

## N-ACETYLCYSTEINE IS EFFECTIVE IN AMELIORATING NEPHROTOXICITY INDUCED BY LOW DOSE, BUT NOT LARGE DOSE OF 5-FLUOROURACIL IN RATS

MOHAMED M. ELBADR <sup>1</sup>; SABAH. M. MOHAMED <sup>2</sup>; KHALED M.A. HASSANEIN <sup>3</sup>; HALA M. ELBADRE <sup>4</sup>; EBTSAM S. ABDEL-LAH <sup>2</sup> AND ESRAA A. AHMED <sup>1</sup>

<sup>1</sup>Department of Medical Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt.  
[mmelbadr@aun.edu.eg](mailto:mmelbadr@aun.edu.eg), [Esraa.mohamed12@med.aun.edu.eg](mailto:Esraa.mohamed12@med.aun.edu.eg)

<sup>2</sup>Department of Pharmacology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.  
[ebtsam\\_saber@aun.edu.eg](mailto:ebtsam_saber@aun.edu.eg)

<sup>3</sup>Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt. [khaledhassanein@aun.edu.eg](mailto:khaledhassanein@aun.edu.eg)

<sup>4</sup>Department of Medical Biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt.  
[hala.elbadre@aun.edu.eg](mailto:hala.elbadre@aun.edu.eg)

**Received:** 4 August 2024; **Accepted:** 1 September 2024

### ABSTRACT

5-Fluorouracil (5-FU) is a common chemotherapy drug with demonstrated effectiveness in treating human cancer. However, hepatotoxic and nephrotoxic side effects limit its therapeutic value. This investigation exhibited the therapeutic influence of N-acetylcysteine (NAC) in 5-FU (20 and 50 mg) kidney injury in rats. For this purpose, 40 male rats were assigned into 5 groups. The 1<sup>st</sup> group was used as a control. The 2<sup>nd</sup> group was injected with 20 mg/kg of 5-FU i.p. for 6 days. The 3<sup>rd</sup> group received 5-FU at a dose of 50 mg/kg i.p. for 6 days. The 4<sup>th</sup> group received 5-FU 20 mg +NAC 40 mg/kg for 6 days. The 5<sup>th</sup> group supplied with 5-FU 50 mg + NAC 40 mg/kg. Biochemical assessment for serum urea, creatinine, uric acid, albumin, and inflammatory markers as TNF- $\alpha$  and IL-1 $\beta$ , and oxidative stress parameters as GSH and MDA were measured. 5-FU nephrotoxicity was noticed by significant elevation of all renal parameters, such as creatinine, urea, uric acid, MDA, TNF- $\alpha$  and IL-1 $\beta$ , with a remarkable decline in albumin and GSH levels. NAC treatment improved the kidney status, especially induced by 20 mg 5-FU. So, NAC exhibited a remarkable effect in protecting nephrotoxicity caused by the low dose of 5-FU rather than using it with a high dose of 5-FU.

**Keywords:** Fluorouracil, Inflammatory markers, N-acetylcysteine, Oxidative stress.

### INTRODUCTION

Because the kidneys' primary role is to concentrate and eliminate poisons and toxic

metabolites of medications, the primary goal of drug-induced toxicity is the kidney's exposure to these substances. One of the main causes (about 8%–60%) of acute kidney damage (AKI) in a hospital setting is nephrotoxicity (Mody *et al.*, 2020). An increased incidence of diabetes and hypertension is accompanied by high rates of chronic kidney disease (CKD), which

Corresponding author: Sabah. M. Mohamed

E-mail address: [vet.sabah@aun.edu.eg](mailto:vet.sabah@aun.edu.eg);

[vet.sabah.abdelrahman@gmail.com](mailto:vet.sabah.abdelrahman@gmail.com)

Present address: Department of Pharmacology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

have increased to roughly 13–15% globally in recent years (Levey *et al.*, 2005).

In our systems, the metabolism of drugs occurs in the liver, the GIT and the kidneys. Renal and extra-renal excretions are the main pathways of drug excretion (Perazella, 2019). Drugs can be eliminated from the body by either of the two routes that lead to renal excretion: tubular secretion traffic from the proximal tubule (PT) into the loop of Henle and subsequently into the distal tubule, or glomerular filtration. Drugs may precipitate, crystallize, or form casts in the most distal portions of the tubules, leading to obstacles to the tubules (Luque *et al.*, 2017). A further pathway involves the occurrence of interstitial nephritis, which is triggered by tubulointerstitial inflammation (Chamarthi *et al.*, 2018).

Nephrotoxicity caused by drugs arises through one of three mechanisms: (1) dose-dependent proximal tubular damage and acute tubular necrosis (ATN); (2) dose-dependent tubular obstruction by drug- and metabolite-containing crystals or casts; and (3) dose-independent interstitial nephritis that results from drug- and metabolite-induced casts (Kwiatkowska *et al.*, 2021).

Improving the cardiac and renal toxicity brought on by the rising, and dose-dependent, usage of anti-cancer medications is a major concern for oncologists (Abdel-Daim *et al.*, 2020). The popular anticancer medication, 5-Fluorouracil (5-FU) is a pyrimidine antimetabolite, and is used extensively in human medicine because of its exceptional anti-cancer properties against several kinds of cancers, including skin, stomach, and colon cancers (Rashid *et al.*, 2013). It is transformed intracellularly into a number of active metabolites that impact both normal and tumor cells' RNA and DNA synthesis. These metabolites include fluorouridine triphosphate, fluorodeoxyuridine triphosphate, and fluorodeoxyuridine monophosphate. After being metabolically converted to 5-fluoro-2-deoxyuridine

monophosphate, it irreversibly inhibits thymidylate synthase (TS), which promotes apoptosis in malignant cells and even in regularly growing cells (Chibber *et al.*, 2011). Because of this, 5-FU has demonstrated exceptional toxicity and negative effects on the kidneys and heart (Arab *et al.*, 2018).

