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Effect of n-acetyl cysteine and moringa oleifera on acute kidney injury induced by cisplatin in rats.

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- Oxidative stress
- Apoptosis
- Cisplatin
- N-acetylcysteine
- Moringa olifera

Abstract

This study aimed to assess the effects of n-acetylcysteine and moringa oleifera on cisplatin-induced acute kidney injury (AKI) in rats. Forty adult male rats were divided into five groups: Group 1 (control), Group 2 (cisplatin, 6 mg/kg IP on the 8th day), Group 3 (N-acetylcysteine, 200 mg/kg/day orally for 12 days + cisplatin), Group 4 (Moringa oleifera, 300 mg/kg/day orally for 12 days + cisplatin), and Group 5 (combined N-acetylcysteine and Moringa oleifera + cisplatin). Blood samples were collected for biochemical analysis, and renal tissue was examined for oxidative stress markers, caspase 3, and aquaporin 2 expression. Results revealed marked histopathological damage and increased caspase 3 expression in Group 2. N-acetylcysteine and Moringa oleifera treatments reduced this damage, with the best outcome in Group 5. Aquaporin 2 expression decreased significantly in Group 2 but improved in the treated groups, particularly Group 5. Serum creatinine, urea, and potassium levels increased significantly in Group 2, whereas treatment with N-acetylcysteine and Moringa oleifera significantly reduced these levels, with the best results in Group 5. Creatinine clearance decreased in Group 2 but improved with treatment, especially in Group 5. Serum KIM-1, serum NGAL, and urine NGAL were elevated in Group 2 and reduced in the treated groups, with the best outcome in Group 5. Oxidative stress markers improved in Groups 3, 4, and 5, with the best results in Group 5. A negative correlation was observed between serum KIM-1 and aquaporin 2. In conclusion, N-acetylcysteine and Moringa oleifera may protect against cisplatin-induced AKI.

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Introduction

Cisplatin is a widely used, potent and effective chemotherapeutic agent. which is used to treat solid tumors such as head, neck, colon, cervix, ovaries, and testicular germ cell tumors. About 20% of cancer patients receive cisplatin therapy. However, because of several adverse effects on different organs, such as the liver, kidneys, inner ear, peripheral nerves, testes, and GIT, its clinical utility has diminished. The kidney's high mitochondrial density makes it more susceptible to cisplatin toxicity than other tissues because it absorbs the drug at higher concentrations than other tissues. Through its conjugation with glutathione, cisplatin can block antioxidant enzymes, resulting in an excess of reactive oxygen species (ROS) in the kidney. Ultimately, ROS causes mitochondrial dysfunction**[1]**.Cisplatin is correlated to high incidence of AKI. Even with just one dose of cisplatin, there is a risk of having AKI.30% of patients treated with cisplatin develop acute kidney injury (AKI) which is *a* global health problem with high morbidity and mortality rates. Even patients that do not develop AKI are at risk long-term decline in renal functions and development of chronic kidney disease**[2].**The cisplatin-induced AKI model is a well-established animal model used to investigate the pathogenesis of AKI**[3].**cisplatin nephrotoxicity involves intracellular stresses including DNA damage, mitochondrial pathology, oxidative stress, endoplasmic reticulum stress, apoptosis, and inflammation. All have key roles in the pathogenesis of cisplatin nephrotoxicity, So, it is essential to minimize the potential side effects induced by cisplatin via co-administration with effective antioxidants that can inhibit free radical generation and apoptosis**[4].**Aquaporins (AQPs) are membrane water channels, with at least 13 aquaporin isoforms (AQP 0 to 12). Among them, AQP1 and AQP2 have been identified in the kidneys, Aquaporin-2 is expressed mainly at the apical membrane and intracellular vesicles in the collecting duct principal cells **[5].**Previous studies using rats have indicated that the renal expression of AQP2 is decreased by treatment with cisplatin, release of AQP2 was decreased dramatically at 24 hours of cisplatin treatment, and the decrease was maintained during the experimental period. This data suggests that AQP2 can be used to detect early renal impairment due to cisplatin**[6].** N-acetylcysteine (NAC), a precursor of glutathione, has been used as a mucolytic drug for treating cystic fibrosis, used to treat acetaminophen poisoning also used for patients with chronic diseases with decrease the glutathione while increasing oxidative stress (like the alcoholic liver disease)**[7].** The use of antioxidant and anti-inflammatory drugs to minimize cisplatin-induced nephrotoxicity has gained considerable interest. NAC has a well-documented antioxidant and anti-inflammatory activity **[8].**Moringa oleifera plant has many medical applications, such as antifungal, antioxidant, anti-inflammatory, hepatoprotective, and neuroprotective effects**[9].**So, this study aims to assess the role of n-acetylcysteine and moringa oleifera treatment in acute kidney injury induced by cisplatin chemotherapy and to assess the possible involvement of renal aquaporin 2.

