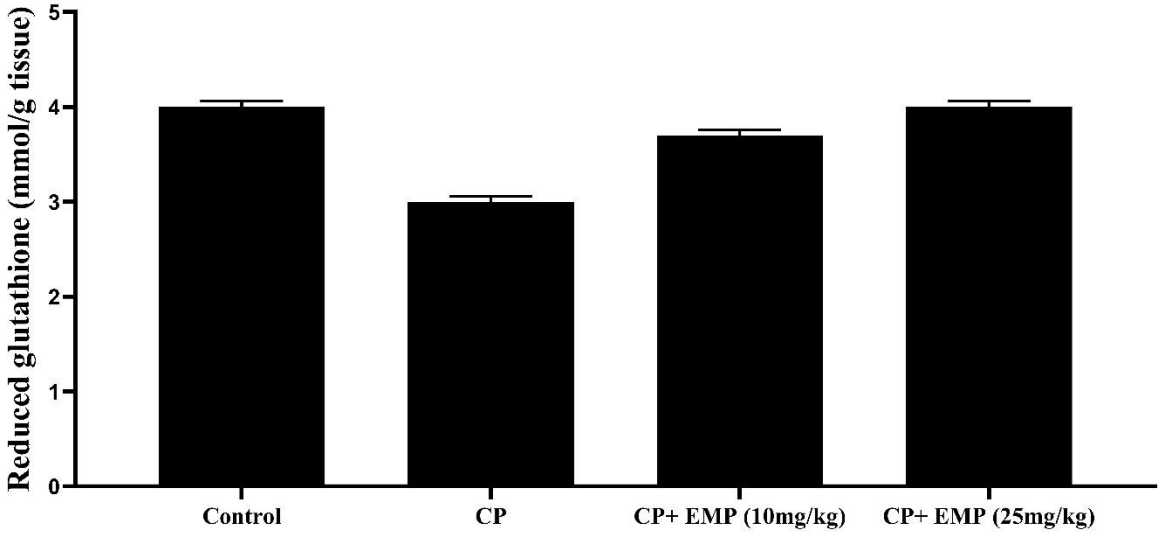

Figure (3): Reduced glutathione (mmol/g tissue) in control and treated rats

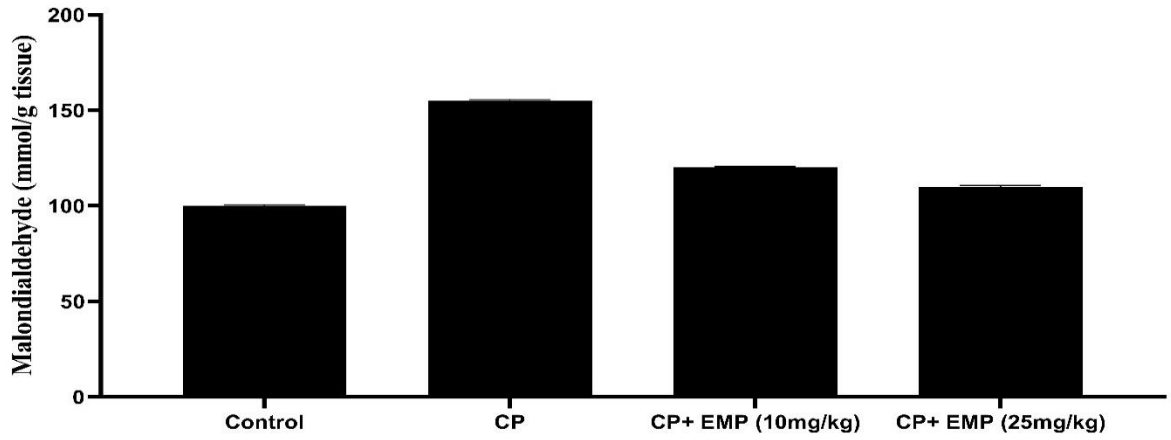

Figure (4): Malondialdehyde (mmol/g tissue) in control and treated rats

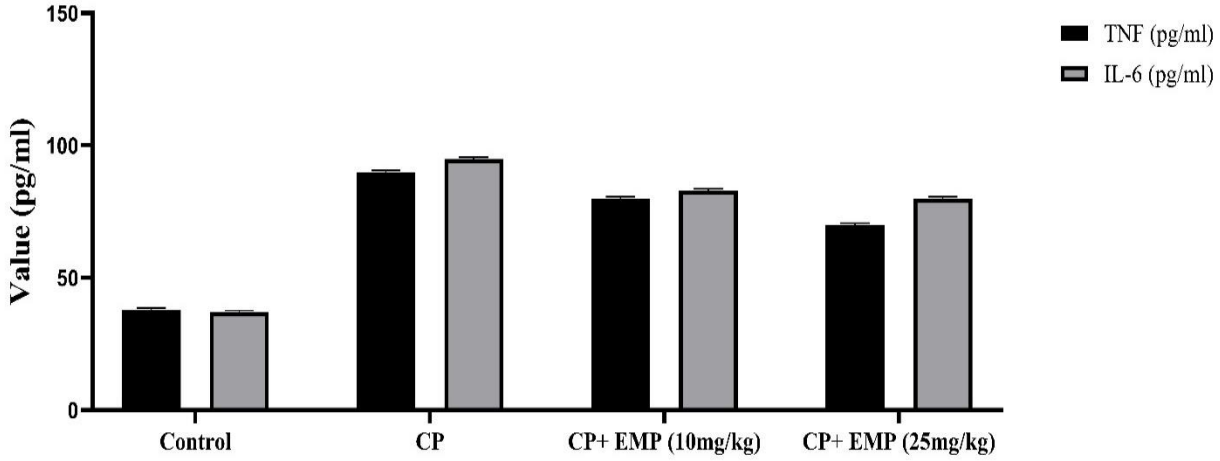

Figure (5): TNF and IL-6 (pg/ml) in control and treated rats

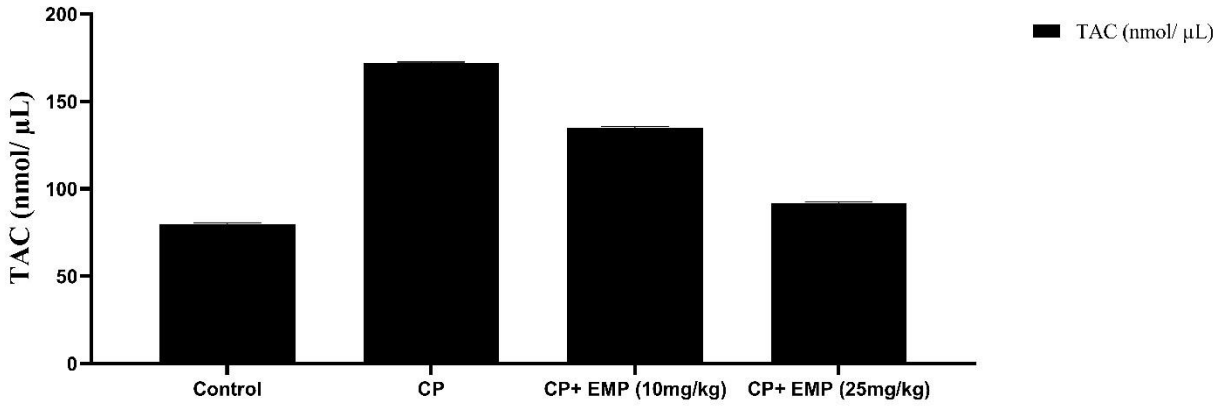

Figure (6): TAC (nmol/µl) in control and treated rats

