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N-acetyl cysteine ameliorates meloxicam induced hepatorenal oxidative stress, inflammation and apoptosis through modulating the levels of caspase-3, Bax and TNF-α pathways in a rat model

Adham Omar Mohamad Sallam1*, Ashraf Abd El-Hakem Ahmed El-Komy1, Enas Abdulrahman Hasan Farag2, Samar Saber Ibrahim3

[1]*Deptartment of Pharmacology, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2] *Department of Pharmacology, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt*

[3] *Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Egypt*

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs), involving meloxicam, are associated with significant hepato-renal toxicity due to their competitive nonelective inhibition of cyclooxygenase enzymes. This study investigates whether N-acetyl cysteine (NAC), with its several pleiotropic effects, such as anti-inflammatory, antioxidant, and anti-apoptosis, could mitigate the hepato-renal toxicity induced by Meloxicam. Fifty albino rats were randomly divided into five groups: a control (saline), a vehicle control (Tween 80), meloxicam-treated, NAC-treated, and a combination treatment group (meloxicam + NAC). The study evaluated biochemical parameters, oxidative stress markers, and histological and immunohistochemical changes in liver and kidney tissues. NAC administration significantly reversed the elevated levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, and creatinine induced by Meloxicam toxicity. Additionally, oxidative stress markers such as malondialdehyde (MDA) were significantly reduced, while antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) showed significant improvement in the NAC-treated groups ($P \leq 0.05$). Histological and immunohistochemical assessment confirmed these findings, showing reduced tissue damage and apoptosis. In conclusion, the NAC protects against Meloxicam-induced hepato-renal toxicity with a significant reduction in oxidative stress and inflammation, supporting its potential therapeutic role in conjunction with NSAID treatment protocols.

1. INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to alleviate inflammation, fever, and pain, treating conditions like rheumatoid arthritis, back pain, and headaches, which helps restore normal tissue structure and organ function (Ungprasert, 2015; Jarrar et al., 2019; Rivera-Velez, 2019). However, NSAIDs can be toxic to vital organs, including the heart, liver, and kidneys, limiting their administration (Ungprasert, 2015; Burukoglu et al., 2016; Jyothi et al., 2023).

The cyclooxygenase (COX) enzyme, particularly COX-1 and COX-2, is implicated in the adverse effects of NSAIDs. COX-1 is consistently present in tissues and is associated with the side effects of NSAIDs due to its role in prostaglandin (PG) production. While COX-2 is activated during tissue damage or inflammation, leading to pain by enhancing nociceptive nerve sensitivity (Uzun et al., 2015; Hernández-Avalos et al., 2020; Jyothi et al., 2023).

The negative side effects of COX-1 selective medications prompted the development of COX-2 selective therapies, such as meloxicam, which exhibits anti-inflammatory, analgesic, and antipyretic properties with a higher affinity for COX-2 at lower doses (Burukoglu et al., 2016; Villalba, 2016). However, at higher doses, meloxicam can inhibit COX-1, leading to potential liver dysfunction, renal failure, cardiotoxicity, and gastrointestinal irritation (Uzun et al., 2015; Jyothi et al., 2023).

Chronic use of NSAIDs can suppress prostaglandins, resulting in nephrotoxicity characterized by acute interstitial nephritis, proteinuria, and acute tubular necrosis (Ingrasciotta, 2015; Macêdo et al., 2019; Jyothi et al., 2022). Moreover, NSAIDs can induce oxidative stress, leading to fulminant liver failure, elevated aminotransferase levels, and hepatitis with jaundice (Sriuttha, 2018; da Silva et al., 2022).

N-acetylcysteine (NAC) possesses pharmacological properties due to its thiol group (-SH), which scavenges free radicals and acts as a precursor for L-cysteine, essential for the synthesis and regeneration of reduced glutathione (GSH), a potent antioxidant (Ommati et al., 2021; Poli et al., 2022; Allam et al., 2022). NAC enhances glutathione levels, detoxifies free radicals, and restores the pro-oxidant/antioxidant balance, exhibiting anti-inflammatory effects by reducing proinflammatory cytokines and inhibiting NF-κB (Gençosman et al., 2022; Korkmaz et al., 2023). Additionally, NAC serves as a mitochondrial protective agent, but the mechanisms underlying its protective effects remain unclear (Galal et

* Correspondence to: amrm50124@gmail.com

al., 2019; Peerapanyasut et al., 2019). Therefore, this study aims to investigate the intracellular pathway of NAC in mitigating meloxicam-induced hepatorenal toxicity by assessing serum biochemicals and tissue oxidative/antioxidant parameters, along with histological and immunohistochemical evaluation of TNF, caspase III, and Bax protein expression across experimental groups.

2. MATERIAL AND METHODS

2.1. Chemicals

N-acetylcysteine (NAC) purchased from EPICO Company, located in 10th of Ramadan City, Egypt. Adwia Pharmaceuticals, located in New Cairo, Egypt, provided meloxicam. NF polysorbate 80 was obtained from Arabcomed Company in Obour City, Egypt, while N-ethylmaleimide was obtained from Sigma-Aldrich Co. In Dokki, Giza, Egypt, Bio diagnostics provided all antioxidant and biochemical assay kits .

2.2. Experimental Animals

The study comprised fifty male albino rats, each weighing 145 ± 7.3 g, obtained from the Laboratory Animal Centre at the Faculty of Veterinary Medicine, Benha University, Egypt. The rats experienced a two-week acclimatization phase before the commencement of the trial. The Ethics Review Committee of the Faculty of Veterinary Medicine at Benha University, Egypt, the experimental design for the study (BUFVTM 14-07-23). Animals in the study were fed conventional laboratory food and had unlimited access to water.