5-FU's systemic action is linked to various adverse reactions that may cause stopping the medication and reduce its effectiveness (de Andrade *et al.*, 2023). According to the majority of published data, organs such as the intestines and bone marrow, that have significant concentrations of proliferative cells, are more likely to have 5-FU adverse effects (Chrysostomou *et al.*, 2023). However, the liver, kidneys, and lungs-essential organs with a low potential for proliferation-are continuously exposed to 5-FU metabolites and may suffer adverse effects as a result (Kuan *et al.*, 1998). The oxidative stress and inflammation caused by 5-FU chemotherapy may be the cause of these pathological processes (Akindele *et al.*, 2018).

In the kidney, glutathione (GSH) is a highly concentrated nucleophilic molecule that contains sulfur. GSH is essential for defending cells against damage caused by free radicals generated by oxygen and other activated hazardous substances (Ross, 1988). As a strong antioxidant, N-acetylcysteine (NAC) might act as an initial step in producing glutathione (Bernard *et al.*, 1984). More GSH is available for the detoxification of oxygen-derived free radicals and other foreign substances after the stimulation of GSH synthesis after NAC administration (Meister and Anderson, 1983).

N-acetylcysteine is a sulfhydryl donor that has a variety of medical uses. It has been shown to function as a lipid peroxidation inhibitor (LPO) and cellular necrosis, a free radical scavenger, and a mitochondrial protector (Samuni *et al.*, 2013). As an initiator in the process of GSH synthesis, it enriches the cellular glutathione (GSH) level

and boosts numerous cellular defense mechanisms (Yalçın *et al.*, 2008). Moreover, NAC is a popular antioxidant against oxidative stress, which can be utilized to restore the balance between pro-oxidants and antioxidants *in vivo* and *in vitro* (Campos *et al.*, 2012).

Considering L-cysteine's acetylated precursor, NAC has been used for a long time as a mucolytic agent and to treat mental problems, doxorubicin-induced cardiotoxicity, acetaminophen intoxication, stable angina pectoris, and acute respiratory distress syndrome. Furthermore, its level of toxicity is really low (Samuni *et al.*, 2013). Because NAC is oxidized by different radicals and acts as a nucleophile, it can decrease the disulfide bridges in proteins, absorb free radicals, and create metal chelation (Atkuri *et al.*, 2007). Moreover, NAC's anti-inflammatory and antiapoptotic qualities are well-known (Zafarullah *et al.*, 2003). In addition to these immediate benefits, NAC also works by elevating GSH levels (Samuni *et al.*, 2013). There hasn't been any research done on how NAC affects acute renal failure brought on by 5-FU.

The objective of this study was to assess any potential protective benefits of NAC in rats, which had both high and low doses of 5-FU-induced nephrotoxicity. Additionally, it looked into how oxidative stress and inflammatory markers might help NAC work its magic on the kidneys.

## MATERIALS AND METHODS

### 1. Drugs and Chemicals

Commercial vial of 5-Fluorouracil (5-FU) obtained from Hikma Specialized Pharmaceutical. N-acetylcysteine (NAC) white powder obtained from AK Scientific, Inc. (USA).

### 2. Animals

We purchased forty mature male Albino Wistar rats from the Animal House of the Faculty of Veterinary Medicine Assiut

University, weighing 200–250 g. For a week, rats were housed in standard, pathogen-free facilities with a temperature of  $24 \pm 2^\circ\text{C}$ , a relative humidity of 60–70%, and a cycle of 12 hours of light and darkness. The animals had unrestricted access to water and were fed a usual pellet meal. The ethics committee of Assiut University's College of Medicine approved the study (approval no: 17300967).

### 3. Induction of nephrotoxicity

5-Fluorouracil (5-FU) 20 mg / Kg (Yousef and Aboelwafa, 2017 and Adikwu *et al.*, 2019) and 50 mg / Kg b.w. (El-Sherbiny *et al.*, 2021) was injected intraperitoneally (i.p.) daily for 6 days to cause toxicity to the kidneys.

### 4. Animal grouping

Animals used in the investigation were divided into 5 groups, each group containing 8 rats. The first control group received distilled water i.p. daily for 6 days. The second and third groups received 5-FU i.p. for 6 days at doses of 20 and 50 mg/kg, respectively. The fourth group was injected with 5-FU at a dose of 20 mg/kg + 40 mg/kg of NAC i.p. for 6 days (Efrati *et al.*, 2007). The fifth group was injected intraperitoneally with 5-FU 50 mg/kg plus NAC 40 mg/kg. After finishing the experiment, the rats received 4% isoflurane anesthesia, and blood samples were collected and kept for 15 minutes in a clean, dry centrifuge tube. The samples were then centrifuged for another 15 minutes at 3000 rpm. Serum was obtained using Eppendorf tubes to evaluate kidney function parameters and inflammatory markers (TNF- $\alpha$  and IL-1 $\beta$ ). The kidneys were separated, cleaned, and weighed individually. A 10% (w/v) homogenate of the kidneys was rapidly produced by homogenizing them in an ice-cold phosphate buffer (pH 7.4) solution. A 10-minute,  $4^\circ\text{C}$ , 1800 g centrifugation was performed on the homogenate. In order to test GSH and MDA, the 10% supernatant was removed and stored at  $-80^\circ\text{C}$  (Elsayed and Zaazaa, 2024).