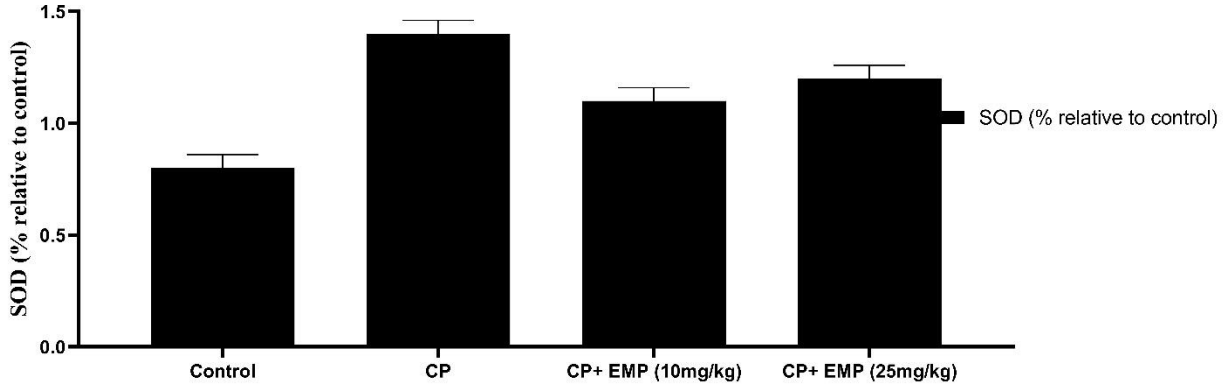

Figure (7): SOD (% relative to control) in control and treated rats

Histological and immune histochemical studies

Excised kidney tissue was fixed by 10% neutral buffered formaldehyde solution then paraffin-embedded for histological and immunohistochemical examination. Five slides from each specimen with 2 levels on each slide were cut at 5-µm thicknesses.