2.3. Experimental Design

Fifty male albino rats were randomly divided into five groups, each containing 10 rats. Group I (Control) received a daily oral dose of saline (the vehicle for NAC). Group II (Vehicle Control) was administered a daily oral dose of a saline solution containing 1% polysorbate 80 (the vehicle for meloxicam). Group III (Meloxicam Toxic Control) received a daily oral dose of meloxicam at a concentration of 1 mg/kg body weight, dissolved in saline with 1% polysorbate 80 (Da Silva et al., 2022). Group IV (N-acetylcysteine) received a daily oral dose of NAC at a concentration of 200 mg/kg body weight, dissolved in saline (Dhillon T. et al., 2022). Group V (Combination Treatment) received both NAC and meloxicam. All treatments were administered orally at 10 AM daily for the 28-day experimental period .

2.4. Sampling

The study involved anesthetizing rats with isoflurane for 24 hours prior to sampling. Blood samples were collected from the retro-orbital plexus into sterile centrifuge tubes, allowed to coagulate at room temperature for 30 minutes, and then centrifuged at 1200 g for 20 minutes. The serum was stored at -20 °C for subsequent biochemical analysis. Following blood collection, the rats were cervical decapitated, and the liver and kidneys were excised, cleaned with physiological saline, and prepared for analysis. To prevent GSH oxidation, each tissue was homogenized on ice with phosphate buffer (pH 7.4), and the homogenates were centrifuged at 1200 × g for 20 minutes at 4°C. Supernatants were collected for oxidative stress biomarker analysis. All rodents and tissue samples were buried in a supervised and sanitary manner after fixation in 10% neutral buffered formalin for 48 hours.

2.5. Serum Biochemical Studies

Analyses of Serum Biochemistry Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Reitman and Frankel, 1957), and alkaline phosphatase (ALP) (Tietz et al., 1983). Also, serum albumin and total protein were measured according to the methods described by Doumas et al. (1971) and Doumas et al. (1981), respectively. Subtracting albumin from total protein allowed us to compute globulin levels. In order to determine kidney function, the levels of serum urea and creatinine were measured using the methodology developed by Coulombe and Favreau (1963) and Bartels et al. (1972). Serum cholesterol was performed by the method described by Meiattini et al. (1978). Estimation of triglycerides and high-density lipoprotein (HDL) was performed as described by Bucolo and David (1973) and Grove (1979), respectively. However, low-density lipoprotein (LDL) level was calculated according to the previous formula of Friedewald et al.(1972) .

2.6. Detection of Oxidative/Antioxidant Cascades

The serum concentrations of catalase activity (CAT) (Aebi, 1984), superoxide dismutase (SOD) (Nishikimi et al. 1972), and malondialdehyde (MDA) (Ohkawa et al., 1979) were measured using diagnostic kits from Bio-diagnostics, Egypt.

2.7. Histological Examination

Liver and kidney specimens from each group were processed in the following steps: fixed in 10% neutral buffered formalin, dehydrated with ethanol at varying percentages, cleared with xylene, and then embedded in paraffin wax. As part of the standard procedure for histological examination, five µm thick slices were cut using a microtome and stained with hematoxylin and eosin (H&E) according to Bancroft et al .(2013) .

Immunohistochemical Studies

Immunohistochemical staining was conducted on 5 µm thick paraffin sections to identify apoptosis and inflammation markers (BAX, Caspase-3, and TNF-α) in liver and kidney tissues. After deparaffinization and rehydration, endogenous peroxidase activity was inhibited with 3% hydrogen peroxide. Antigen retrieval involved heating in citrate buffer at 90°C. Tissues were blocked with 10% bovine serum albumin to prevent nonspecific binding. Specific primary antibodies were utilized from Abcam (rabbit anti-TNF-α, anti-BAX, and anti-Caspase-3) and detected them using a biotinylated secondary antibody and the avidin-biotin complex method. Hematoxylin served as a counterstain, with diaminobenzidine (DAB) used as the chromogen.

2.8. Statistical Analysis

SPSS (version 20.0, SPSS Inc., Chicago, IL, USA) was used to statistically analyze the data. One-way ANOVA, accompanied by Duncan's post hoc test, was employed to compare group means. The data were expressed as (Mean ± S.E), with a P-value < 0.05 deemed statistically significant.

3. RESULTS

3.1. Serum Biochemical Studies

Meloxicam toxicity and NAC treatment effects on biochemical markers of the liver (Albumin, total protein, ALP, ALT, and AST) and kidneys (Creatinine, Urea) in rats were illustrated in table 1. When meloxicam was given

to rats, serum ALT, AST, and ALP activities were significantly ($P \leq 0.05$) higher than in control rats. In the same way, Meloxicam raised the levels of creatinine and urea by a significant amount ($P \leq 0.05$). On the other hand, when meloxicam was given to rats, blood total protein and albumin levels dropped significantly ($P \leq 0.05$) compared to rats that were not given the drug. When N-acetyl cysteine was given with Meloxicam, Serum levels of AST, ALT, and ALP were considerably lower than those observed in the non-treated control group, although they were still higher. Additionally, there was a large rise in serum total protein and albumin levels, as well as a considerable drop in serum urea and creatinine values, which were then almost returned to the comparable parameters in the control group. (Table 1).

Meloxicam administration significantly ($P \leq 0.05$) increases serum cholesterol, triglycerides, and LDL levels with decrease in HDL level (Table 2).

The adverse effect was of meloxicam on lipid profile can be improved by administration of NAC.