## 5. Biochemical investigations

The evaluation of renal function was carried out spectrophotometrically using kits (Sigma Diagnostics, Pvt. Ltd, Baroda, India), including serum concentrations of urea, creatinine, uric acid, and albumin.

## 6. Determination of oxidative stress markers in kidney tissue

### 6.1. Determination of the renal reduced glutathione (GSH)

Sedlak and Lindsay (1968) approach was used to assess the levels of GSH in the kidneys. Spectrophotometric measurements of the color absorbance were made at 412 nm using colorimetric kits (Biodiagnostic, Giza, Egypt).

### 6.2. Determination of malondialdehyde (MDA)

The MDA level in kidney homogenate was determined using the methodology outlined by Ohkawa and his group in 1979. The employed measurement method was spectrophotometric, using colorimetric kits (Biodiagnostic, Dokki, and Giza, Egypt), depending on a colorimetric reaction with thiobarbituric acid.

## 7. Determination of inflammatory markers

### 7.1. Determination of the level of tumor necrosis factor- $\alpha$ (TNF- $\alpha$ )

Following the manufacturer's instructions, the AssayPro USA rat TNF- $\alpha$  ELISA kit was used to measure the levels of TNF- $\alpha$  in rat serum. Using an automated ELISA reader set to 450 nm, the response was monitored (Elsayed and Zaazaa, 2024).

### 7.2. Determination of the level of interleukin-1 $\beta$ (IL-1 $\beta$ )

The rat IL-1 $\beta$  ELSIA kit (Dynatech Microplate Reader Model MR 5000, Canada) was used to measure the amount of IL-1 $\beta$  in the rat serum, following the manufacturer's instructions. It took five minutes for the ELISA reader to read the absorbance at 450 nm (Gelen *et al.*, 2018).

## 8. Statistical analysis

The data were statistically evaluated using Graph Pad Prism version 9 for Windows, 2007 by Graph Pad software, Inc. The mean  $\pm$  standard error of the means (SE) was used for presenting the data. For multiple comparisons, the one-way ANOVA was utilized; if needed, the Bonferroni test was then done. Significant variances were defined as those with  $P < 0.05$ . The data and statistical analysis adhere to the standards for the design and analysis of experiments in pharmacology.

## RESULTS

### 1. Effect of NAC treatment on renal parameters in nephrotoxicity caused by 5-FU in rats:

The rats that received 20 mg and 50 mg of 5-FU had significantly increased uric acid, creatinine, and urea levels ( $P < 0.05$ ) with a remarkable drop in albumin concentration, compared to the negative control group. The biochemical variations were higher with the high dose of 5-FU (Table 1).

The results revealed a significant improvement in renal parameters ( $P < 0.05$ ) after using NAC. More positive results were seen when rats received a low dose of 5-FU rather than a high dose (Table 1).

**Table 1:** The effect of N-acetylcysteine on renal parameters in cases of renal injury induced by 5-FU (20, 50 mg/kg):

Groups	Control	5-FU (20 mg)	5-FU (50 mg)	NAC (40 mg/kg) + 5-FU (20 mg)	NAC (40 mg/kg) + 5-FU (50 mg)
<b>Creatinine conc. (mg/dl)</b>	0.8 ± 0.03	2.0 ± 0.08 <sup>\$</sup>	4.4 ± 0.24 <sup>\$*</sup>	0.9 ± 0.04 <sup>*#</sup>	3.0 ± 0.14 <sup>\$*#</sup>
<b>Urea conc. (mg/dl)</b>	25.3 ± 1.37	56.9 ± 0.51 <sup>\$</sup>	94.7 ± 1.39 <sup>\$*</sup>	37.5 ± 1.13 <sup>\$*#</sup>	84.2 ± 1.07 <sup>\$*#</sup>
<b>Uric acid conc. (mg/dl)</b>	3.06 ± 0.30	7.8 ± 0.29 <sup>\$</sup>	11.0 ± 0.47 <sup>\$*</sup>	4.3 ± 0.12 <sup>*#</sup>	7.8 ± 0.29 <sup>*#</sup>
<b>Albumin conc. (g/dl)</b>	3.7 ± 0.22	2.3 ± 0.09 <sup>\$</sup>	1.3 ± 0.05 <sup>\$*</sup>	3.3 ± 0.18 <sup>*#</sup>	2.3 ± 0.27 <sup>\$#</sup>

Values were expressed as mean ± SE of 8 rats. 5-FU: 5-Fluorouracil, NAC: N-acetylcysteine.

<sup>\$</sup> Significant difference at p < 0.05 vs. negative control group.

\* Significant difference at p < 0.05 vs. 5-FU (20 mg) group.

# Significant difference at p < 0.05 vs. 5-FU (50 mg) group.

## 2. Effect of NAC treatment on renal GSH and MDA in 5-FU-induced renal damage in rats:

The findings showed that the GSH concentration dropped and the MDA level rose significantly (p < 0.05) in rats given 20 mg of 5-FU. The rats given 50 mg of 5-FU showed more alterations than the negative

control group. On the other hand, the rats that received NAC treatment showed a substantial (p < 0.05) rise in GSH and a substantial drop in MDA concentration. Rats receiving 20 mg/kg of 5-FU as opposed to 50 mg/kg showed better treatment of oxidative stress indicators with NAC (Table 2).