Haematoxylin and Eosin (H&E) stain: For general morphological and structural study. A minimum of 10 fields for each slide were examined and for severity of changes they scored semi quantitatively the scoring was done as none, mild, moderate and severe changes. **(Carson et al. 2009).**

Immunohistochemical Study:

For analysis of caspase-3 expression, tissue sections were incubated in 60°c for 30 minutes, and then the sections were deparaffinized in xylene and rehydrated by alcohol. Antigen retrieval was performed by citrate solution in pH= 9 at 121°C for 20 minutes. Endogenous peroxidase activity was blocked by 10-min incubation in 6% hydrogen peroxide solution in distilled water. Sections were incubated with A polyclonal rabbit anti-human Caspase-3 antibody (diluted 1:200, Pharmingen, San Diego, CA, USA) overnight at 4°C. The slides were washed two times in PBST (for 5 min each time) and incubated in streptavidin-peroxidase (Dakocytomation) diluted 1:150 in PBST for 1 hr at room temperature. After two PBST washes of 5 min, bound peroxidase was identified using the Novared TR system (Abcys; Paris, France). Nuclear counterstaining was performed with 1/2-diluted Harris hematoxylin. Cellular localization in caspase immunoreactivity was cytoplasmic. Sections treated without primary antibodies served as negative controls. For positive controls, adenoma of the large intestine was processed in the same way as renal tissue **(Brown and Richard, 2009).**

1.1. Morphometric Analysis

The image analyzer (ImageJ 1.46r) was used to measure the mean apoptotic index of the tubular and glomerular cells in Caspase-3 stained sections. The previous measurements were estimated in five non-overlapping fields/section in five serial sections/specimen from each animal in each group at 400x magnification.

1.2. Statistical Analysis

All statistical analyses were performed via Paleontological Statistics Version 3.0 (PAST 3.0) statistical software **(Hammer et al., 2001).**

The obtained data were expressed as mean standard deviation (SD) and analyzed using analysis of variance (ANOVA). Statistical significance level was defined as $p<0.05$.

2. Results

2.1. Histological Results

Group I (control): Microscopic examination of H&E stained sections showed normal appearance of glomeruli, tubules and tubulointerstitial cells. (fig.1 A).

Group II treated group (cisplatin):The results obtained by H&E staining showed obvious pathological lesions with glomerular atrophy, widening of the glomerular capsular space, extensive degeneration of the renal tubular epithelial cells with sloughing of cells into lumina, with occasional interstitial mononuclear inflammatory cell infiltrate(fig.1 B)

Group III and Group IV protective group (Cisplatin + Empagliflozin): The severity of pathological damage was reduced in protective group in comparison to treated group The pathological changes were lighter than those of the treated group,

renal tubular epithelial cells degeneration and necrosis were also sporadically observed(fig.1 C-D)..

4.2Histochemical results:

The immunohistochemical results of the protective groups showed mild and focal cytoplasmic immunopositivity to caspase 3 in PCT and scattered glomerular cells (fig.1). Apoptotic index in the treated (Cisplatin) group was (2.49±0.01) and showed marked decrease after Empagliflozin co-adminstration (0.86±0.01) (table 1).

