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COMPARATIVE ASSESSMENT OF THE PROTECTIVE EFFECT OF N-ACETYL CYSTEINE AND CAPTOPRIL AGAINST CYCLOPHOSPHAMIDE-INDUCED NEPHROTOXICITY IN WISTAR RATS

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This work was executed to assess and compare the protective effect of n-acetyl cysteine (NAC) and captopril (CPL) in the nephrotoxicity induced by cyclophosphamide (CYP) in rats. Thirty-six adult male Wistar rats were separated into six groups (6 rats each). The control group received normal saline. NAC and CPL treated groups received NAC (200 mg/kg) and CPL (60 mg/kg) respectively for six consecutive days. Whereas CYP treated group received CYP (150 mg/kg) on the sixth day of the experiment. NAC+CYP and CPL+CYP treated groups received NAC and CPL respectively for 6 days then administered a single dose of CYP on the sixth day of the experiment. The intraperitoneal route is the method of administration of all drugs. CYP treated group showed a significant rise in levels of serum urea, creatinine, uric acid, and cystatin C, as also kidney MDA and IL-6 levels. Furthermore, it showed a significant decrease in kidney SOD, GSH, and IL-10 levels. Furthermore, the level of the Bcl_2 gene was downregulated. Histopathological changes in the kidney exhibited marked tubular degenerative changes. Additionally, there was a significant increase in caspase-3 within the epithelium of the renal tubules. On the other hand, NAC+CYP and CPL+CYP treated groups protected against abnormal biochemical and histological changes and restored the Bcl_2 gene. These data suggested that the administration of NAC and CPL could protect against CYP- induced nephrotoxic effect through their antioxidant, anti-inflammatory and anti-apoptotic effects.

Keywords: *Cyclophosphamide, N-acetyl cysteine, Captopril, Oxidative stress, Gene expression, Anti-inflammatory, Nephrotoxicity*

INTRODUCTION

The human body's primary excretory organ is the kidney. It is crucial for controlling the equilibrium of ions, salt, and water in the body. Numerous medications can seriously harm the kidneys over time[1]. Cyclophosphamide (CYP) is a vastly used anticancer drug. CYP has numerous side effects such as cardiotoxicity, nephrotoxicity, and hepatotoxicity.

Cyclophosphamide nephrotoxicity is related to free radical-induced oxidative stress because CYP has a pro-oxidant character, it causes oxidative stress, which decreases the activity of the antioxidant defense system and increases lipid peroxidation in many tissues[2]. CYP produced two active metabolites phosphoramide and acrolein, and through interfering with cellular DNA, they inhibit the proliferation of cancer cells. Acrolein is a highly reactive aldehyde, which is able to interact with the antioxidant defense system, it can bind to cysteine, a constituent amino acid of GSH, and reduced its renal level. Moreover, acrolein can hinder the antioxidant system by increasing the production of free radicals, which is accountable for oxidative stress and initiating cell death, apoptosis[3].

Therefore, searching for antioxidant and free radical scavenging agents is important in alleviating these harmful CYP nephrotoxic effects.

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N-Acetyl cysteine (NAC) is one of the most significant antioxidant defenses, it's a thiol-containing antioxidant and free radical scavenger utilized to generate glutathione to safeguard against the harmful effects of various toxins[4]. Through enhancing antioxidant capacity and energy metabolism, NAC decreases organ toxicity like the liver, heart, and brain[5-7]. Moreover, it is utilized as an antidote for paracetamol overdose[8], NAC has long been employed as a mucolytic drug. Besides, it has the capacity to reduce inflammation and prevent apoptosis[9]. As a result of its antioxidant and anti-inflammatory characteristics, NAC possesses nephroprotective benefits in different studies[10-12].

Angiotension-converting enzyme inhibitor captopril (CPL) is frequently administered to treat hypertension and congestive heart failure. The beneficial effect of CPL might be through the antioxidant mechanisms, which are reportedly correlated to the drug's sulfhydryl group (SH) that directly scavenges ROS[13]. Besides, CPL increases the antioxidant enzymes and nonenzymatic antioxidant defenses[14]. In addition, El-Sayed et al. [15] investigated the nephroprotective effect of CPL on nephrotoxicity induced by the anticancer drug, cisplatin.

The aim of the current work was to assess and compare the protective effect of NAC and CPL in the nephrotoxicity induced by CYP in Wistar rats.

MATERIALS AND METHODS

Animals

Thirty-six adult male Wistar rats (weighing 180-200g) from the animal dwelling, Faculty of Medicine, Sohag University, Egypt were used. Rats were preserved in a controlled setting with standard temperature (25 ± 2ºC) and 12-hrs cycles of light and dark. Food and water were freely available to all rats. The experimental plan was authorized by the Medical Research Ethics Committee (MREC) of the Faculty of Medicine, Sohag University, Egypt under IBR: Soh-Med-21-12-38.

Drugs and chemicals

Cyclophosphamide was purchased as Endoxan vials from Baxter Oncology GmbH., Germany. N-acetyl cysteine and captopril were purchased from Panreac AppliChem., Italy and Sigma-Aldrich., England respectively. Kits for determination of superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) were obtained from Bio-diagnostic Company, Egypt. While cystatin C and interleukin 6 (IL-6) were purchased from Elabscience., USA. Interleukin 10 (IL-10) was purchased from Cusabio., USA.