MaterialsandMethods

Experimental

Animals:

This study was conducted on forty adult (8 to12 weeks old) male Sprague Dawley rats of average weight, (200 – 250 g) housed in the animal house of Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University. Animals were kept in controlled environmental conditions. The Animal care and ethics committee of Mansoura faculty of medicine approved our experimental protocol. Code number: MU-ACUC(MED.MS.24.01.25).

Drugs and chemicals:

Cisplatin ampoules[1 mg/ml] was purchased from Mylan Pharmaceuticals, France.

N-acetylcysteinepowderwas purchased from Sigma Drugs-Aldrich (St. Louis, MO, USA).

Moringa oleifera leaves powder extract were purchased from moringa sales at the National Research Centre (NRC), Egypt.

Experimental model of cisplatin-induced acute kidney injury(AKI):

AKI was induced by a single intraperitoneal (IP) injection of cisplatin (6 mg/kg) on the 8th day of the experimental period which lasted for 12 days**[10].**

Experimental Groups:

Forty adultmale Sprague Dawleyrats were divided into 5main groups with 8ratsineachgroup.Group1: received1 mL of 0.9%saline and 1 mL of 2% carboxymethylcellulose orally for 12 consecutive days. CMC was used as a vehicle for the experimental drug in other groups, and it was administered to the control group to ensure that all groups received the same volume and formulation, thus eliminating any effects that could result from the vehicle itself.

Group 2: received 0.9 % saline orally for 12 consecutive days and only a single IP injection ofcisplatin (6 mg/kg) on the 8th day**[10].**Group 3: received n-acetylcysteine (200 mg/kg/day) dissolved in 0.9 % saline orally for 12 days, and only single IP injection ofcisplatin on the 8th day**[11].**Group 4: received moringa oleifera (300mg/kg/day) dissolved in 0.9 % saline orally for 12 days, followed by single IP injection of cisplatin on the 8th day**[12]**.Group 5: received n-acetylcysteine(200 mg/kg/day) and moringa oleifera(300mg/kg/day) dissolved in 0.9 % saline orally and single IP injection ofcisplatin on the 8th day.

Bythe end of 12th day for each group, the following investigations were done:

Blood samples collection and obtaining samples of renal tissue:

By the end of the study, rats were euthanized by IP injection of overdose of thiopental sodium at a dose of 800 mg /kg. Blood samples were obtained by puncturing the heart after the rats were sedated, put on a surgical board, and cleaned with ethanol. The abdomen of rat was then opened by making an incision in the center of the xiphoid process. Rats were perfused via inferior vena cava with normal saline then kidney was extracted from the abdomen. Blood samples were collected via cardiac puncture because it allows for the collection of a larger volume of blood with minimal hemolysis, which is important for accurate biochemical analysis. Although the inferior

vena cava was used for saline perfusion to clear the circulatory system before tissue collection, cardiac puncture was performed immediately before hand under deep anaesthesia to ensure proper sample quality without compromising animal welfare. Serum was obtained by centrifugation ofblood at 2000 rounds per minute for 10 minutes. After that, sera were frozen and kept at-20ºC for biochemical analysis. One kidney was kept in formalin (10%) for histopathological and immunostaining investigations while the other kidney was kept in liquid nitrogen to measure oxidative stress markers. After necropsy, syringes were put in safety boxes and all carcasses were placed in body bags and labelled with following information ; IACUC method used to ensure death, date and initials of person disposing the carcass. After euthanasia , animals were sent to the incinerator.

Biochemicalassay:

Serum creatinine, creatinine clearance ,serum urea , and serum potassium levels:

Using commercial kits from Bio-diagnostic, Egypt (CAT. No. CR 12 50) for creatinine, (CAT. No. UR 21 10) for urea,(CAT. No. K298.001)for potassium. serum levels of creatinine, urea and potassium were determined in accordance with instructions of manufacturer.

Fractional excretion of sodium (FENa%):

Using commercial kits from Bio-diagnostic, Egypt (CAT. No. CR 12 50) for creatinine and(CAT. No. Na 303 001) for sodium.

Serum KIM-1 assay:

Using rat KIM 1 ELISA kit purchased from EAGLEBIOSCIENCES(USA)CAT#RKM29-K01, levels of serum KIM 1 were determined under instructions of manufacturer.

Serum and urine NGAL assay:

Using a rat NGAL ELISA kit purchased from EAGLE BIOSCIENCES (USA) CAT# ST001, levels of serum and urine NGAL were determined according to the manufacturer's instructions.