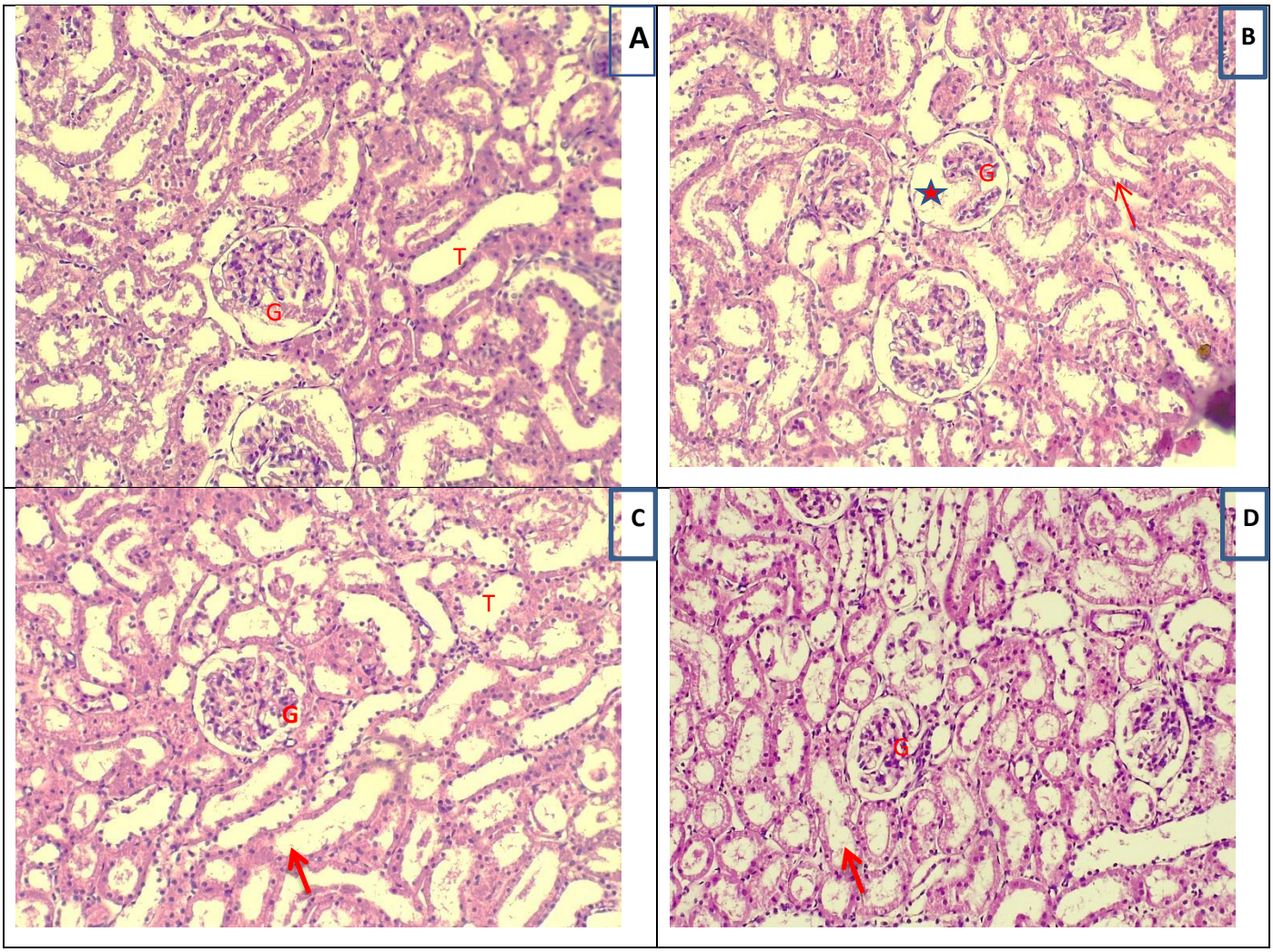

Fig 1 : photomicrograph of sections in kidneys of:
A- Control adult rat kidney showing normal Architecture of glomerulus (G) and normal renal tubules (T) with intact epithelium**. B-** (cisplatin group) showing atrophy of glomerulus (G), with wide space (star), vacuolar degeneration of the tubular epithelium with loss of brush border (arrow). **C-D** section of the kidney (protective group) showing normal appearance of the tubules (T) and glomeruli (G) still some tubules showing flattened epithelium with wide Lumina (arrow) **(H&E x200).**