Experimental design

The animals were split into six groups at random, each with six animals. **Control group:** received normal saline daily for six consecutive days. **NAC treated group:** received NAC 200 mg/kg [16] daily for six consecutive days. **CPL treated group:** received CPL 60 mg/kg[15] daily for six consecutive days. **CYP treated group:** received saline for six consecutive days then a single dose of CYP (150 mg/kg)[17], on the sixth day of the experiment. **NAC+CYP treated group:** received NAC for 6 consecutive days then a single dose of CYP on the sixth day of the experiment, two hrs after the last NAC dose. **CPL+CYP treated group:** received CPL for 6 consecutive days then a single dose of CYP on the sixth day of the experiment, two hrs after the last CPL dose. All medications in all groups are administered via the intraperitoneal route. 24 hrs after the end of the experiment, rats were euthanized. Blood and kidney tissue samples were gathered. Each animal's right kidney was taken out, preserved in 10% formalin, and processed for histological and immunohistochemical analysis. Part of the left kidney was immersed in liquid nitrogen and used to study gene expression. The remaining portion of the left kidney was weighed before being homogenized in 10 ml of ice-cold phosphate buffer saline (pH 7.4) using a motor-driven homogenizer and then centrifuged for 15 min at 4000 rpm at 4◦C. Biochemical analysis was carried out using the supernatant.

Biochemical measurements

Measurement of urea, creatinine, and uric acid

Serum urea, creatinine, and uric acid levels were determined by a colorimetric method using standardized commercially available kits.

Measurement of cystatin C level

Serum cystatin C level was measured using an Enzyme-linked immunosorbent assay (ELISA) kit. Microplate reader Stat Fax 2200 (AWARENESS, USA) was used to calculate the results which were expressed as ng/mL.

Measurement of SOD, GSH, and MDA levels

In kidney homogenate, the levels of SOD, GSH, and MDA were assayed by a colorimetric method according to Nishikimi et al [18]. Beutler et al. [19] and Ohkawa et al. [20] respectively. At 560 nm, 405 nm, and 534 nm respectively, changes in the absorbance were detected. The level of SOD was expressed as U/g tissue. While GSH was expressed as mg/g tissue, and nmol/g tissue is for MDA level expression.

Measurement of IL-6 and IL-10 levels

Using ELISA assay, the levels of IL-6 and IL-10 were measured in the kidney tissue homogenate according to the manufacturer's guidelines. Results were determined by an ELISA microplate reader. The level of both IL-6 and IL-10 was expressed as pg/g tissue.

Estimation of Bcl_2 gene expression

B-cell lymphoma 2 (Bcl_2) gene expression was analyzed using the Applied Biosystems 7500, (USA) instrument. The whole RNA was isolated from kidney tissue using the Ribozol™ reagent (AMRESCO, USA). Template cDNA was synthesized using GoTaq® 1-Step RT-qPCR system with BRYT-Green fluorescence dye (Promega Corporation, USA). The PCR amplification was 5 min initial denaturation at 95° c, then 40 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 30 s, and extension at 72°C for a further 30 s. The gene expression level was normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping gene. The comparative threshold cycle (Ct) method was used to calculate relative fold changes in gene[21]. The primers used were as follows:

Gene	**(Primer sequence) 5–3′**
GAPDH	Forward: 5′- GGC ACA GTC AAG GCT GAG AAT G-3′
Reverse:	5′- ATG GTG GTG AAG ACG CCA GTA-3′
Bcl_2	Forward: 5′- GCA GAG ATG TCC AGT CAG C-3′
Reverse:	5′- CCC ACC GAA CTC AAA GAA GG-3′

Histological and immunohistochemical studies

The formalin-fixed kidney specimens were processed for histological and immunohistochemical studies as follows:

Histological studies

After being fixed for 24 hrs, kidney tissues were gathered, dehydrated in upgraded concentrations of alcohol, cleaned in xylene, and then embedded in paraffin. Hematoxylin and eosin (H&E)-stained sections of 5 μm thickness were made and inspected under a light microscope[22].

Immunohistochemical studies

The immunohistochemical staining procedure was performed in accordance with Saber et al.[23]. For antigen retrieval, sections were dewaxed, rehydrated, and then incubated in a boiling 0.05 M citrate buffer solution at pH 6.8 for 10 min. These sections were then treated with 0.3% H2O2 and protein blocking. Then, sections were incubated with polyclonal anti-caspase-3 antibodies (Invitrogen, dilution 1/100). After that, slides were incubated with a goat anti-rabbit secondary antibody (EnVision+™ System Horseradish Peroxidase Labelled Polymer; Dako) at room temperature for 30 min after being washed with phosphate-buffered saline. DAB kit (3,3′-Diaminobenzidine) was used for staining tissue sections before being counter-stained with Mayer's hematoxylin. The immunolabelling index of caspase-3 was measured by calculating the positive cells per 1000 kidney cells.

Statistical analysis

All statistical analyses were conducted using the SPSS program (software package, version 26). The data were statistically evaluated using one-way analysis of variance (ANOVA), and the Tukey post hoc test was performed to analyze the variations across different groups. When $P<0.05$, the difference was deemed significant. Data were expressed as the Mean ± SE of the values.

RESULTS AND DISCUSSION

Results

Effects of NAC and CPL on urea, creatinine, and uric acid levels

The IP injection of CYP led to a significant (P<0.05) elevation of BUN, serum creatinine, and uric acid levels compared to the control group. Both NAC and CPL attenuated the CYP -induced nephrotoxicity where BUN, serum creatinine, and uric acid levels were significantly (P<0.05) reduced in the NAC+ CYP group and CPL+CYP group compared to CYP treated group **(Table 1)**. No significant (P>0.05) difference between the NAC+ CYP group and the CPL+CYP group.

NAC and CPL treated groups did not show significant (P>0.05) change in the previous parameters compared to the control group but, the parameters were significantly (P<0.05) reduced compared to CYP treated group.

Effects of NAC and CPL on cystatin C level

As shown in **Fig. 1**, serum cystatin C level was significantly (P<0.05) increased in CYP treated group compared to the control group. Both NAC+CYP and CPL+CYP treated groups significantly (P<0.05) reduced serum cystatin C level compared to CYP treated group, with insignificant (P>0.05) difference between them.