Assessment of markers of oxidative stress in renal tissue:

A small part of the renal tissue was homogenized in a solutionof 50 mMole potassium phosphate, 1mM EDTA, pH7.5 and aliquoted into several ependorffs. Malondialdehyde (MDA), a lipid peroxidation marker, and reduced glutathione (GSH), an antioxidant, were assessed using colorimetric kits from Bio-Diagnostics , Egypt, in accordance with instructions of manufacturer. (CAT. No. MD 25 29) for MDA and company (CAT. No. GR 25 11) for GSH.

Histopathologicalexaminationand immunohistochemistry for caspase 3 and Aquaporin 2 :

Kidneyswere washed with saline andthen fixed in 10% formalin solution. After that, renal tissues wereembedded in paraffin, sectioned, stained with hematoxylin and eosin (H&E) and then examined by light microscope for histopathological evaluation. Deparaffinization, rehydration, washing, immersion in 3% H2O2, pepsin digestion for antigen retrieval, and overnight incubation with rabbit monoclonal antibody class IgG were all perform tissue sections. Diaminobenzidine/ peroxidase substrate produced a brown-coloured signal. Phosphate buffered solution was used to replace primary antibody and adjacent sections were used as negative control. Caspase 3 and

aquaporin 2 were quantified by calculating the percentage of renal area occupied by positive staining by use of image j software.

Computer Assisted digital image analysis (Digital morphometric study):

Olympus® digital camera installed on an Olympus® microscope with 1/2 X photo adaptor was used to photograph our slides, using 40 X objective. Image analysis was performed by image j software.

Statisticalanalysis:

Data was entered and statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 23.Quantitative data was described as means(SD)after testing for normality by Shapiro-Wilk test. One way ANOVA (analysis of variance) test, with LSD post-hoc multiple comparisons, was used for comparison between groups.Correlation between parametric continuous variables was done using Pearson's correlation. "p value ≤0.05" was considered to be statistically significant.

Results

Biochemicalanalysis:

Table (1) showed a significant elevation in the serum levels ofcreatinine, urea ,potassium , KIM-1, NGAL and urine NGAL in cisplatin group in comparison to control group($p< 0.05$). Also, cisplatin+ n-acetylcysteine and cisplatin+moringa oliferagroups displayed a significant increasein these parameters comparedtocontrol group ($p< 0.05$). These parameters decreasedwith moringa olifera , n-acetylcysteine and combinedgroups respectively in comparison to cisplatin group ($p < 0.05$).n-acetylcysteine and combined groups showed a decline inthese parameters which are better than moringa olifera group ($p<0.05$). Combined group demonstrated a significantdecline inthese parameters compared to moringa olifera group and n-acetylcysteine group separately ($p < 0.05$).As regard creatinine clearance, There is a significant decrease in in cisplatin group in comparison to control group($p< 0.05$).creatinine clearance increasedwith moringa olifera , n-acetylcysteine and combinedgroups respectively in comparison to cisplatin group ($p < 0.05$).n-acetylcysteine and combined groups showed an increase in creatinine clearance inwhich are better than moringa oliferagroup ($p<0.05$).Combined group demonstrated a significant increase in creatinine clearance compared to moringa olifera group and n-acetylcysteine group separately ($p < 0.05$).As regard FENa% , in cisplatin group was >2% indicating that AKI due to cisplatin is typically intrinsic type.FENa% decreased with moringa olifera , n-acetylcysteine and combined groups respectively in comparison to cisplatin group ($p < 0.05$).

Table 1: Values of serum creatinine, creatinine clearance, blood urea nitrogen (BUN), serum potassium , FENa% , serum KIM-1 , urine NGAL and serum NGAL among study groups.
F: One Way ANOVA test. *****Statistically significant P value < 0.05
a significant with the control group
b significant with the cisplatin group
c significant with the NAC group
d significant withthe Mo group
e significant with the NAC+Mogroup