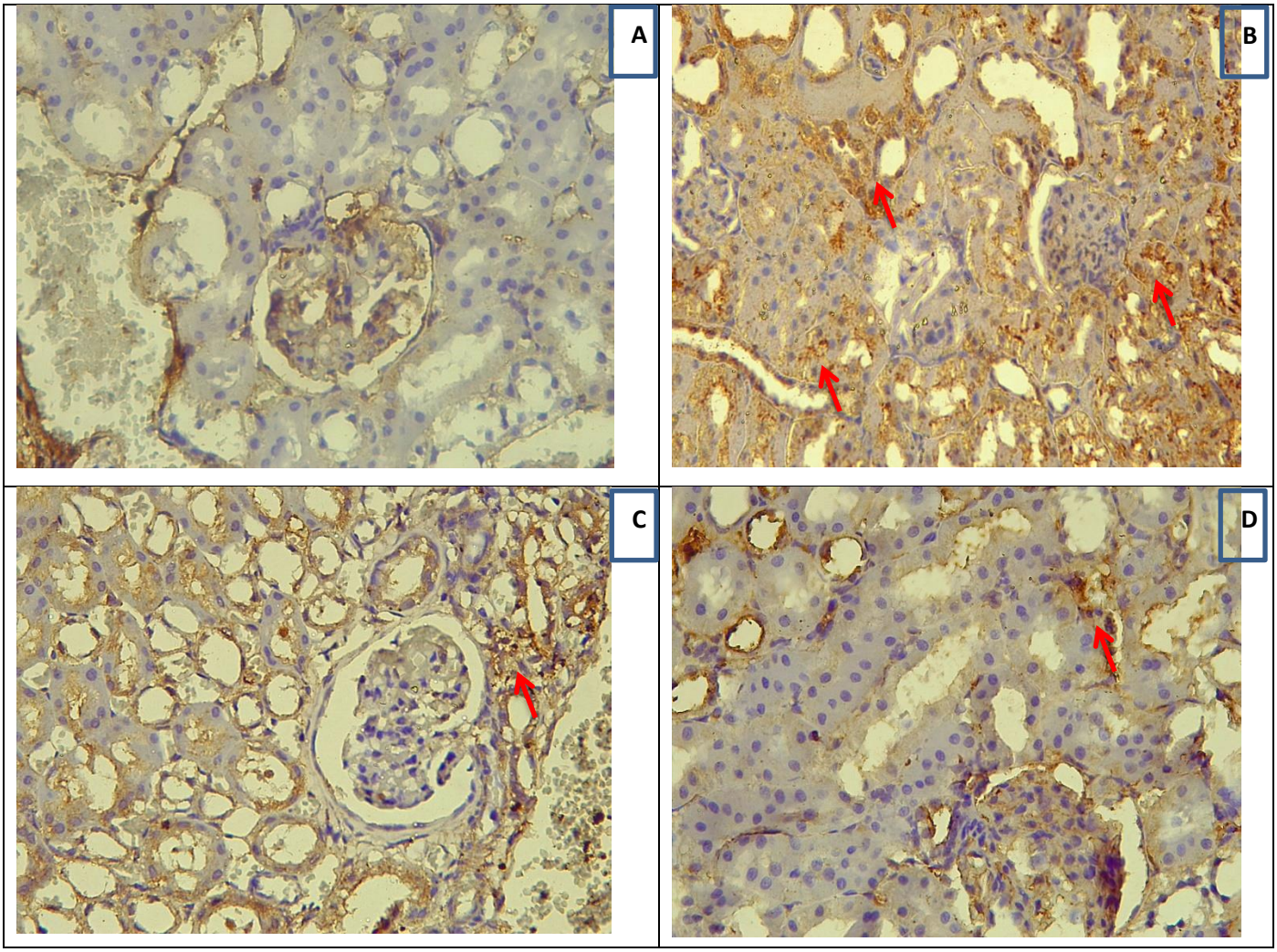

Fig 2 : photomicrograph of Sections in kidneys of:
A- Control adult rat kidney showing immunonegativity to caspas 3 stain.
B-rat kidney of (cisplatin group) shows strong and diffuse immunopositivity to caspas 3 in the apoptotic cells.
C-D rat kidneys of protective group shows focal and mild immunoreactivity to caspas 3 (Caspase3, x400).

Table 1. Optical Density of Caspase-3+ expressions in the in the kidney tissues for the different groups of the study expressed as mean ± SD.

Parameters **Study Groups**	**Optical Density of the Caspase-3+ve expression in the kidney tissues.**
Group 1(C)	0.27±0.01
Group 2(Cisplatin treated)	2.49±0.01
Group 3 (Protected)	0.89±0.01
Group 4 (Protected)	0.84±0.01