3.2. Oxidative/Antioxidant Cascades

In Meloxicam intoxicated rats, the levels of oxidants and antioxidants were elevated, while the levels of SOD and CAT were decreased compared to the control non-treated group. While rats given Meloxicam plus NAC had lower levels of MDA and higher levels CAT in the liver and kidneys compared to the group that was given meloxicam alone (Table 3).

Table 1: Effect of NAC treatment on liver and kidney biochemical tests in Meloxicam intoxicated rats.

Parameters	Control (saline)	Tween 80	Meloxicam	NAC	Meloxicam + NAC
AST (U/L)	37.20±2.46^{c}	34.80±1.80^{c}	131.80±1.46^{a}	35.60±2.20^{c}	78.40±3.08^{b}
ALT (U/L)	33.76±3.84^{c}	29.07±3.56^{c}	140.33±12.14^{a}	29.87±3.57^{c}	63.29±5.09^{b}
ALP (U/L)	174.68±23.41b^{c}	133.68±23.08^{c}	408.99±8.59^{a}	145.34±21.98^{c}	227.72±6.39^{b}
Total protein (gm / dl)	10.32±0.38^{a}	10.15±0.26^{a}	6.05±0.20^{c}	10.08±0.04^{a}	9.00±0.03^{b}
Albumin (gm/dl)	4.14±0.11^{a}	4.36±0.11^{a}	2.29±0.13^{c}	4.07±0.17^{a}	3.67±0.09^{b}
Creatinine (mg/dl)	0.71±0.04^{c}	0.76±0.07^{c}	2.10±0.12^{a}	0.75±0.09^{c}	1.30±0.06^{b}
Urea (mg/dl)	2.27±0.10^{c}	2.39±0.09^{c}	5.22±0.06^{a}	2.45±0.15^{a}	3.33±0.09^{b}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at ($P<0.05$).

Table 2: Effect of NAC on serum Cholesterol, Triglycerides, HDL, and LDL in Meloxicam intoxicated rats.

Parameters	Control saline	Tween 80	Meloxicam	NAC	Meloxicam + NAC
Cholesterol (mg/dl)	138.76 ± 7.40^{c}	144.54 ± 1.53^{c}	231.54 ± 6.43^{a}	136.70 ± 7.93^{a}	166.70 ± 4.05^{b}
Triglycerides (mg/dl)	146.68 ± 13.66^{c}	146.48 ± 11.00^{c}	334.68 ± 9.01^{a}	139.82 ± 5.94^{a}	181.58 ± 4.48^{b}
HDL (mg/dl)	56.94 ± 2.85^{a}	53.28 ± 3.29^{a}	23.00 ± 1.16^{c}	54.14 ± 2.22^{a}	46.06 ± 1.16^{b}
LDL (mg/dl)	52.49 ±7.98^{c}	61.96 ± 2.73^{c}	141.60 ± 6.17^{a}	54.60 ± 8.84^{c}	84.32 ± 4.39^{b}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at ($P<0.05$).

Table 3: Effects of NAC treatment on oxidative stress markers in liver and kidney tissues in rats induced Meloxicam toxicity.

Parameters	Organ	Control saline	Tween 80	Meloxicam	NAC	Meloxicam + NAC
CAT (U/gm)	Liver	531.15 ± 16.93^{a}	531.50 ± 20.68^{a}	229.67 ± 16.46^{c}	520.83 ± 14.80^{a}	462.26 ± 10.16^{b}
SOD (U/gm)	Liver	294.91 ± 15.21^{a}	283.92 ± 11.73^{a}	111.84 ± 7.63^{c}	292.16 ± 13.73^{a}	233.68 ± 20.17^{b}
MDA (nmol/gm)	Liver	129.94 ± 10.05^{c}	130.46 ± 8.85^{c}	362.93 ± 20.78^{a}	121.41 ± 11.30^{c}	185.42 ± 21.61^{b}
CAT (U/gm)	Kidney	540.81 ± 7.56^{a}	551.31 ± 5.96^{a}	343.98 ± 6.12^{c}	540.22 ± 9.50^{a}	466.54 ± 15.19^{b}
SOD (U/gm)	Kidney	219.44 ± 11.58^{a}	222.45 ± 8.86^{a}	99.39 ± 4.77^{c}	212.36 ± 5.97^{a}	185.85 ± 8.50^{b}
MDA (nmol/gm)	Kidney	156.86 ± 12.83^{c}	161.56 ± 6.68^{c}	318.60 ± 7.68^{a}	170.04 ± 5.31^{c}	207.07 ± 16.98^{b}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at ($P < 0.05$).

3.3. Histopathological Results

The histopathological changes of liver and kidney tissues in the experimental groups were illustrated in (Fig. 1). The liver tissues in the non-treated Control group demonstrated a normal hepatic histoarchitecture, with well-maintained hepatocytes, clear sinusoids, and central veins, with no evidence of inflammation, necrosis, or fibrosis. Similarly, the vehicle Control group exhibited a histologically normal liver, without any signs of inflammation, or necrosis. The N-acetylcysteine (NAC) group also showed well preserved intact hepatic architecture with no observable damage. On contrast, the Meloxicam-treated group displayed significant liver injury, characterized by centrilobular necrosis, hepatocellular vacuolization, and inflammatory cell infiltration, along with congested central veins and sinusoidal dilation. These changes indicated marked hepatocellular damage due to Meloxicam. However, the combination treatment (Meloxicam + NAC) group demonstrated a notable reduction in liver damage, with improved hepatocyte preservation, reduced necrosis, diminished inflammatory infiltrate, and decreased sinusoidal dilatation. These findings suggest the hepatoprotective effect of N-acetylcysteine in ameliorating Meloxicam-induced liver injury.