In addition, NAC and CPL-treated groups show insignificant (P>0.05) change in cystatin C level compared to the control group but, its level was significantly (P<0.05) reduced compared to CYP treated group.

Table 1: Effect of intraperitoneal NAC (200 mg/kg/day) and CPL (60 mg/kg/day) on serum urea, creatinine, and uric acid levels in CYP -induced nephrotoxicity in rats.

Groups	**urea** (mg/dl)	**creatinine** (mg/dl)	**uric acid** (mg/dl)
Control	16.17±1.35	0.53±0.04	3.03±0.27
NAC	16.33±1.52	0.52±0.03	3.10±0.21
CPL	16.17±1.35	0.54±0.04	3.12±0.20
CYP	31.33±2.19*	1.09±0.09*	10.83±0.69*
NAC+ CYP	19.83±1.51[a]	0.56±0.03[a]	4.67±0.33[a]
CPL+ CYP	19.17±1.01[a]	0.57±0.03 [a]	4.70±0.39[a]

Data represent mean ± SE (n=6). NAC= N-Acetyl cysteine, CPL= Captopril, CYP= Cyclophosphamide.
*Significant vs. control group (P < 0.05).
[a] Significant vs. CYP group (P < 0.05).

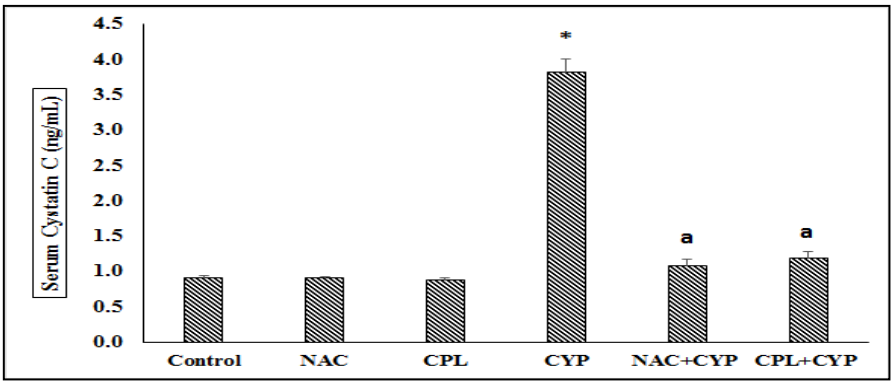

Fig. 1: Effect of intraperitoneal NAC (200 mg/kg/day) and CPL (60 mg/kg/day) on serum cystatin C level in CYP -induced nephrotoxicity in rats.
Data represent mean ± SE (n=6). NAC= N-Acetyl cysteine, CPL= Captopril, CYP= Cyclophosphamide.
*Significant vs. control group (P < 0.05).
[a] Significant vs. CYP group (P < 0.05).

Effects of NAC and CPL on SOD, GSH, and MDA levels

As seen in **Table 2**, CYP significantly (P<0.05) reduced kidney SOD and GSH levels compared to the control group. NAC and CPL attenuated CYP -induced oxidative stress as they significantly increased SOD and GSH levels in the NAC+CYP and CPL+CYP groups respectively when compared to the CYP group. Besides, NAC+CYP increase SOD and GSH levels in the kidney compared to the CPL+CYP group and restored GSH level back to the control level, but with a statistically insignificant (P>0.05) difference between them.

Kidney MDA was significantly (P<0.05) increased in the CYP group compared to the control group. Besides, compared to the CYP group, the results revealed a significantly (P<0.05) decreased kidney tissue MDA level in NAC+CYP and CPL+CYP groups. There was no significant (P>0.05) difference between NAC+CYP and CPL+CYP groups, a few differences were observed but statistically were negligible.

On the other hand, NAC and CPL-treated groups did not show significant (P>0.05) change in SOD, GSH, and MDA levels compared to the control group but, significantly (P<0.05) reduced them compared to CYP treated group.

Effects of NAC and CPL on IL-6 and IL-10 levels

Interleukin 6 significantly (P<0.05) elevated in the CYP group compared to the control group. NAC+CYP and CPL+CYP groups significantly (P<0.05) decreased IL-6 level compared to the CYP group. As regard IL-10, CYP significantly (P<0.05) decreased IL-10 level compared to the control group, and both NAC+CYP and CPL+CYP groups significantly (P< 0.05) increased it compared to the CYP group **(Figs.2&3)**. No statistically significant (P>0.05) difference between NAC+CYP and CPL+CYP groups concerning both IL-6 and IL-10 levels.

Again, both NAC and CPL-treated groups did not show significant (P>0.05) change in IL-6 and IL-10 levels compared to the control group but, significantly (P<0.05) reduced them compared to CYP treated group.

Table 2: Effect of intraperitoneal NAC (200 mg/kg/day) and CPL (60 mg/kg/day) on kidney SOD, GSH, and MDA levels in CYP -induced nephrotoxicity in rats.

Groups	**SOD** (U/g)	**GSH** (mg/g)	**MDA** (nmol/g)
Control	264.5±6.6	301.48±3.36	149.17±3.00
NAC	261.7±7.9	304.25±7.33	152.50±3.82
CPL	263.5±4.2	305.90±5.54	151.17±3.59
CYP	149.2±8.8*	197.85±6.95*	315.80±7.03*
NAC+ CYP	257.7±5.8[a]	301.77±7.97[a]	159.17±3.27[a]
CPL+ CYP	246.3±6.6[a]	287.60±3.61[a]	178.33±7.03[a]

Data represent mean ± SE (n=6). NAC= N-Acetyl cysteine, CPL= Captopril, CYP= Cyclophosphamide, SOD= Superoxide dismutase, GSH= Reduced glutathione, MDA= Malondialdehyde

*Significant vs. control group (P < 0.05).