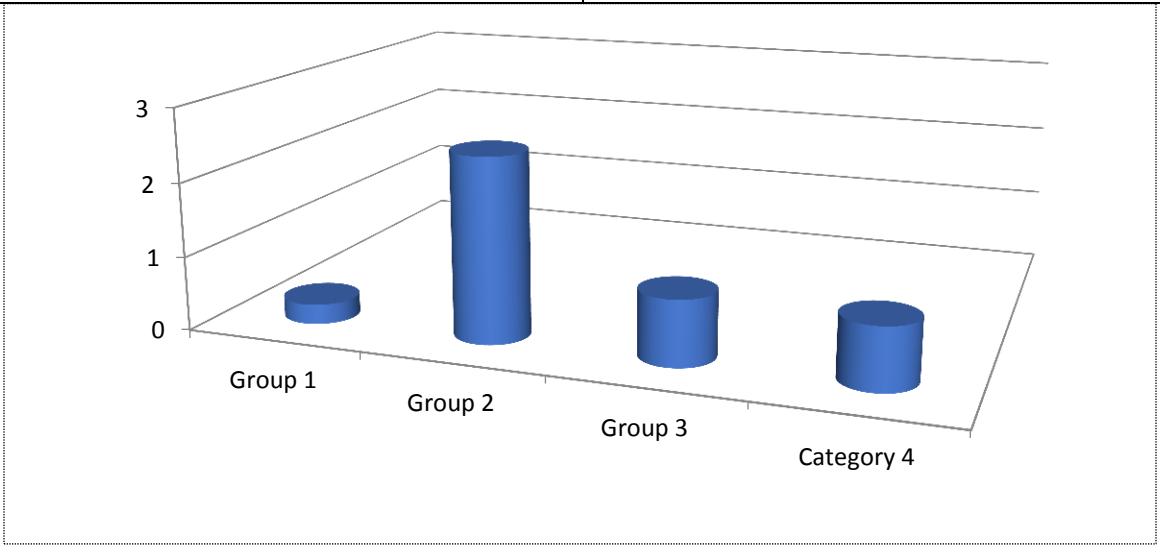

Fig. (3): The Optical Density of the the Caspase-3+ve expression in the kidney tissues. For the different groups of the study in comparison to the control group.

Discussion

Empagliflozin is an antidiabetic agent used in adult patients with type 2 diabetes mellitus. It was FDA-approved in 2014. Empagliflozin can be used as a single agent or as a combination agent with other antidiabetic products. Combination products include empagliflozin and linagliptin and empagliflozin in combination with metformin. These newer agents can be more expensive for patients, thus impeding the clinician's ability to prescribe them based on patient financial considerations. However, the American Diabetes Association (ADA) is calling for agents with proven mortality reduction for use as second-line therapy after metformin. Such agents include empagliflozin and liraglutide (**Fitchett et al., 2019**).

The primary treatment for diabetes is lifestyle management and exercise, followed by metformin use. Per Standards of Medical Care in Diabetes, if the A1c is greater than 9%, then combination therapy with metformin is recommended. In 2016, the FDA (United States Food and Drug Administration) approved a new indication for empagliflozin, which was to reduce the risk of cardiovascular death in adult patients with type 2 diabetes and cardiovascular disease. Empagliflozin has been shown to reduce hospitalizations for heart failure and death from cardiovascular causes. Patients are at an increased

risk of cardiovascular mortality with type 2 diabetes, so prescribers should be made aware of the benefits of empagliflozin. In a nutshell, empagliflozin may be effective in the following settings (**Schwaiger et al., 2019**).

The chemotherapy drug cisplatin is effective against a variety of solid tumors. However, the emergence of nephrotoxicity, which prohibits high therapeutic dosages, limits its usage. Therefore, it is crucial to discover an adjuvant medication to lessen Cisplatin-induced nephrotoxicity. Today's prevention methods include drinking enough water and using diuretics, however increased kidney toxicity still happens (**Miller et al., 2010**).

The goal of this study was to determine whether SGLT2 inhibitor Empagliflozin, a medication used to treat type 2 diabetes, has a potential protective or therapeutic effect on acute kidney injury brought on by Cisplatin. It also sought to elucidate the drug's mechanism of action, particularly in relation to its impact on oxidative stress, and modulators of inflammation and cell differentiation via nerve growth factor assay. Its impacts on various biochemical indicators of renal function were also evaluated.

Due to its clinical relevance 30% of patients are exposed to acute kidney damage following a single dose of cisplatin and repeatability, a single dose model of acute kidney toxicity to cisplatin was used for this work (**Humanes et al., 2012**).

Cisplatin significantly reduced the body weight of rats, which is consistent with the findings of **Ali et al. (2011)** and **Humanes et al. (2012)**. This reduction in body weight may be due to decreased food intake brought on by nausea and the anorexia it caused, as well as the induction of polyurea and the loss of nutrients in urine (**Humanes et al., 2012**).

In the current investigation, a single dosage of Cisplatin significantly raised blood levels of urea and creatinine, which is explained by a decrease in glomerular filtration rate brought on by acute tubular necrosis and damage. Renal cell death, inflammation, fibrogenesis, and tissue remodeling are caused by the accumulation of cisplatin and its conversion to an active metabolite in renal tubular cells (**Dugbartey et al., 2016**). The major cause of this harm is oxidative stress because chloride ions found in cisplatin are hydrolyzed and generate hydroxyl radicals. However, the application of antioxidant techniques showed conflicting results and reduced cisplatin's anti-tumor properties.