**Table 2:** Effect of N-acetylcysteine on renal GSH and MDA in kidney injury caused by 5-Fluorouracil in rats (20, 50 mg/kg):

Groups	Control	5-FU (20 mg)	5-FU (50 mg)	NAC (40 mg/kg) + 5-FU (20 mg)	NAC (40 mg/kg) + 5-FU (50 mg)
<b>GSH conc. (mmol/g)</b>	4047 ± 217.5	2244 ± 124.6 <sup>\$</sup>	816.8 ± 0.24 <sup>*\$</sup>	3406 ± 135 <sup>\$*#</sup>	2264 ± 47.04 <sup>\$#</sup>
<b>MDA conc. (mmol/g)</b>	9.5 ± 0.50	70.0 ± 2.40 <sup>\$</sup>	93.3 ± 3.07 <sup>*\$</sup>	18.5 ± 0.59 <sup>\$*#</sup>	44.4 ± 0.44 <sup>\$*#</sup>

Values were expressed as mean ± SE of 8 rats. 5-FU: 5-Fluorouracil, NAC: N-acetylcysteine.

<sup>\$</sup> Significant difference at p < 0.05 vs. control group.

\* Significant difference at p < 0.05 vs. 5-FU (20 mg) group.

# Significant difference at p < 0.05 vs. 5-FU (50 mg) group.

## 3. Effect of NAC treatment on TNF-α and IL-1β in 5-FU induced nephrotoxicity in rats:

The outcomes showed, in contrast to the control group, that the concentrations of TNF-α and IL-1β were substantially (p <

0.05) higher in the 5-FU groups (20 mg and 50 mg). TNF-α and IL-1β concentrations significantly (p < 0.05) decreased in NAC-treated groups. There was a greater reduction in case of the lower dose, 20 mg/kg of 5-FU, compared to 50 mg/kg (Table 3).

**Table 3:** The effect of N-acetylcysteine on serum TNF- $\alpha$  and IL-1 $\beta$  in nephrotoxicity induced by 5-Fluorouracil in rats (20, 50 mg/kg):

Groups	Control	5-FU (20 mg)	5-FU ( 50 mg )	NAC (40 mg/kg) +5-FU (20 mg)	NAC (40 mg/kg) +5-FU (50 mg)
TNF- $\alpha$ conc. (ng/ml)	262.2 $\pm$ 5.43	422.2 $\pm$ 3.18 <sup>\$</sup>	521.3 $\pm$ 6.06 <sup>\$*</sup>	334.5 $\pm$ 15.8 <sup>\$*#</sup>	468.0 $\pm$ 1.16 <sup>\$*#</sup>
IL-1 $\beta$ conc. (pg/ml)	204.8 $\pm$ 4.88	333.8 $\pm$ 17.34 <sup>\$</sup>	494.7 $\pm$ 2.73 <sup>\$*</sup>	216.5 $\pm$ 4.77 <sup>*#</sup>	387.9 $\pm$ 2,28 <sup>\$*#</sup>

Values were expressed as mean  $\pm$  SE of 8 rats. 5-FU: 5-Fluorouracil, NAC: N-acetylcysteine.

\$ Significant difference at  $p < 0.05$  vs. negative control group.

\* Significant difference at  $p < 0.05$  vs. 5-FU (20 mg) group.

# Significant difference at  $p < 0.05$  vs. 5-FU (50 mg) group.

## DISCUSSION

In recent times, cancer has been an important worldwide public health concern (Siegel *et al.*, 2019). Chemotherapy, radiation, and surgery are some methods used in cancer treatment. One drug to treat many kinds of cancers is 5-fluorouracil (5-FU). Despite several benefits, organ toxicity, thymine synthesis suppression, and DNA damage have restricted 5-FU (Gelen *et al.*, 2018). Considering 5-FU's method of action, it impacts the S phase of the cell cycle by inhibiting thymidylate synthase, which stops DNA synthesis and ultimately results in cell death (Sethy and Kundu, 2021). According to AIDosari *et al.* (2023), 5-FU has serious adverse effects, including DNA damage, by inhibiting tumors and normal cells in patients from proliferating, inducing apoptosis, and causing cell death. Only a small percentage of 5-FU is eliminated by the kidney, while the majority is eliminated by the liver (Longley *et al.*, 2003). Drug-induced hepatotoxicity and nephron-toxicity have become more common (Sengul *et al.*, 2021).

This study confirmed that 5-FU is a clear model of nephrotoxicity (LD50 of 5-FU is 250 mg/kg) in experimental rats at dosages of 20 and 50 mg/kg, supported by the renal function measures. The 5-FU group showed significantly higher levels of creatinine, urea, and uric acid than the control group. The higher the 5-FU dosage, the greater the kidney damage, suggesting

that the nephrotoxicity caused by 5-FU is dose-dependent. Rats treated with 5-FU also exhibited a significant drop in albumin levels. These outcomes agreed with those of Badawoud *et al.* (2017), who examined the potential protective effect of L-arginine against 5-fluorouracil-induced nephron-toxicity (189 mg/rat/week) in male albino rats for four weeks. They found a remarkable decline in albumin and an increase in total proteins, creatinine and urea levels, compared to the control group.