Regarding the Kidney tissue, the untreated control group exhibited normal renal architecture, with well-preserved glomeruli and intact tubular structures, without any signs of inflammation or necrosis. The vehicle control group also showed similar normal kidney histology. The renal architecture remained intact in the N-acetylcysteine (NAC) control group, with no evidence of tubular necrosis, interstitial inflammation, or fibrosis. In contrast, the meloxicam-treated group showed severe kidney damage, including tubular necrosis, glomerular shrinkage, and interstitial inflammation. However, the combination treatment group (meloxicam + NAC) demonstrated a marked reduction in tubular necrosis and inflammation, along with improved glomerular integrity and minimal interstitial cell infiltration, suggesting the protective role of N-acetylcysteine in mitigating meloxicam-induced renal damage.

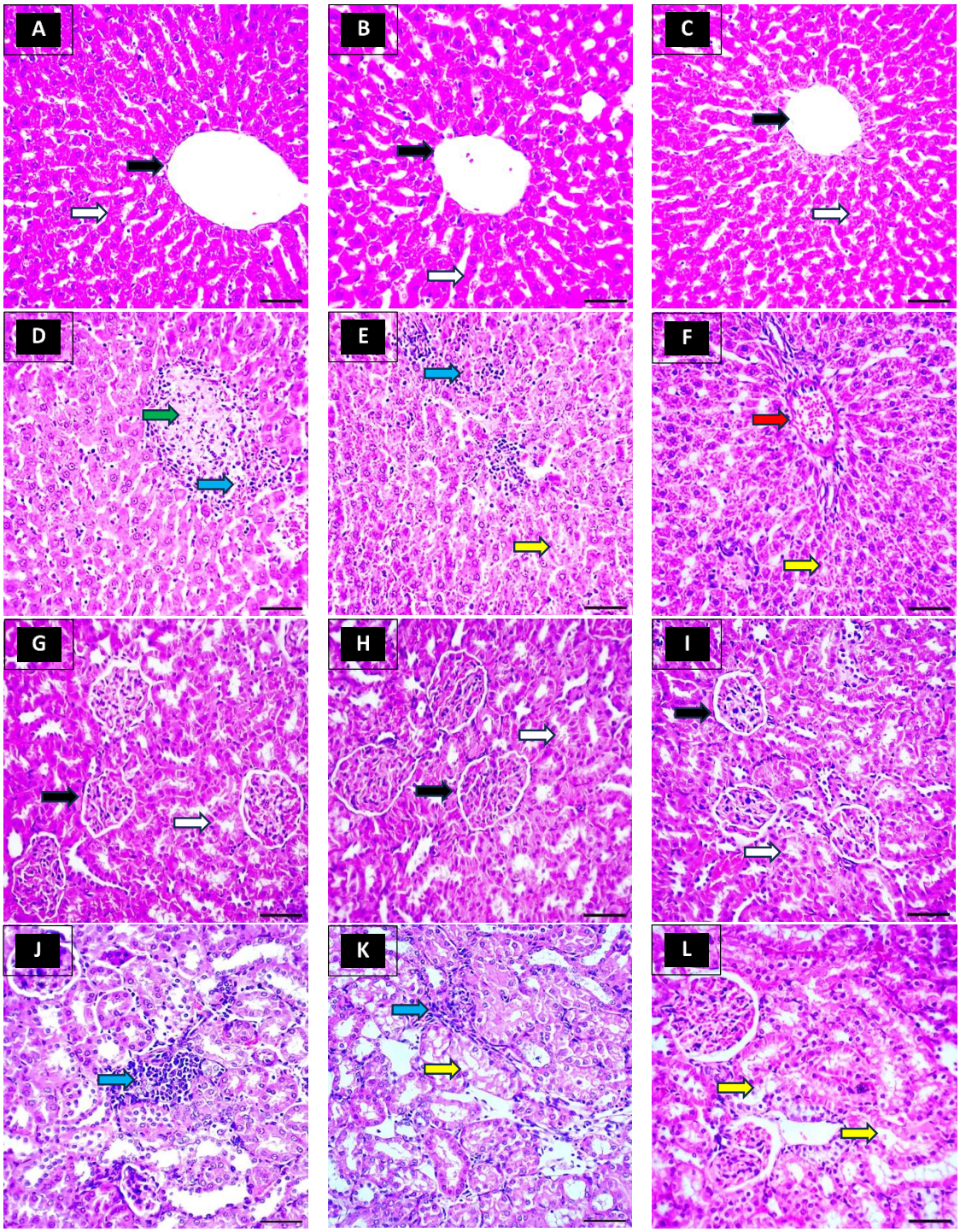

Figure 1 showing the histopathological changes of liver and kidney tissues from various treatment groups. The untreated Control (A, G) and vehicle Control (B, H) groups displaying normal tissue architecture with well-preserved hepatocytes (white arrow) and intact kidney structures (black arrow). The N-acetylcysteine (NAC) (C, I) also exhibiting relatively preserved hepatic architecture and glomerular integrity. In contrast, the Meloxicam-treated group (D, J, E, and K) revealing substantial tissue damage, characterized by hepatocellular as well as renal epithelium degeneration (yellow arrow), necrosis (green arrow), inflammatory cell infiltration (blue arrow) in the hepatic parenchyma as well as in the renal interstitium, and congested portal veins (red arrow). The combination Treatment group (F, L) demonstrates considerable improvement in both liver and kidney histology, showing reduced necrosis (green arrow), inflammation (blue arrow), and sinusoidal congestion.