Empagliflozin restored the oxidant/antioxidant equilibrium, as seen by enhanced reduced glutathione levels and superoxide dismutase activity, as well as decreased malondialdehyde and total nitrite/nitrate contents. Empa (10 and 25 mg/kg) also significantly decreased the high levels of tumor necrosis factor-alpha, interleukin-6, apoptosis signal-regulating kinase1, c-Jun N-terminal kinase, BCL2 associated X protein, and caspase-3 in hepatic tissues and lowered the levels of interleukin-10 in the liver (**Dalia et al; 2023**).

There was no statistically significant difference in uric acid level across the several research groups in the current investigation, even though cisplatin tends to raise uric acid level due to inadequate excretion, as previously documented by (**Silici et al., 2011**).

A neurotrophic molecule called nerve growth factor keeps the delicate balance between cell differentiation, survival, and death during growth and adulthood. Tyrosine kinase receptor A (TrkA) and p75 neurotrophic receptor are two of the two types of receptors it operates on, and it is expressed not just in nervous tissue but also in numerous mammal cells.

The former is involved in the activation of apoptosis and cell death, whereas the latter is connected to cell differentiation, survival, and hence protection. It has a significant connection to inflammation and tissue healing and is also a powerful inducer of fibroblasts and epithelial cells (**Micera et al., 2007**). According to research (**Vizza et al., 2015**), it may contribute to renal fibrosis by increasing epithelial-mesenchymal transition through activation of TGF- signaling.

With the presence of *NGF-B* and TrkA receptors in tubular and glomerular cells, as well as p75 in interstitial and mesangial cells, it was shown that *NGF-B* signaling is significant in human kidney and glomerular response to injury (**Bono- figlio et al., 2007**). In the current study, cisplatin significantly decreased the level of *NGF-B* in renal tissue.

TrkA receptors are found in tubular cells, and tubulointerstitial apoptosis and renal dysfunction have been linked to up-regulation of proapoptotic cytokines like *TNF-α* and *TGF-β* and down-regulation of survival factor (**Teteris et al., 2007**), which was demonstrated in this study.

Oxidative stress caused by phospholipid damage, mitochondrial dysfunction, and lysosomal hydrolase inhibition, which results in the buildup of reactive oxygen species, is one of the key causes of cisplatin-induced acute kidney injury. According to **Hosohata, 2016;** the activation of Cisplatin also results in the consumption of reduced glutathione, which increases the level of ROS and cellular damage. In this investigation, Cisplatin administration resulted in a decrease in reduced glutathione and an increase in the products of lipid peroxidation.

It is now understood that the apoptosis or necrosis that Cisplatin causes in tubular and tumor cells is

dose-dependent; a low dosage may be associated with apoptosis, but a large dose causes necrosis, particularly in renal tubules, and this necrosis may activate the inflammatory and immunological responses.

As a result of Cisplatin's interaction with many cellular organelles that have associated necrosis and apoptosis pathways, tubular cells may also experience necroptosis, also known as actively programmed necrosis (**Sancho-Martnez et al., 2012**).

Cisplatin builds up inside the numerous mitochondria in the proximal tubules, which causes them to malfunction and reduce ATP production. Cisplatin-induced DNA damage results in *p53* activation, an increase in reactive oxygen species, and endoplasmic reticular stress. These events work in concert to activate caspase 9 and, in turn, caspases 3, 6, and 7, which support apoptosis and necrosis (**Miller et al., 2010**).

With its ability to control hyperglycemia, lower blood pressure and body weight, as well as reduce inflammation and glomerular hyperfiltration, empagliflozin, an inhibitor of the SGLT2 transporter in the kidney, has been shown to have reno-protective effects in diabetic nephropathy (**Perrone-Filardi et al., 2017; Wanner, 2017**).

In tests of cardioprotection by these drugs, it was also demonstrated that it may have a direct anti-inflammatory, antiapoptotic, anti-mitochondrial dysfunction, and anti-oxidative action that appears to be independent of its effect on blood glucose level (**Lahnwong et al., 2018**).

In contrast to studies that showed decreased visceral adiposity by empagliflozin (**Kusaka et al., 2016; Xu et al., 2017**), in our work, administration of empagliflozin orally in a dose of 10 and 25 mg/kg daily increased body weight compared to Cisplatin group. However, did not differ from control group (usual weight gain was preserved).