Additionally, in rats given 20 mg/kg of 5-FU i.p. daily for five days, Adikwu and colleagues (2019) investigated the protective effect of selenium in nephrotoxicity caused by 5-fluorouracil. They demonstrated that statistically significant increases in creatinine, urea, uric acid, and MDA levels were indicative of nephrotoxic action of 5-FU. In addition, 5 FU-treated rats exhibited a significant reduction in the levels of catalase, superoxide dismutase, GSH, potassium, sodium, chloride, bicarbonate, and GSH peroxidase.

The misincorporation of fluoronucleotides into RNA and DNA as well as the suppression of the enzyme thymidylate synthase, which synthesizes nucleotides, have been identified as the mechanisms of 5-FU's cytotoxicity (Longley *et al.*, 2003).

According to numerous studies, oxidative stress is a major factor in the nephrotoxicity caused by 5-FU (Rashid *et al.*, 2014). Excessive formation of free radicals can

result from the experimental administration of 5-FU, which might cause DNA damage and death in the cell (Xia *et al.*, 2016). A decrease in kidney GSH levels plus rises in MDA levels supported this conclusion.

This result indicates oxidative stress and agrees with a previous study, where nephrotoxicity and hepatotoxicity were induced by injecting 20 mg/kg of 5-FU i.p. in rats for six days (Gelen *et al.*, 2018). The researchers found a rise in MDA concentration, in addition to a lowering in SOD and GSH levels in the kidney.

In contrast to the control group, the 5-FU groups (20 mg and 50 mg) showed significantly higher concentrations of TNF- $\alpha$  and IL-1 $\beta$ , as reported by Elsayed and Zaazaa (2024). They investigated l-carnitine nanoparticles protective effects against renal injury caused by 5-Fluorouracil (25 mg/kg, i.p.) three times a week for three weeks in mice bearing Ehrlich ascites carcinoma (EAC) and proposed that MCP-1 and TNF- $\alpha$  showed a slight but significant increase in the 5-FU treated group relative to the EAC group. This result revealed that mice fed 5-FU had inflammation in their kidneys. Furthermore, transcription factor-mediated immune response regulation through genes that control and activate pro-inflammatory indicators, NF- $\kappa$ B shows an important function in inflammation illness in many organs, contributing to tissue damage pathogenesis and the production of edema that leads to tissue damage injury (Caglayan *et al.*, 2018). Furthermore, Elghareeb *et al.* (2021) reported that rats treated with 5-FU (five injections at a dose of 40 mg/kg b.w./IV) had significantly higher blood concentrations of IL-1 $\beta$  and TNF- $\alpha$ , and significantly lower levels of IL10, IL12, IgM, and IgG.

Antioxidants, both nonenzymatic and enzymatic, such as vitamin C and reduced glutathione (GSH), counteract the damage caused by intracellular ROS by either directly scavenging oxygen radicals like hydroxyl radical (OH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hypochlorous acid (HOCl), or

by repairing the oxidative damage (Bachowski *et al.*, 1998). N-acetylcysteine has an antioxidant effect that protects against oxidative stress and inhibits the generation of lipoprotein peroxide by scavenging free radicals in cellular membranes. It is a glutathione precursor (GSH) and medicine with a sulfhydryl-donor (Cotgreave, 1996). N-acetylcysteine has been reported to enhance renal microcirculation and redox state, as well as regenerate glutathione and endothelium-derived relaxing factor (Heyman *et al.*, 2003).

The results of the current investigation indicated that NAC had a renoprotective effect (LD50 of NAC in rats is 1205 mg/kg) in the two 5-FU model doses. However, the 5-FU 20 mg group treated with NAC showed more effectiveness, in contrast to those of the control group. Albumin levels considerably rose as a marker of renal protection, while NAC usage decreased kidney function parameters (creatinine, urea, and uric acid). Nouri and Heidarian (2019) also noted that 100 mg/kg bw/day by oral treatment of NAC for five days led to a substantial decline in the levels of glutamic-oxaloacetic transaminase (GOT), urea, creatinine, and uric acid in the serum, compared to the control group. The administration of two doses (300 mg/kg) of NAC effectively lowered the elevation in BUN and creatinine levels, while also greatly improving the histological damage induced by folic acid delivery (Aparicio-Trejo *et al.*, 2019).

However, Alexandropoulos *et al.* (2017) used a rat design of intestinal ischemia-reperfusion (I/R) to show the protective effects of NAC 160 mg/kg and atorvastatin in kidney and liver injury. Additionally, they noticed that the I/R and therapy groups' serum creatinine and urea levels were identical.

Compared to the 20 and 50 mg 5-FU treated groups, the NAC-treated groups exhibited a large rise in GSH and a considerable reduction in MDA (a good predictor of lipid

peroxidation), indicating a link between oxidative stress and apoptosis. This was reliable with an article published in 2011 by Fitri *et al.*, which showed that the H<sub>2</sub>O<sub>2</sub> and MDA levels were raised by 2, 4, and 8 µM dosages of NAC, but the GSH level was lowered. Additionally, Beceren *et al.* (2017) reported that rats given 150 mg/kg/day i.p. of NAC for five days saw a large rise in tissue glutathione, a considerable drop in liver malondialdehyde, and a significant reduction in endosulfan-induced nephrotoxicity.

Compared to the 5-FU-treated group, the TNF-α and IL-1β concentrations in the NAC-treated groups decreased considerably (P<0.05). This demonstrated NAC's capacity to treat the inflammatory state brought by 5-FU (20 and 50 mg). Beceren *et al.* (2017) investigated the protective influence of NAC (150 mg/kg/day i.p. for 5 days) on endosulfan-induced liver and kidney toxicity in rats. The investigation found that TNF-α, IL-1β, and IL-6, were significantly elevated in the endosulfan-treated animals compared to the control group, and this increase was avoided by using NAC.