3.4. Immunohistochemical Results

Figure (2-4) showing the analysis of Caspase-3, Bax, and TNF-α expression through immunohistochemical analysis across the experimental groups. Distinct patterns were associated with tissue damage and treatment effects. In the untreated control group, minimal immunoreaction for Caspase-3, Bax, and TNF-α was observed in both liver and kidney tissues, indicating low levels of apoptosis and inflammation. Similarly, the vehicle Control group exhibited low expression of these markers, consistent with the absence of significant apoptotic or inflammatory activity. The N-acetylcysteine (NAC) group also demonstrated minimal expression of Caspase-3, Bax, and TNF-α, confirming that NAC did not induce apoptosis or inflammatory responses.

The group treated with Meloxicam showed a significant increase in the expression of Caspase-3 and Bax, particularly in areas of tissue damage, indicating heightened apoptotic activity. TNF-α expression was also elevated, reflecting increased inflammation in both liver and kidney tissues. However, in the combination Treatment (Meloxicam + NAC) group, there was a notable reduction in immunoreaction for Caspase-3, Bax, and TNF-α compared to the Meloxicam-intoxicated group, demonstrating a hepatorenal protective effect of N-acetylcysteine in reducing apoptosis and inflammation induced by Meloxicam.

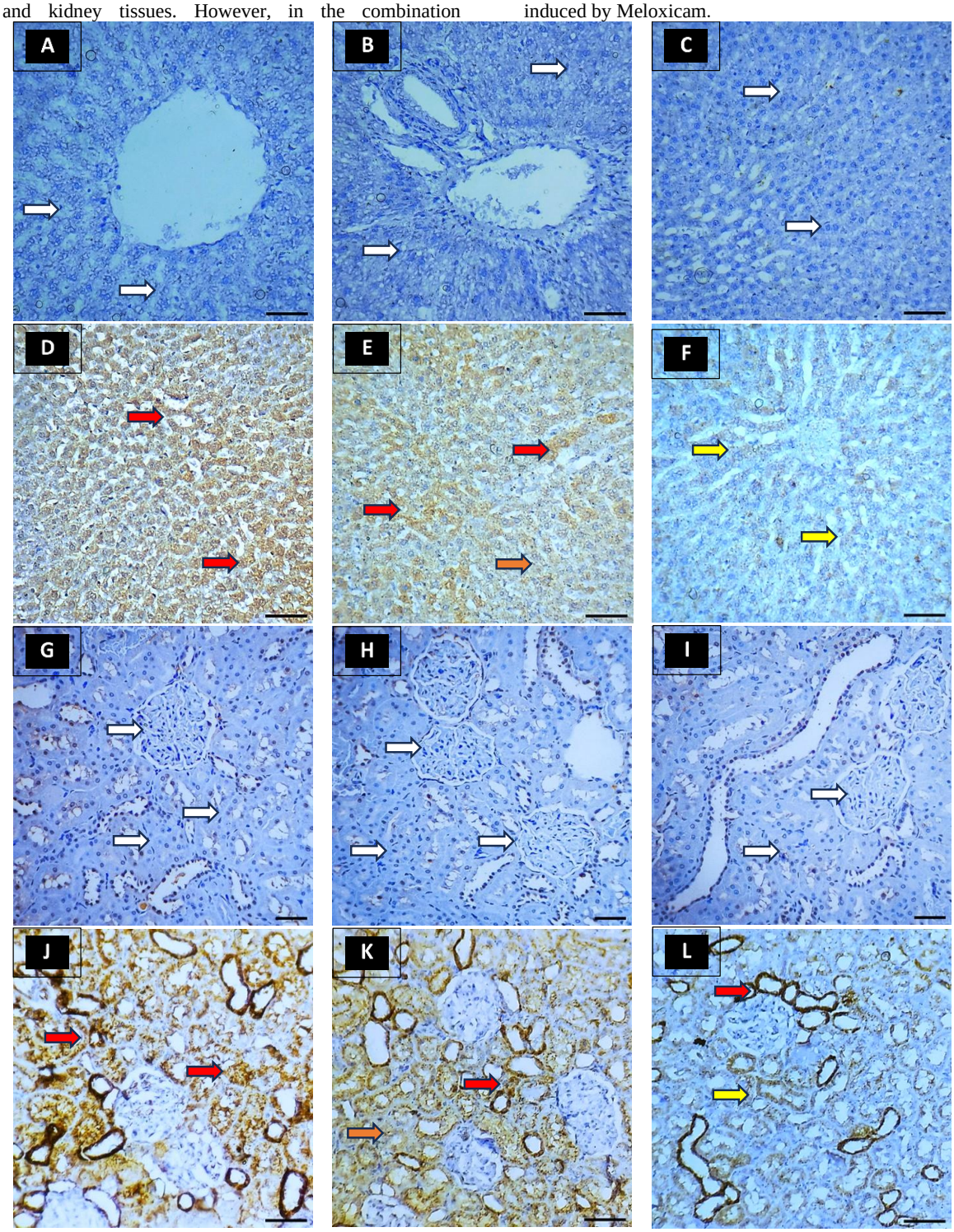

Figure 2 illustrating immunohistochemical staining for Bax in liver and kidney tissues across various treatment groups. The Control (A, G) and Vehicle Control (B, H) groups exhibiting "Negative" Bax expression (white arrow), indicating no detectable activity. In the N-acetylcysteine (NAC) group (C, I), liver tissues remain "Negative" for Bax, while one kidney field showing slight reaction. In contrast, the Meloxicam group (D, E; J, K) displays "Strong" Bax expression (red arrow) in both liver and kidney tissues. The Combination Treatment (F, L) showing "Moderate" Bax expression (orange arrow) in liver tissues and "Weak" expression (yellow arrow) in kidneys.