[a] Significant vs. CYP group (P < 0.05).

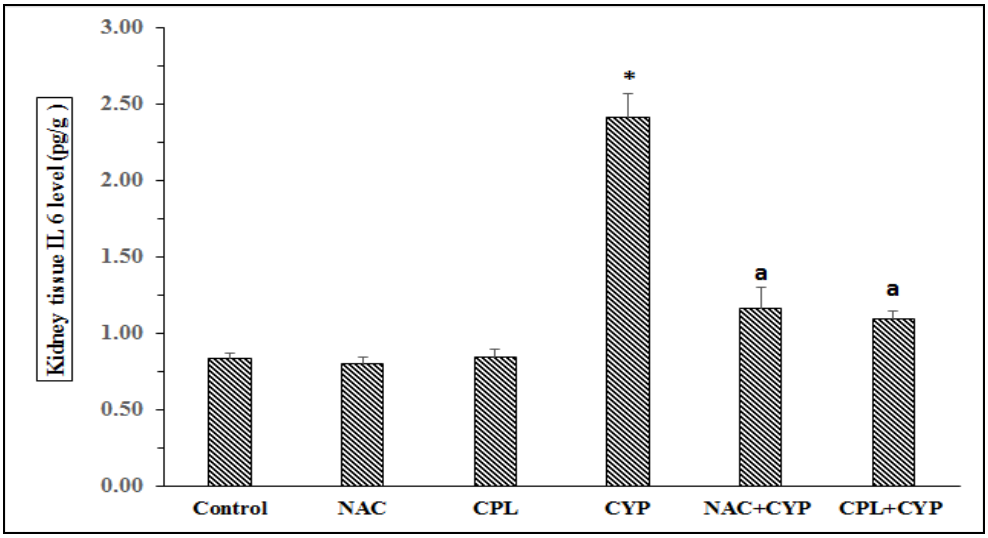

Fig. 2: Effect of intraperitoneal NAC (200 mg/kg/day) and CPL (60 mg/kg/day) on kidney IL-6 level in CYP -induced nephrotoxicity in rats.

Data represent mean ± SE (n=6). NAC= N-Acetyl cysteine, CPL= Captopril, CYP= Cyclophosphamide, IL-6 =Interleukin 6

*Significant vs. control group ($P < 0.05$).

[a] Significant vs. CYP group ($P < 0.05$).

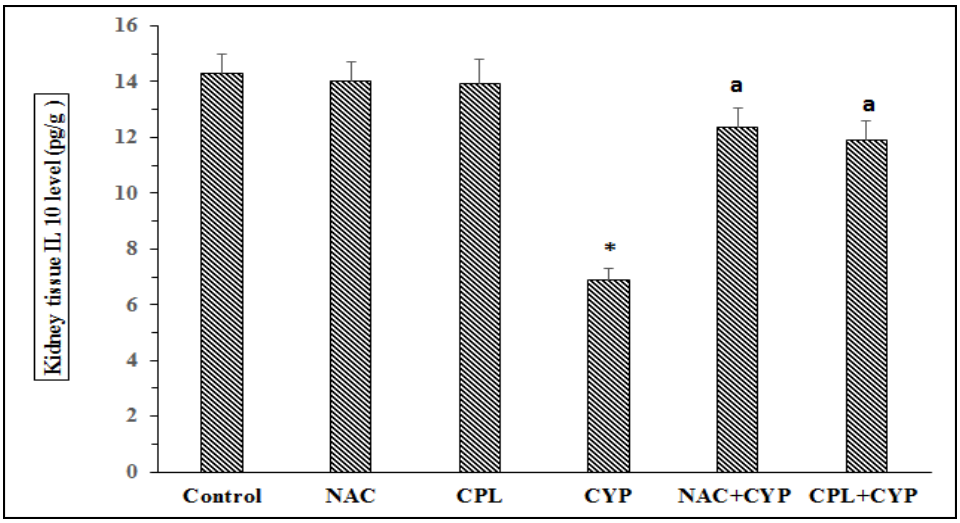

Fig. 3: Effect of intraperitoneal NAC (200 mg/kg/day) and CPL (60 mg/kg/day) on kidney IL-10 level in CYP -induced nephrotoxicity in rats.

Data represent mean ± SE (n=6). NAC= N-Acetyl cysteine, CPL= Captopril, CYP= Cyclophosphamide, IL-10 =Interleukin 10

*Significant vs. control group ($P < 0.05$).

[a] Significant vs. CYP group ($P < 0.05$).

Effects of NAC and CPL on Bcl_2 gene expression

There was a significant decrease ($P<0.01$) in the kidney Bcl_2 gene expression in CYP-treated rats in comparison to the control group. However, both NAC+CYP and CPL+CYP groups significantly ($P<0.05$) increased Bcl_2 gene expression compared to the CYP group, with no statistically significant ($P>0.05$) difference between them **(Fig.4)**.

Both NAC and CPL treated groups did not show significant ($P>0.05$) change in Bcl_2 gene expression compared to the control group but, significantly ($P<0.05$) reduced them compared to CYP treated group.