This study has some limitations, such as the duration of the study, and doses of NAC and needs more parameters to be investigated.

## CONCLUSIONS

The outcomes of this experiment showed that NAC considerably improved the nephrotoxicity caused by injecting 5-FU. It showed that the antioxidant properties of NAC, along with its reduction of inflammation, are the reasons behind its renoprotective efficiency. It was found that the NAC was more effective with low doses, rather than with high doses of 5-FU. So it is recommended to add NAC in patients using a low dose of 5-FU, but patients using a high dose need another renoprotective strategy rather than NAC.

## ACKNOWLEDGMENTS

The Assiut University Faculty of Veterinary Medicine is acknowledged by the authors for its assistance in the research.

## REFERENCES

- Abdel-Daim, M.M.; Mahmoud, O.M.; Al Badawi, M.H.; Alghamdi, J.; Alkahtani, S. and Salem, N.A. (2020): Protective effects of Citrus limonia oil against cisplatin-induced nephrotoxicity. *Environmental Science & Pollution Research*, 27, 41540-41550. <https://doi.org/10.1007/s11356-020-10066-x>.
- Adikwu, E.; Ebinyo, N. and Amgbare, B. (2019): Protective Activity Of Selenium Against 5-Fluorouracil-Induced Nephrotoxicity In Rats. *Cancer Translational Medicine*, 5, 50-55. [https://doi.org/10.4103/ctm.ctm\\_26-19](https://doi.org/10.4103/ctm.ctm_26-19).
- Akindele, A.J.; Oludadebo, G.O.; Amagon, K.I.; Singh, D. and Osiagwu, D.D. (2018): Protective effect of carvedilol alone and coadministered with diltiazem and prednisolone on doxorubicin and 5-fluorouracil-induced hepatotoxicity and nephrotoxicity in rats. *Pharmacology Research & Perspectives*, 6, e00381. <https://doi.org/10.1002/prp2.381>.
- ALDosari, S.M.; Banawas, S.; Ghafour, H.S.; Tlili, I. and Le, Q.H. (2023): Drug release using nanoparticles in the cancer cells on 2-D materials in order to target drug delivery: A numerical simulation via molecular dynamics method. *Engineering Analysis with Boundary Elements*, 148, 34-40. <https://doi.org/10.1016/j.enganabound.2022.12.020>.
- Alexandropoulos, D.; Bazigos, G.V.; Doulamis, I.P.; Tzani, A.; Konstantopoulos, P.; Tragotsalou, N.; Kondi-Pafiti, A.; Kotsis, T.; Arkadopoulos, N. and Smyrniotis, V. (2017): Protective effects of N-acetylcystein and atorvastatin against renal and hepatic injury in a rat model





- microbiota modulation of efficacy and toxicity of cancer chemotherapy and immunotherapy. *Gastroenterology*, 164, 198-213. <https://doi.org/10.1053/j.gastro.2022.10.018>.
- Cotgreave, I.A. (1996): N-acetylcysteine: Pharmacological considerations and experimental and clinical applications. *Advances in Pharmacology*, 38, 205-227. [https://doi.org/10.1016/S1054-3589\(08\)60985-0](https://doi.org/10.1016/S1054-3589(08)60985-0).
- De Andrade, G.L.; Da Silva Souza, B.; Araújo, D.D.; De Freitas, C.D. and De Oliveira, J.S. (2023): Protective Effect of Plumeria Pudica Latex Proteins on Intestinal Mucositis Induced by 5-Fluorouracil. *Mini Reviews in Medicinal Chemistry*, 23, 298-306. <https://doi.org/10.2174/1389557522666220817112943>.
- Efrati, S.; Berman, S.; Siman-Tov, Y.; Lotan, R.; Averbukh, Z.; Weissgarten, J. and Golik, A. (2007): N-acetylcysteine attenuates NSAID-induced rat renal failure by restoring intrarenal prostaglandin synthesis. *Nephrology Dialysis Transplantation*, 22, 1873-1881. <https://doi.org/10.1093/ndt/gfm113>.
- El-Sherbiny, M.; Fahmy, E.K.; Eisa, N.H.; Said, E.; Elkattawy, H.A.; Ebrahim, H.A.; Elsherbiny, N.M. and Ghoneim, F.M. (2021): Nanogold Particles Suppresses 5-Fluorouracil-Induced Renal Injury: An Insight into the Modulation of Nrf-2 and Its Downstream Targets, HO-1 and  $\gamma$ -GCS. *Molecules*, 26, 7684. <https://doi.org/10.3390/molecules26247684>.
- Elghareeb, M.M.; Elshopakey, G.E.; Hendam, B.M.; Rezk, S. and Lashen, S. (2021): Synergistic effects of Ficus Carica extract and extra virgin olive oil against oxidative injury, cytokine liberation, and inflammation mediated by 5-Fluorouracil in cardiac and renal tissues of male albino rats. *Environmental Science & Pollution Research*, 28, 4558-4572. <https://doi.org/10.1007/s11356-020-10778-0>.
- Elsayed, H. and Zaazaa, A.M. (2024): Effectiveness of l-carnitine Nanoparticles against Nephrotoxicity Induced by 5-Fluorouracil in Ehrlich ascites Carcinoma Bearing Mice. *Egyptian Academic Journal of Biological Sciences, B. Zoology*, 16, 31-46. <https://doi.org/10.21608/eajbsz.2024.340550>.
- Fitri, L.; Sardjono, T.; Simamora, D.; Sumarno, S. and Setyawati, S. (2011): High dose of N-acetylcysteine increase H<sub>2</sub>O<sub>2</sub> and MDA levels and decrease GSH level of HUVECs exposed with malaria serum. *Tropical Biomedicine*, 28, 7-15.
- Gelen, V.; Şengül, E.; Yıldırım, S. and Atila, G. (2018): The protective effects of naringin against 5-fluorouracil-induced hepatotoxicity and nephrotoxicity in rats. *Iranian Journal of Basic Medical Sciences*, 21, 404-410. <https://doi.org/10.22038/IJBMS.2018.27510.6714>.
- Heyman, S.N.; Goldfarb, M.; Shina, A.; Karmeli, F. and Rosen, S. (2003): N-acetylcysteine ameliorates renal microcirculation: studies in rats. *Kidney International*, 63, 634-641. <https://doi.org/10.1046/j.1523-1755.2003.00783.x>.
- Kuan, H.-Y.; Smith, D.E.; Ensminger, W.D.; Knol, J.A.; Deremer, S.J.; Yang, Z. and Stetson, P.L. (1998): Regional pharmacokinetics of 5-fluorouracil in dogs: role of the liver, gastrointestinal tract, and lungs. *Cancer Research*, 58, 1688-1694.
- Kwiatkowska, E.; Domański, L.; Dziejewski, V.; Kajdy, A.; Stefańska, K. and Kwiatkowski, S. (2021): The mechanism of drug nephrotoxicity and the methods for preventing kidney damage. *International Journal of Molecular Sciences*, 22, 6109. <https://doi.org/10.3390/ijms22116109>.
- Levey, A.S.; Eckardt, K.-U.; Tsukamoto, Y.; Levin, A.; Coresh, J.; Rossert, J.; Zeeuw, D.D.; Hostetter, T.H.; Lameire, N. and Eknoyan, G. (2005): Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving

- Global Outcomes (KDIGO). *Kidney International*, 67, 2089-2100. <https://doi.org/10.1111/j.1523-1755.2005.00365.x>.
- Longley, D.B.; Harkin, D.P. and Johnston, P.G. (2003): 5-fluorouracil: mechanisms of action and clinical strategies. *Nature Reviews Cancer*, 3, 330-338. <https://doi.org/10.1038/nrc1074>.
- Luque, Y., Louis, K.; Jouanneau, C.; Placier, S.; Esteve, E.; Bazin, D.; Rondeau, E., Letavernier, E.; Wolfroth, A. and Gosset, C. (2017): Vancomycin-associated cast nephropathy. *Journal of the American Society of Nephrology: Journal of the American Society of Nephrology*, 28, 1723-1728. <https://doi.org/10.1681/ASN.2016080867>.
- Meister, A. and Anderson, M.E. (1983): Glutathione. *Annual Review of Biochemistry*, 52, 711-760. <https://doi.org/10.1146/annurev.bi.52.070183.003431>.
- Mody, H.; Ramakrishnan, V.; Chaar, M.; Lezeau, J.; Rump, A.; Taha, K.; Lesko, L. and Ait-Oudhia, S. (2020): A Review on Drug-Induced Nephrotoxicity: Pathophysiological Mechanisms, Drug Classes, Clinical Management, and Recent Advances in Mathematical Modeling and Simulation Approaches. *Clinical Pharmacology in Drug Development*, 9, 896-909. <https://doi.org/10.1002/cpdd.879>.
- Nouri, A. and Heidarian, E. (2019): Ameliorative effects of N-acetyl cysteine on diclofenac-induced renal injury in male rats based on serum biochemical parameters, oxidative biomarkers, and histopathological study. *Journal of Food Biochemistry*, 43, e12950. <https://doi.org/10.1111/jfbc.12950>.
- Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): Assay for lip. id peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95, 351-358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3).
- Perazella, M.A. (2019): Drug-induced acute kidney injury: diverse mechanisms of tubular injury. *Current Opinion in Critical Care*, 25, 550-557. <https://doi.org/10.1097/MCC.0000000000000653>.
- Rashid, S.; Ali, N.; Nafees, S.; Ahmad, S.T.; Hasan, S.K. and Sultana, S. (2013): Abrogation of 5-fluorouracil induced renal toxicity by bee propolis via targeting oxidative stress and inflammation in Wistar rats. *Journal of Pharmacy Research*, 7, 189-194. <https://doi.org/10.1016/j.jopr.2013.03.003>.
- Rashid, S.; Ali, N.; Nafees, S.; Hasan, S.K. and Sultana, S. (2014): Mitigation of 5-Fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in wistar rats. *Food & Chemical Toxicology*, 66, 185-193. <https://doi.org/10.1016/j.fct.2014.01.026>.
- Ross, D. (1988): Glutathione, free radicals and chemotherapeutic agents: mechanisms of free-radical induced toxicity and glutathione-dependent protection. *Pharmacology & Therapeutics*, 37, 231-249. [https://doi.org/10.1016/0163-7258\(88\)90027-7](https://doi.org/10.1016/0163-7258(88)90027-7).
- Samuni, Y.; Goldstein; S.; Dean; O.M. and Berk; M. (2013): The chemistry and biological activities of N-acetylcysteine. *Biochimica et Biophysica Acta-General Subjects*; 1830; 4117-4129. <https://doi.org/10.1016/j.bbagen.2013.04.016>.
- Sedlak; J. and Lindsay; R.H. (1968): Estimation of total; protein-bound; and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*; 25; 192-205. [https://doi.org/10.1016/0003-2697\(68\)90092-4](https://doi.org/10.1016/0003-2697(68)90092-4).
- Sengul, E., Gelen, V. and Gedikli, S. (2021): Cardioprotective activities of quercetin and rutin in Sprague Dawley rats treated with 5-fluorouracil. *JAPS: Journal of Animal & Plant Sciences*; 31; 423-431. <https://doi.org/10.36899/JAPS.2021.2.0231>.