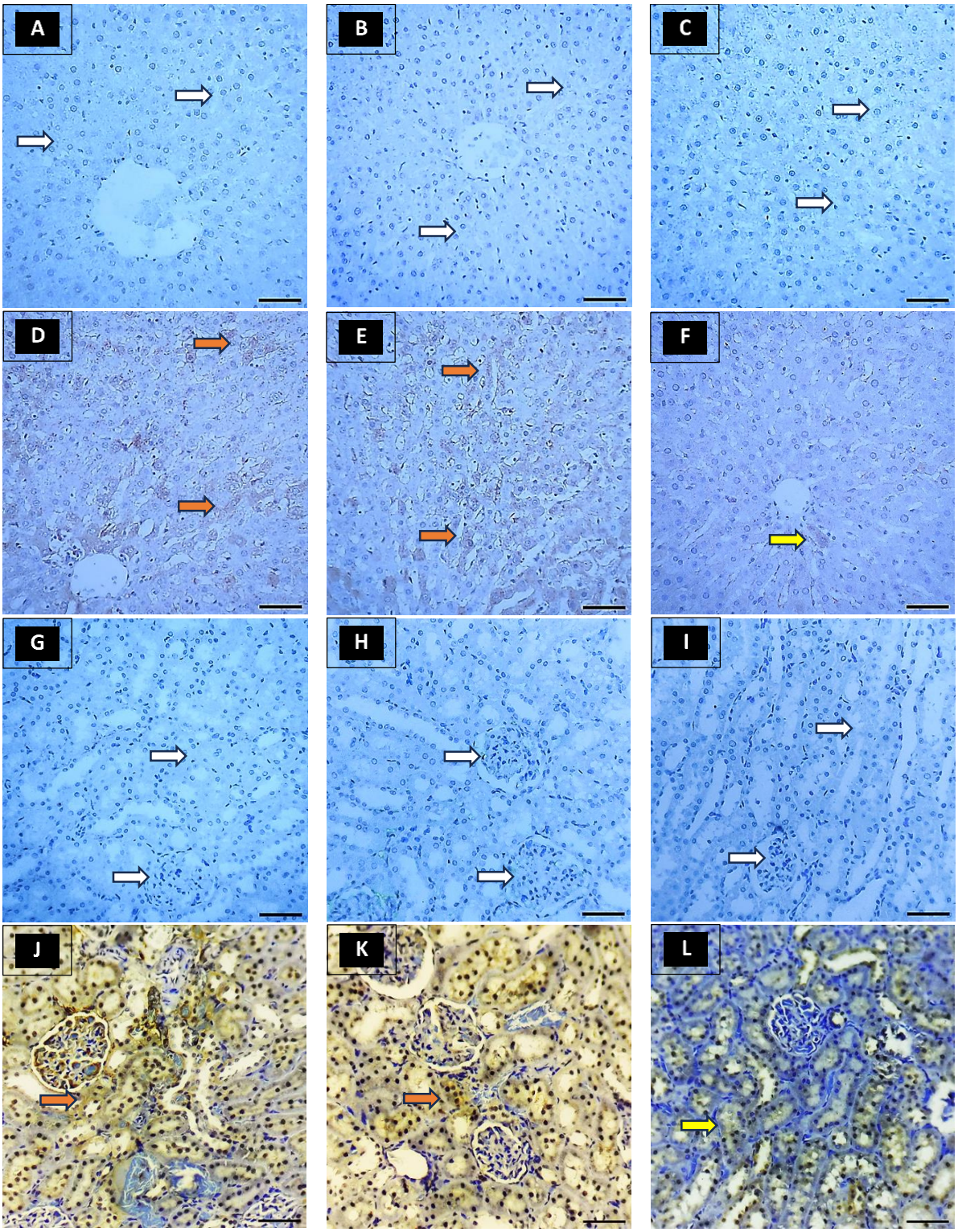

Figure 3. Immunohistochemical staining for TNF-α in liver and kidney tissues across different treatment groups. fig (A) and (G) showing "Negative" TNF-α expression in the control group (white arrow), while fig (B) and (H) illustrating similar results in the Vehicle Control group, indicating no detectable TNF-α activity. In the N-acetylcysteine (NAC) group (C) and (I), TNF-α expression remains "Negative," consistent with the control findings. fig (D) and (E) for liver and (J) and (K) for kidney tissues treated with Meloxicam display a "Moderate" for TNF-α, reflecting a moderate level of expression (orange arrow). In contrast, the combination of Meloxicam and N-acetylcysteine (NAC) (F) and (L) results in a "Weak" for TNF-α in both tissues, demonstrating week expression compared to Meloxicam alone (yellow arrow).