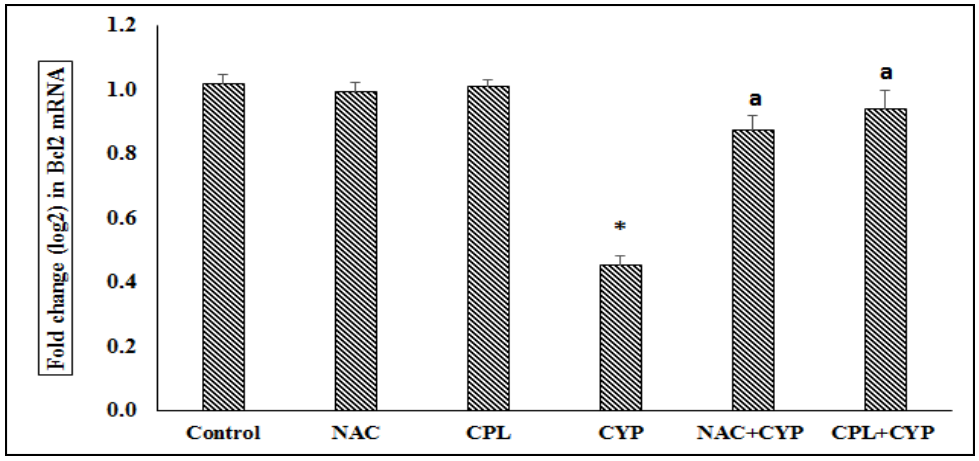

Fig. 4: Effect of intraperitoneal NAC (200 mg/kg/day) and CPL (60 mg/kg/day) on kidney Bcl_2 mRNA expression in CYP -induced nephrotoxicity in rats.

Data represent mean ± SE (n=6). NAC= N-Acetyl cysteine, CPL= Captopril, CYP= Cyclophosphamide, IL-10 =Interleukin 10, Bcl_2= B-cell lymphoma 2

*Significant vs. control group ($P < 0.05$).

[a] Significant vs. CYP group ($P < 0.05$).

Histological and immunohistochemical changes

Histological changes

Sections of the kidneys of control rats showed preserved cortex and medulla. The glomeruli have normal glomerular tufts, and the tubules are lined by a single layer of cuboidal cells. The interstitial tissue showed minimal focal congestion with no inflammatory reaction **(Fig.5 A)**. Besides, sections of NAC and CPL-treated groups showed normal glomeruli and tubules similar to the control group **(Fig.5 B & C)**.

Sections of rats treated with CYP (CYP treated group) showed tubular degenerative changes including a detachment of tubular epithelium from the basement membrane with the shedding of the cells into the tubular lumen. Renal tubules showed frequent luminal hemorrhage and luminal eosinophilic material. The lining epithelium of the tubules is attenuated, and the cells have deep eosinophilic cytoplasm with focal cloudy swelling. Apoptosis of tubular lining epithelium was also seen. The glomeruli showed mild histological changes in the form of focal relative widening of bowman`s space and mild congestion of glomerular capillary tufts. The stroma showed a patchy inflammatory reaction mainly to neutrophils and lymphocytes with focal attacking of lining epithelium by neutrophils. Congestion of stromal capillaries was observed **(Fig.5 D)**. However, sections of NAC+CYP and CPL+CYP groups showed almost similar changes. Renal tissue in both groups showed minimal damage compared to tissue sections of cyclophosphamide-treated rats. The tubular epithelium is restored at least focally with reduced intra-tubular hemorrhage. The glomeruli looked unremarkable apart from mild congestion of capillary tuft. Residual interstitial inflammatory reaction and atrophy of renal tubules were noticed **(Fig.5 E& F)**.

Immunohistochemical changes

Examination of immune stained sections for caspase-3 of the control group showed mild cytoplasmic expression of caspase-3 within the renal tubular epithelium **(Fig. 6A)**. Kidney sections for caspase-3 of NAC and CPL treated groups showed scanty cytoplasmic expression of caspase-3 within the renal tubular epithelium **(Fig. 6 B& C).** In CYP treated group, there was marked cytoplasmic and nuclear expression of caspase-3 within the renal tubular epithelium compared to the control group **(Fig. 6 D)**, while sections of NAC+CYP and CPL+CYP groups showing a marked decrease of cytoplasmic and nuclear caspase-3 expression within the renal tubular epithelium **(Fig. 6 E& F)** compared to the CYP treated group.