- Sethy, C. and Kundu, C.N. (2021): 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: Implication of DNA repair inhibition. *Biomedicine & Pharmacotherapy*; 137; 111285. <https://doi.org/10.1016/j.biopha.2021.111285>.
- Siegel, R.L.; Miller, K.D. and Jemal, A. (2019): Cancer statistics; 2019. *CA: A Cancer Journal For Clinicians*; 69; 7-34. <https://doi.org/10.3322/caac.21551>.
- Xia, L.; Chen, S.; Dahms, H.-U.; Ying, X. and Peng, X. (2016): Cadmium induced oxidative damage and apoptosis in the hepatopancreas of *Meretrix meretrix*. *Ecotoxicology*; 25; 959-969. <https://doi.org/10.1007/s10646-016-1653-7>.
- Yalçın, S.; BILGİLİ, A.; Onbaşlar, İ.; ERASLAN, G. and ÖZDEMİR, M. (2008): Synergistic action of sodium selenite and N-acetylcysteine in acetaminophen-induced liver damage. *Human & Experimental Toxicology*; 27; 425-429. <https://doi.org/10.1177/0960327108094612>.
- Yousef, H.N. and Aboelwafa, H.R. (2017): The potential protective role of taurine against 5-fluorouracil-induced nephrotoxicity in adult male rats. *Experimental & Toxicologic Pathology*; 69; 265-274. <https://doi.org/10.1016/j.etp.2017.01.012>.
- Zafarullah, M.; Li, W.; Sylvester, J. and Ahmad, M. (2003): Molecular mechanisms of N-acetylcysteine actions. *Cellular & Molecular Life Sciences*; 60; 6-20. <https://doi.org/10.1007/s000180300001>.

## ان-استيل سيستايين فعال في تحسين السمية الكلوية الناجمة عن جرعة منخفضة وليس جرعة كبيرة من 5-فلورويوراسيل في الجرذان

محمد مصطفى البدر ، صباح محمد محمد ، خالد محمد احمد حسانين ، هاله مصطفى البدر ،  
ابتسام صابر عبداللاه ، اسراء عبدالخالق احمد

Email: [vet.sabah.abdelrahman@gmail.com](mailto:vet.sabah.abdelrahman@gmail.com) Assiut University website: [www.aun.edu.eg](http://www.aun.edu.eg)

5-فلورويوراسيل هو دواء شائع الاستخدام للعلاج الكيميائي وله فعالية مثبتة في علاج السرطان البشري. ومع ذلك، فإن الآثار الجانبية الكلوية والكبدية والكلوية تحد من قيمته العلاجية. بحثت هذه الدراسة في التأثير العلاجي ل-ان-استيل سيستايين ضد السمية الكلوية للفلورويوراسيل باستخدامه بجرعتي 20 و 50 ملجم في ذكور الجرذان. ولهذا الغرض، تم تقسيم 40 جرذاً ذكراً إلى 5 مجموعات. كانت المجموعة الأولى بمثابة مجموعة للتحكم والمقارنة بين باقي المجموعات. حقنت المجموعة الثانية 20 ملجم/كجم من الفلورويوراسيل بالحقن داخل الغشاء البريتوني لمدة 6 أيام. حقنت المجموعة الثالثة الفلورويوراسيل لمدة 6 أيام بجرعة 50 ملجم/كجم من وزن الجسم. حقنت المجموعة الرابعة ان-استيل سيستايين 40 ملجم/كجم + 20 ملجم/كجم من الفلورويوراسيل لمدة 6 أيام. بينما حقنت المجموعة الخامسة ان-استيل سيستايين 40 ملجم/كجم + 50 ملجم/كجم من الفلورويوراسيل. تم قياس التقييم الكيميائي الحيوي لليوريا في الدم، والكرياتينين، وحمض البوليك، والألبومين، والسيتوكينات الالتهابية مثل TNF- $\alpha$  و IL-1 $\beta$  ومضادات الأكسدة، ودلائل الإجهاد التأكسدي مثل GSH و MDA. وقد لوحظت السمية الكلوية للفلورويوراسيل من خلال الارتفاع الكبير في جميع الدلائل الكلوية مثل الكرياتينين واليوريا وحمض البوليك و MDA و TNF- $\alpha$  و IL-1 $\beta$  مع انخفاض كبير في مستويات الألبومين و GSH. أدى علاج ان-استيل سيستايين إلى تحسين حالة الكلى بشكل خاص أثناء استخدام 20 مجم 5-الفلورويوراسيل. لذلك، توصي هذه الدراسة باستخدام ان-استيل سيستايين في علاج السمية الكلوية الناتجة عن الجرعة المنخفضة من 5-فلورويوراسيل أفضل من استخدامه مع الجرعة العالية.