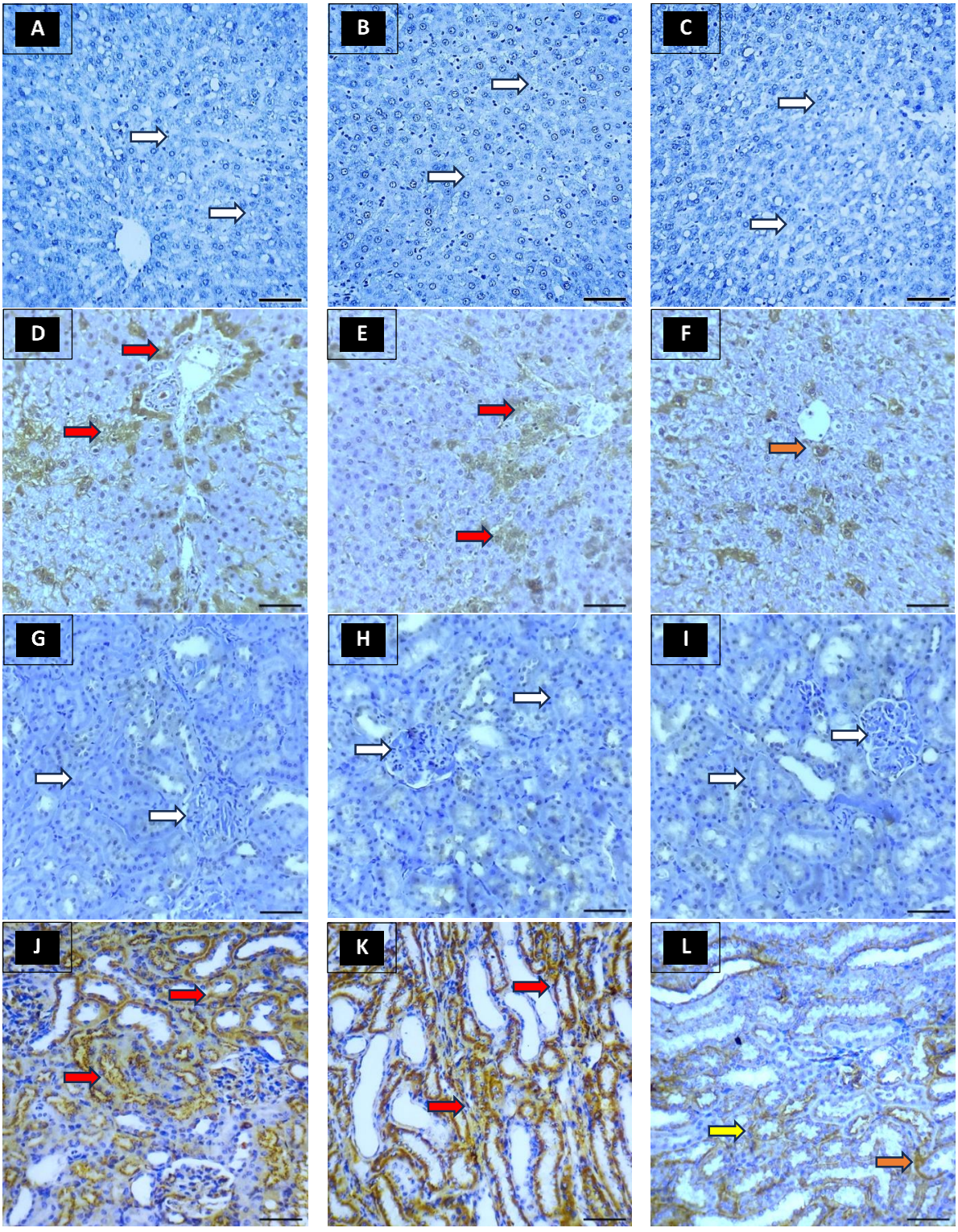

Figure 4 illustrating the immunohistochemical reaction for Caspase-3 in liver and kidney tissues across various treatment groups. The Control (A, G) and Vehicle Control (B, H) groups exhibiting "Negative" Caspase-3 activity, with no detectable activity across all fields. The NAC group (C, I) also shows "Negative" activity in liver tissues, consistent with controls, while kidney tissues remain "Negative". In contrast, Meloxicam treatment (D, E) and (J, K) results in moderate Caspase-3 activation, with most fields exhibiting either "Strong" (red arrow) or "Moderate" (orange arrow) activity. The combination of Meloxicam and NAC (F, L) shows limited Caspase-3 activity in hepatic tissue, while kidney tissues display moderate immunohistochemistry.

4. DISCUSSION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed for managing pain and inflammation (Ungprasert, 2015; Rivera-Velez, 2019). Meloxicam is an NSAID that selectively inhibits COX-2, reducing pro-inflammatory prostaglandin synthesis. Studies indicated that oral meloxicam significantly reduces paw edema and joint inflammation in models of adjuvant-induced arthritis (Crofford et al., 2000) and monosodium urate crystal-induced joint inflammation (Nishida et al., 2004). It also alleviates carrageenan-induced paw edema (Gupta et al., 2007) and decreases lipopolysaccharide-induced inflammatory markers in rat models (Villa-Vázquez et al., 2011). At lower doses, meloxicam primarily inhibits COX-2, but at higher doses, it also inhibits COX-1, which

is associated with adverse effects such as gastrointestinal, renal, and liver toxicity (Villalba, 2016; Jyothi, 2023). Nephrotoxicity can be commonly associated with hematuria, proteinuria, and acute tubular necrosis, as well as acute interstitial nephritis and other rare renal injuries (Ingrasciotta, 2015; Jyothi, 2023). Consequently, the use of meloxicam is limited due to these potential adverse effects (Burukoglu, 2016 ; Ungprasert, 2015).

In the present study, Meloxicam treatment significantly elevated serum levels of liver enzymes (ALT, AST, and ALP), as well as urea, creatinine, cholesterol, triglycerides, and LDL, while reducing total protein, albumin, and HDL compared to controls. These changes normalized with the addition of N-acetyl cysteine (NAC). Meloxicam also increased MDA levels and decreased antioxidant enzymes catalase and superoxide dismutase. In contrast, the Meloxicam + NAC group showed higher GSH and CAT levels and lower MDA levels than the meloxicam-only group, with these biochemical alterations confirmed by immunohistochemical and histopathological analyses.