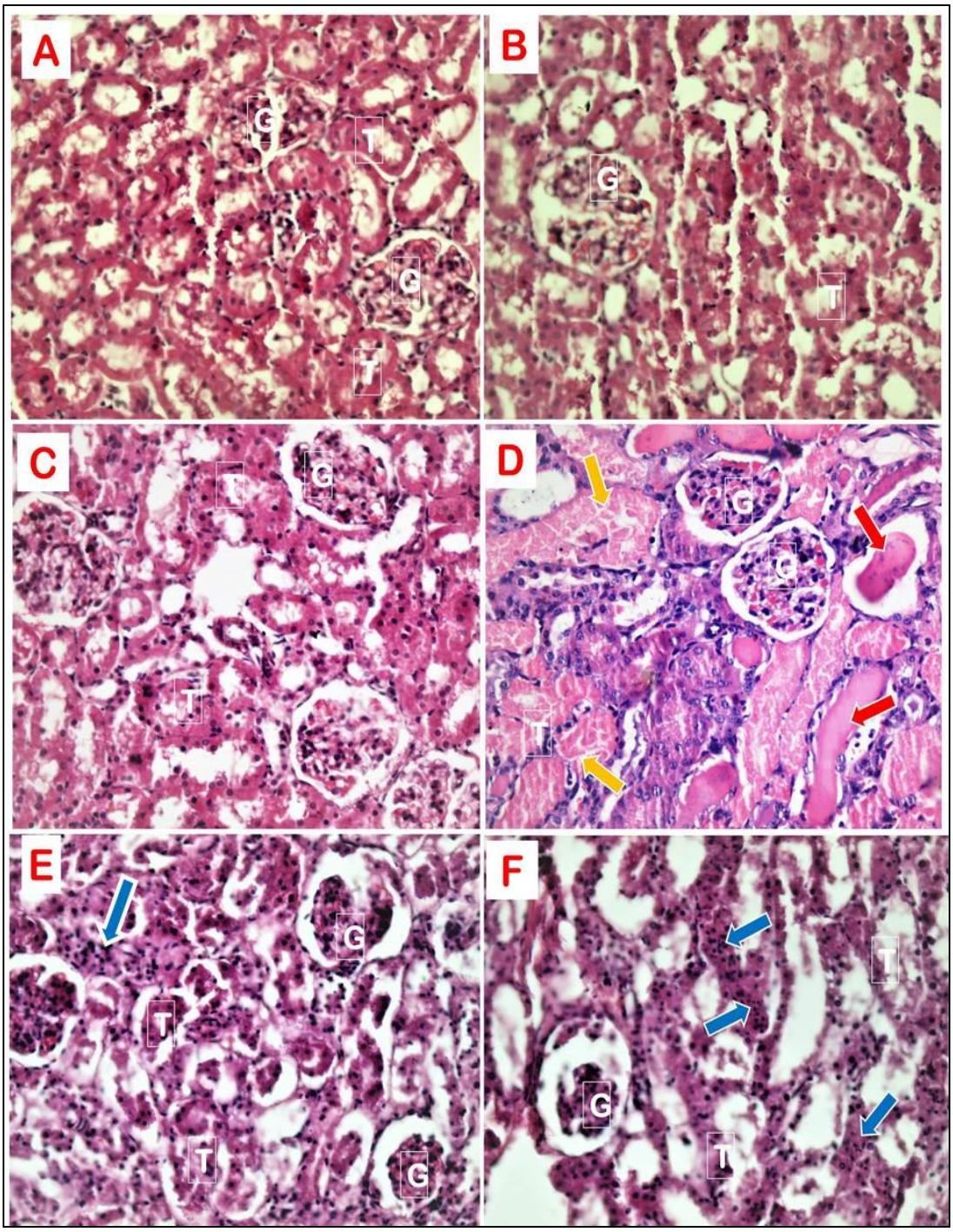

Fig. 5: Photomicrographs of sections in the kidney showed: rather normal glomeruli and tubules **A:** Control group**, B:** NAC treated group**, C:** CPL treated group. **D:** Renal tissue of the CYP-treated group showed frequent shedding of tubular lining, hemorrhage into tubular lumina (orange arrows), and luminal hyaline cast (red arrows) with congestion of glomeruli. **E:** NAC+CYP treated group showed atrophy of tubular lining with no shedding. Residual stromal inflammatory reaction (Blue arrows) was noticed. **F:** CPL+CYP treated group showed atrophy of the tubular lining with no shedding. Residual stromal inflammatory reaction (Blue arrows) was noticed. Magnification is x400 for all, G for Glomeruli, and T for tubules.

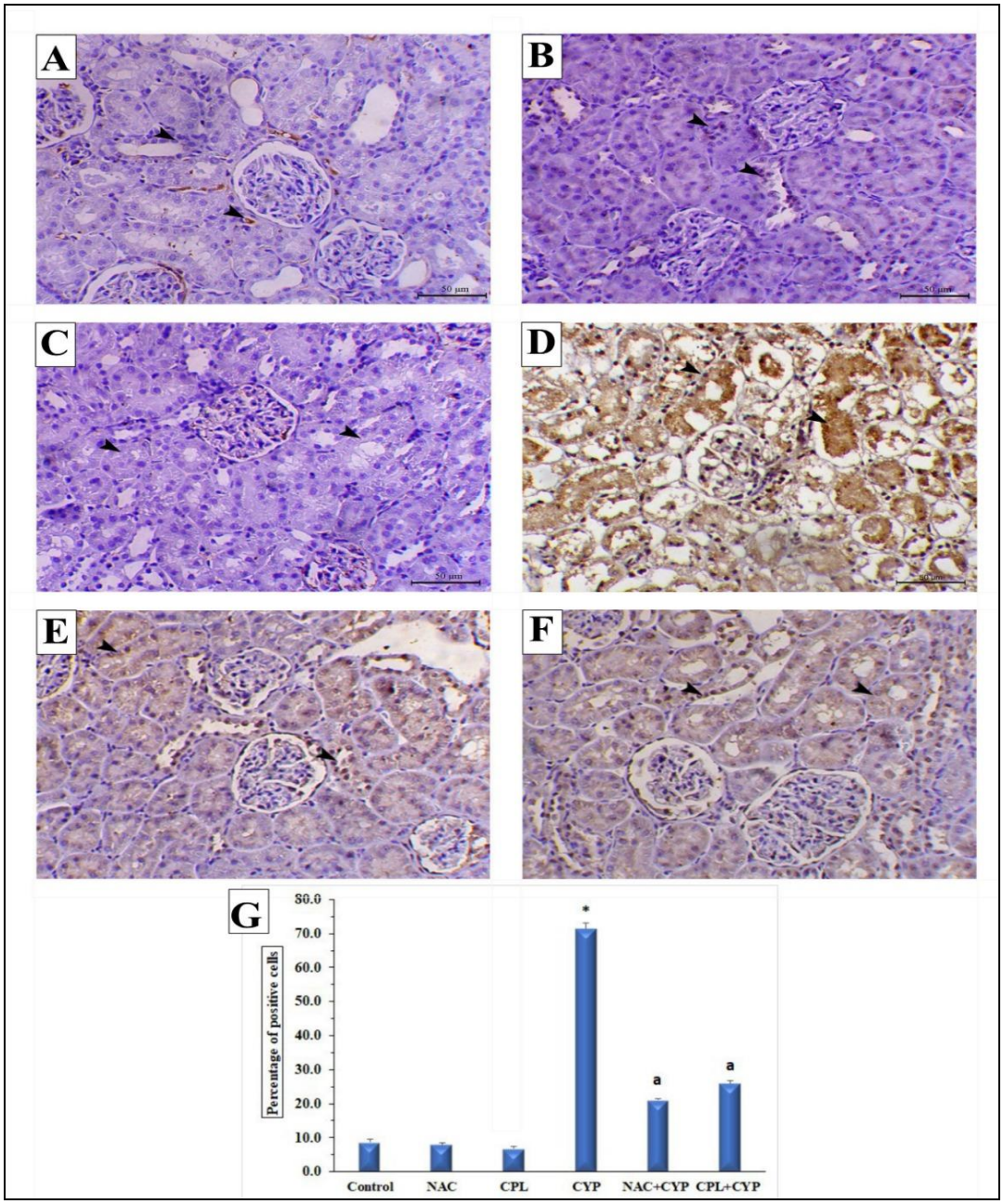

Fig. 6: Photomicrographs of sections in the kidney showed:

A: The kidney of the control group showed mild cytoplasmic expression of caspase-3 within the renal tubular epithelium (arrowheads).