This may be explained by the fact that Empagliflozin was administered for a longer period in these trials than it was in the current investigation. However, the group using Empagliflozin alone may have experienced the induction of hyperphagia and fluid intake in response to glucosuria, preserving weight gain.

Empagliflozin treatment reduced blood creatinine, uric acid, calcium, and urea levels while having no effect on the level of albumin. In the group receiving empagliflozin, a higher blood glucose level was found, which may have been caused by the previously reported compensatory hyperphagia. The difference between the Empagliflozin alone group and the control group in terms of uric acid levels was similarly significant (**Chino et al., 2014**). This difference may be explained by an increase in uric acid excretion brought on by glucosuria.

The kidney function of 247 patients with kidney damage who were treated with cyclosporine or cisplatin in combination with the SGLT2 inhibitor empagliflozin showed a significant ($P<0.001$) improvement, as well as a decline in creatinine and uric acid, markers of kidney damage, in **Yi et al.'s** retrospective study from **2023**.

Despite *NGF-B*'s critical role in cell survival, reductions in *NGF-B* were seen in the preventive and therapeutic Empagliflozin groups as well, indicating that *NGF-B* 's protective impact on the kidney is not due to Cisplatin.

The SGLT family of glucose transporters are expressed in the kidney in two different ways: SGLT 2 transporters are expressed in the S1 segment of the proximal convoluted tubules and mediate 90% of glucose absorption, while SGLT 1 transporters are present in the S3 portion and are responsible for reabsorption of the remaining glucose that SGLT2 transporters were unable to absorb (**Szablewski, 2017**).

According to certain research (**Debnam et al., 1995**), a diabetic condition may reduce Cisplatin nephrotoxicity by upregulating SGLT1, which may suggest a cytoprotective impact on the kidney. As a protective mechanism, SGLT1 transporters become more active because of empagliflozin's inhibition of SGLT2 transporters in the kidney (**Rieg et al., 2014**). As previously established (**Ikari et al., 2005a**), the SGLT1 transporters are engaged in the restoration of tight junction and plasma membrane integrity, this may have cytoprotective effects.

Additionally, SGLT1 expressed in S3 partly inhibited the formation of peroxynitrite, protecting against the nephrotoxic effects of cisplatin (**Ikari et al., 2005b**). Therefore, while not explored, this may account for the renoprotective effect of empagliflozin in this investigation.

Malondialdehyde, interleukin 1, and tumor necrosis factor raised levels were lowered by empagliflozin, according to research by Mohammad et al. from 2022. Nuclear factor erythroid 2-related factor 2 and PPARG coactivator 1 alpha, which control antioxidant defence and mitochondrial biogenesis, respectively, reduced in expression because of SGLT2 suppression.

Additionally, empagliflozin significantly raised the LC3-II/LC3-I and bcl2/bax ratios, demonstrating the drug's positive effects on autophagy activation and apoptosis inhibition. Empagliflozin did not in this research activate the Sestrin2/AMP-activated protein

kinase pathway despite its effects on diabetic nephropathy (**Moein et al; 2022**).

Conclusion

In conclusion, the current study showed that pretreatment with Empagliflozin had a protective effect against acute kidney injury brought on by Cisplatin primarily through the reduction of apoptosis and the elevation of reduced glutathione and had anti-inflammatory anti-oxidative stress but not through the reduction of nerve growth factor beta, which was seen in the Cisplatin group. Given that most mechanisms relating to renal toxicity and tumor effects are interconnected, more study is required to determine whether empagliflozin interferes with the antitumor effect of cisplatin.

Limitation of the study

The rapid rate of cisplatin absorption in renal tubular cells causes cisplatin buildup, tubular cell damage, and eventual tubular cell death, which results in acute renal failure (**Miller et al. 2010**). According to earlier research (**Kimoto et al. 2013**), several drugs can lessen the accumulation of cisplatin in the kidney, hence reducing the CP-induced nephrotoxicity. Since we did not evaluate the concentration of cisplatin, we cannot rule out the possibility that some of the protective effects were caused by an increase in cisplatin clearance and a decrease in the buildup of cisplatin.

Declarations

Ethics approval and consent to participate

Approval of the study was obtained from the Institutional Review Board (IRB), Damietta Faculty of Medicine, Al-Azhar University and the research is acceptable according to the guidelines and declaration of Helsinki and our committee standard operating procedure guidelines

Consent for publication

Not applicable.

Availability of data and materials

All data and materials are fully presented in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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