Oxidative stress plays a significant role in the process of tissue damage. It occurs when an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize or repair their harmful effects arises (Aboubakr et al., 2023a; Aboubakr et al., 2023b; Elsayed et al., 2024). Similarly, in a previous study, meloxicam toxicity was associated with a significant decrease in antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) and a notable increase in (MDA levels, indicating oxidative stress (Abd-Elsalam et al., 2019). Histological examinations revealed hepatic steatosis and degenerative changes in hepatocytes, along with degenerative alterations in renal tubular epithelium due to meloxicam use. Additionally, meloxicam treatment resulted in hepatotoxicity, marked by elevated serum liver enzymes (AST, ALT, and ALP), increased MDA levels, along with necrosis, and inflammatory cell infiltration in the liver and kidneys (Ahmed et al., 2015.(

N-acetylcysteine (NAC), derived from the amino acid L-cysteine, possesses antioxidant properties, reduces inflammation, and protects cells from damage, making it a valuable adjunct in mitigating meloxicam-induced toxicity (Peerapanyasut et al., 2019; Morgan et al., 2023). In the present study, NAC significantly reduced serum levels of liver enzymes (ALT, AST, ALP), urea, and creatinine elevated by meloxicam toxicity. Additionally, oxidative stress markers like MDA were notably decreased, while antioxidant enzymes such as SOD and CAT were increased significantly in the NAC-treated groups ($P < 0.05$). Histological and immunohistochemical analyses supported these findings, as the hepatorenal damage and apoptosis were markedly reduced.

These protective effects of NAC are attributed to its thiol group (-SH), which scavenges free radicals and serves as a precursor for L-cysteine, essential for the synthesis and regeneration of reduced glutathione (GSH), a key antioxidant that protects tissues from reactive oxygen species (ROS) during oxidative stress (Ommati et al., 2021; Poli et al., 2022; Allam et al., 2022).

NAC enhanced cytosolic antioxidant activity, inhibited lipid peroxidation, and reduced antibody formation against oxidative adducts (Ronis et al., 2005). Wong et al. (2003) demonstrated NAC's protective efficacy against hepatotoxicity induced by carbon tetrachloride (CCl_4) and trichloroethylene in rats, where significant reductions in liver enzyme levels were recorded. Moreover, NAC also alleviated azathioprine-induced hepatotoxicity in humans by elevating cytosolic GSH levels (Menor et al., 2004). Furthermore, Maheswari et al. (2014) reported that CBZ produced histopathological alterations that were ameliorated by NAC by increasing glutathione (GSH) levels, reducing lipid peroxidation, and elevating glutathione (GSH). Oxidative stress caused by CBZ's hazardous metabolite may cause its hepatotoxicity.

NAC also has a role as a mitochondrial protective agent for organ preservation, where the mitochondrial protection by NAC has been demonstrated in several in vivo and in vitro study models. Even so, how NAC protects the mitochondria and the mechanisms responsible for its actions are yet unknown (Galal et al., 2019; Peerapanyasut et al., 2019).

The histological and immunohistochemical observations of our current study are in harmony and confirmed the biochemical and oxidant/antioxidant parameter alterations among the experimental groups. The meloxicam-treated group displayed significant liver injury, characterized by centrilobular necrosis, hepatocellular vacuolization, and inflammatory cell infiltration, along with congested central veins and sinusoidal dilation. These changes indicated marked hepatocellular damage due to Meloxicam administration. Regarding the kidney tissue, the meloxicam-treated group showed severe kidney damage, including tubular necrosis, glomerular shrinkage, and interstitial inflammation, along with mononuclear cell infiltration. However, our findings revealed the occurrence of apoptosis and an inflammatory response in Meloxicam-intoxicated rats, as evidenced by increased caspase3, bax, and TNF-α expression levels in the liver and kidney. Treatment with the antioxidant NAC reversed Meloxicam-induced apoptotic responses in hepatic and renal tissue, revealing the anti-apoptotic competence of NAC against Meloxicam-induced tissue injury. The results of the present study agreed with the study of Elsayed et al. (2021) that showed that NAC had a protective effect against nephrotoxicity and hepatotoxicity, indicated by attenuation of the CP-induced degenerative changes in the liver and kidney.

In previous studies, NAC's antioxidant and anti-inflammatory effects are potentially useful in the treatment of various liver and kidney injuries as it prevents tissue damage, enhances endothelial function, improves cellular antioxidant ability, enhances energy metabolism, increases tissue oxygenation, and reduces inflammation, fibrosis, nephrotoxicity, and liver injury (Koc et al., 2021; Gençosman et al., 2022). N-acetylcysteine exhibits its anti-inflammatory effect through the reduction of proinflammatory cytokine synthesis, matrix metalloproteinases, and the inhibition of NF-κB (Gençosman et al., 2022; Korkmaz et al., 2023).

5. CONCLUSIONS

This study highlights the significant protective effects of N-acetyl cysteine (NAC), a compound with anti-inflammatory, antioxidant, and anti-apoptotic properties, against Meloxicam-induced hepato-renal toxicity in rats, evidenced through improvements in biochemical, histopathological, and immunohistochemical markers. NAC administration reduced oxidative stress markers, improved antioxidant enzyme activities (SOD, CAT), restored oxidant/ antioxidant balance, and alleviated liver and kidney damage. It modulated key apoptotic and inflammatory markers, including Caspase-3, Bax, and TNF-α, reducing apoptosis and inflammation. These

findings suggest that NAC may be advised as a promising adjunct therapy with NSAIDs, enhancing the safety of these drugs for a longer protocol regimen. Further research is needed to validate these protective effects in human models, identify optimal dosing regimens, and explore NAC long-term efficacy and safety. This study provides strong evidence for advancing NAC as a potential therapeutic agent to mitigate NSAID-induced toxicity in veterinary and human practices.

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Conflicts of Interest :
The authors declare that no conflict of interest exists among them .

Contributions of the authors :
All authors participated equally to this study, including funding acquisition, project administration, and assistance in writing and revising the publication.

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