B: NAC-treated group showed scanty cytoplasmic expression of caspase-3 within the renal tubular epithelium (arrowheads).

C: Kidney of CPL treated group showing scanty cytoplasmic expression of caspase-3 within the renal tubular epithelium (arrowheads).

D: Kidney of CYP treated group showing marked cytoplasmic and nuclear expression of caspase-3 within the renal tubular epithelium (arrowheads).

E: Kidney of NAC+CYP treated group showing a marked decrease of cytoplasmic and nuclear caspase-3 expression within the renal tubular epithelium (arrowheads).

F: Kidney of CPL+CYP treated group showing a marked decrease of cytoplasmic and nuclear caspase-3 expression within the renal tubular epithelium (arrowheads).

*All photomicrographs showed Caspase-3 IHC, X200, bar= 50 µm

G: Graphical presentation of the mean number of caspase-3 positive cells in the different groups.

Discussion

A healthy kidney safeguards the body from numerous toxins by acting as a toxin-clearance device. Renal injury's drawbacks include a reduction in the kidney's capacity for clearance[24].

The present work assesses and compares the protective effect of n-acetyl cysteine (NAC) and captopril (CPL) in the nephrotoxicity induced by cyclophosphamide (CYP) in rats. CYP is an alkylating agent, used for dealing with various types of malignancies. Several toxic side effects have been reported with CYP used, and nephrotoxicity is the chief toxic effect. Moreover, the progression of renal toxicity after CYP administration is characterized by an increase in kidney function biomarkers. In the present study CYP nephrotoxicity was indicated by its significant increase in serum urea, creatinine, uric acid, and cystatin C levels. These results agree with several previous results [17&25&26&27] and reflected damage generated in kidney tubules. In the present study, NAC and CPL pretreatment reduced CYP-induced nephrotoxicity, because serum urea, creatinine, uric acid, and cystatin C levels were restored to nearly the control levels. However, an insignificant difference between them was detected. This indicates that NAC and CPL have a protective potential against CYP-induced nephrotoxicity.

In the present investigation, CYP administration decreased SOD, and GSH levels and increased MDA level in the renal tissue. This result is supported by the result of Alshahrani et al.[27] and. Abraham et al.[28]. Besides, El-Kholy et al.[29] demonstrated that CYP increased oxidative stress through its marked increase in MDA levels in lung and liver tissues together with a decline in GSH levels and SOD activity.

Furthermore, Rashed et al.[30] reported that increased ROS production by CYP can activate the p38 MAPK (mitogen-activated protein kinases), which regulates many apoptotic and inflammatory pathways. Enhanced ROS production ultimately ignites DNA damage and starts the mitochondrial apoptotic cascade by raising the pro-apoptotic protein expression and lowering the anti-apoptotic protein expression[31-33]. Inflammation plays a crucial role in CYP -induced renal toxicity. CYP-induced oxidative stress promotes NF-κB transcription in the nucleus, NF-κB is a crucial transcription factor that is necessary for the induction of several pro-inflammatory cytokines, including TNF-α and IL-6 which are the major cytokines involved in triggering acute inflammatory responses after CYP injection [29]. In accordance with previous research, the kidneys of CYP -treated rats in the present study exhibited an increased IL-6 level, and decreased IL-10 level. Besides, kidney Bcl_2 gene expression was significantly decreased compared to the control group. Moreover, histological and morphological changes revealed CYP-induced tubular degeneration, frequent luminal hemorrhage, inflammation, and apoptosis of tubular lining epithelium. The glomeruli showed focal relative widening of bowman`s space and mild congestion of glomerular capillary tufts. The stroma showed a patchy inflammatory reaction. In addition, the result of the present study showed marked expression of caspase-3 within the renal tubular epithelium. These changes are a sign of nephrotoxicity and provided supportive affirmation for the biochemical parameters of the present study. These results agree with Rehman et al.[3], who demonstrated that in the cortical and medullary regions of the kidneys of CYP-administered mice, epithelial cells exhibit tubular necrosis and desquamation, as well as an invasion of inflammatory cells.

Pretreatment of the rats with NAC and CPL in the present study were able to restore the antioxidant defense system by increasing SOD, and GSH levels and decreasing MDA level in the renal tissue. As well as, NAC and CPL decreased IL-6 level, and increased IL-10 level. Besides, kidney Bcl_2 gene expression was significantly increased compared to the CYP-treated group. Insignificant differences between NAC and CPL were detected. The histopathological abnormalities were improved and there was a marked decrease in the expression of caspase-3 inside the renal tubular epithelium. The aforementioned results are in accordance with previous results[10&12&15]. Moreover, these results indicated that both NAC and CPL played a significant role in the protection against CYP-induced nephrotoxicity, and these protective effects correlated with the regulation of oxidative stress, inflammation, and apoptosis.

N-Acetylcysteine is an acetylated precursor of the amino acid L-cysteine, it is a thiol-containing antioxidant which acts as a direct scavenger of free radicals. In addition, NAC is a source of sulfhydryl groups, thereby encourages GSH production and restores the intracellular GSH concentration that is decreased during oxidative stress and inflammation. The nephroprotective effect of NAC is associated with its marked antioxidant and anti-inflammatory action, it suppresses pro-inflammatory mediators and oxidative enzymes[34]. Besides, NAC increases the anti-apoptotic Bcl-2 protein expression while decreasing the pro-apoptotic Bax protein expression in the renal tubular cells[35].

Captopril has been thought to be a free radical scavenger because of its terminal sulfhydryl group. CPL also has antioxidant effects by enhancing the activity of antioxidant enzymes including glutathione peroxidase and superoxide dismutase[36]. Moreover, de Cavanagh et al.[37] reported that CPL can increase enzymatic and non-enzymatic antioxi-dant defense mechanisms and reduce oxidative/ nitrosative stress and nitric oxide production by decreasing angiotensin II level. Also, angiotensin II activates nicotinamide-adenine dinucleotide phosphate oxidase (NADPH oxidase) which has a fundamental role in ROS formation and oxidative stress[38&39]. Moreover, angiotensin II activated pro-inflammatory cytokines which have a role in CYP-induced renal toxicity. CPL has anti-inflammatory properties; it suppresses NF-κB transcription. CPL reduced IL-1 beta and IL-6 and promotes the production of anti-inflammatory IL-10 in the different inflammatory rat models[40&41]. Besides, Sahin & Ergul[42] mentioned that CPL elevated Bcl-2 and decreased Bax expression after oxidative damage induced H_2O_2.

As mentioned above, acrolein can bind to cysteine, a constituent amino acid of GSH, and reduce its renal level. Thus, sulfhydryl-containing molecules have been reported to be good candidates for acrolein scavenging[3]. Hence, the acrolein-trapping abilities of both NAC and CPL could contribute to their protective effects. Besides, the level of intracellular thiol is crucial for estimating the degree of cellular damage brought on by anticancer drug and GSH level is critical to the well-being of the renal cells. In addition, the ability of thiols to scavenge ROS can protect the tubular epithelium from caspase activation and so from cell death[43]. NAC and CPL in the present study increased the renal GSH, and these elevated GSH levels can protect from the nephrotoxic effect of CYP.

Conclusion

According to the biochemical findings that are corroborated by the histological and immunohistochemical examination, the results of the present work emphasized that CYP induced a nephrotoxic effect in rats. Treatment with NAC and CPL could protect against this effect through their antioxidant, anti-inflammatory, and anti-apoptotic effects. These results suggested that using antioxidants can protect patients undergoing chemotherapy with cyclophosphamide against renal damage.

Conflict of interest

No competing interests are disclosed by the authors.

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نشرة العلوم الصيدلية
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تقييم مقارن للتأثير الوقائي لـــ ان اسيتايل سيستايين و كابتوبريل ضد السمية الكلوية التي يسببها سيكلوفوسفاميد في جرذان ويستار

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تم تنفيذ هذا العمل لتقييم ومقارنة التأثير الوقائي لـــ ان اسيتايل سيستاين و كابتوبريل في السمية الكلوية التي يسببها سيكلوفوسفاميد في الحرذان. تم تقسيم ستة وثلاثين من ذكور جرذان ويستار إلى ست مجموعات (٦ جرذان لكل مجموعة). تلقت المجموعة الضابطة محلول ملحي عادي. تلقت المجموعات المعالجة بـــ ان اسيتايل سيستاين ٢٠٠ مجم / كجم و المعالجة بـكابتوبريل ٦٠ مجم / كجم لمدة ستة أيام متتالية. بينما تلقت المجموعة المعالجة بـــالسيكلوفوسفاميد ١٥٠ مجم / كجم السيكلوفوسفاميد في اليوم السادس من التجربة. تلقت المجموعات المعالجة بـــ ان اسيتايل سيستاين + سيكلوفوسفاميد و كابتوبريل + سيكلوفوسفاميد بـــ (ان اسيتايل سيستاين ٢٠٠ مجم / كجم و كابتوبريل ٦٠ مجم / كجم) على التوالي لمدة ٦ أيام ثم أعطيت جرعة واحدة من السيكلوفوسفاميد في اليوم السادس من التجربة. الطريق داخل الصفاق هو طريقة إعطاء جميع الأدوية. أظهرت المجموعة المعالجة بـــالسيكلوفوسفاميد ارتفاعًا كبيرًا في مستويات اليوريا في الدم والكرياتينين وحمض البوليك وسيستاتين C ، وكذلك مستويات مالوندالديهيد و انترلوكين-٦ في الكلى. علاوة على ذلك ، فقد أظهر انخفاضًا كبيرًا في مستويات سوبر أوكسيد ديسموتيز و الجلوتاثيون و انترلـوكين-١٠ في الكلى. علاوة على ذلك ، تم تخفيض مستوى جين Bcl2. أظهرت التغيرات النسيجية المرضية في الكلى تغيرات تنكسية أنبوبية ملحوظة. بالإضافة إلى ذلك ، كانت هناك زيادة كبيرة في كاسباس- ٣ داخل الأنابيب الكلوية. من ناحية أخرى ، فإن المجموعات المعالجة بـــ ان اسيتايل سيستاين + سيكلوفوسفاميد و كابتوبريل + سيكلوفوسفاميد حمايه ضد التغيرات الكيميائية الحيوية والنسيجية غير الطبيعية واستعادة جين Bcl2. تشير هذه البيانات إلى أن ان اسيتايل سيستاين و كابتوبريل يمكن أن تحمي من التأثير السام للكلية الناجم عن السيكلوفوسفاميد من خلال آثارها المضادة للأكسدة والمضادة للالتهابات ومضادات موت الخلايا المبرمج.