Measure Mean(SD)	Control	Cisplatin	NAC	Mo	NAC+Mo	Test of significance ANOVA
Serum Creatinine (mg/dl)	0.513 ±0.06 bcd	1.79 ±0.38 acde	0.754 ±0.046 abde	1.02 ±0.16 abce	0.593 ±0.09 bdc	F=56.38 P=0.001*
Creatinine Clearance (ml/min)	1.153 ±0.096 bcde	0.449 ±0.08 acde	0.909 ±0.06 abde	0.709 ±0.214 abce	1.05 ±0.014 abcd	F=19.10 P=0.001*
Serum Urea (mg/dl)	25.85 ±4.52 bcd	100.0 ±13.63 acde	39.10 ±6.88 abde	68.49 ±15.09 abce	27.29 ±1.96 bcd	F=76.05 P=0.001*
Serum Potassium (mmol/l)	3.96 ±0.44 bcd	7.56 ±0.39 acde	5.21 ±0.48 abde	6.36 ±0.55 abce	4.58 ±0.56 bcd	F=69.44 P=0.001*
FENa %	0.825 ±0.08 bcd	2.19 ±0.35 acde	1.17 ±0.31 abd	1.49 ±0.42 abce	0.974 ±0.18 bd	F=26.87 P=0.001*
Serum KIM 1 (mg/dl)	0.400 ±0.019 bcd	2.17 ±0.29 acde	1.08 ±0.24 abde	1.59 ±0.11 abce	0.510 ±0.12 bcd	F=130.71 P=0.001*
Urine NGAL (ng/ml)	65.21 ±14.63 bcde	165.51 ±15.21 acde	93.55 ±18.02 abde	123.88 ±34.95 abce	82.66 ±17.44 abcd	F=27.11 P=0.001*
Serum NGAL (ng/ml)	34.29 ±15.71 bcde	122.93 ±12.62 acde	49.52 ±10.59 abde	87.60 ±32.15 abce	44.04 ±11.78 abcd	F=32.24 P=0.001*

Oxidativestressmarkers:

Table (2) showed that cisplatin significantly reduced GSH level and elevated MDA level compared to the control group indicating thatoxidative stress state wasworsened. Both n-acetylcysteine and moringa oliferatreated rats showed a significantly elevated GSH level and significantly reduced MDA level respectively compared to cisplatin group. Combined group significantly improved oxidative state observed by significant elevationofGSHlevelandsignificantreduction of MDA level compared to cisplatin group, n-acetylcysteine group and moringa olifera group.

Table2:Values of markersoftissueoxidativestress;malondialdehyde(MDA),andreducedglutathione (GSH) among study groups.

Measure Mean(SD)	Control	Cisplatin	NAC	Mo	NAC+Mo	Test of significance ANOVA
MDA (nmol /g tissue)	30.37 ±6.67 bcd	76.66 ±14.63 acde	43.77 ±6.36 abd	55.41 ±3.58 abce	36.61 ±8.12 bcd	F=35.28 P=0.001*
Reduced Glutathione (nmol /g tissue)	2.09 ±0.086 bcde	0.688 ±0.18 acde	1.54 ±0.12 abde	1.19 ±0.12 abce	1.87 ±0.08 abcd	F=160.31 P=0.001*

F: One Way ANOVA test. *****Statistically significant P value < 0.05
a significant with the control group **b** significant with the cisplatin group
c significant with the NAC group **d** significant with the Mo group
e significant with the NAC+Mogroup

Histopathologicalexamination:

Figure(1,2)represents the result of histopathological examination of the kidney, with normal renal tissue with intactgglomeruli and tubules in control group. cisplatin group showed marked tubular necrosis with desquamated epithelial cells, vacuolation, tubular dilatation and hyaline cast. cisplatin + n-acetylcysteine group demonstrated normal structure of most of renal tubules with few scattered hyaline casts indicating decrease of structural damage compared to cisplatin group . cisplatin+ moringa oleifera group showed reduction of structural damage compared to cisplatin group by minimal hyaline cast and minimal vacuolation. Combined group(cisplatin + n-acetylcysteine + moringa oleifera) demonstrated normal structure of glomeruli and tubules ,no hyaline cast and absence of epithelial degeneration and that results indicate best outcome by combination of n-acetyl cysteine andmoringa oleifera**.**

Immunohistochemistry for renal caspase3:

Figure (3,4) showed a significant increase in level of caspase 3 in cisplatin group compared to control group as well as to all other treated groups ($p < 0.05$). Level of caspase 3 in cisplatin&moringa oleifera group showed significant increase compared to control and significant decrease compared to cisplatin group($p<0.05$).In cisplatin& n-acetylcysteine group, level of caspase 3 showed significant increase compared to control and significant decrease compared to cisplatin group andcisplatin&moringa oleifera group.Caspase3 levelin combined group showed significant decrease compared to groups (2,3,4) ($p < 0.05$) also significant increase compared to group 1 .

ImmunohistochemistryforrenalAquaporin2:

Figure (5,6) showed a significant decrease in level ofAquaporin 2 in cisplatin group compared to control group as well as to all other treated groups ($p < 0.05$). Level of Aquaporin2 in cisplatin&moringa oleifera group showed significant decrease compared

to groups (2,3,5) and significant increasecomparedtocisplatingroup($p<0.05$).In cisplatin&n-acetylcysteinegroup, level of Aquaporin 2 showed significant decrease compared to control and significant increase compared to cisplatin group and cisplatin &moringa oleifera group. Aquaporin 2 level in combined group showed significant increase compared to group (2.4) ($p < 0.05$) , significant decrease compared to group 1 and non-significant increase compared to group 3.

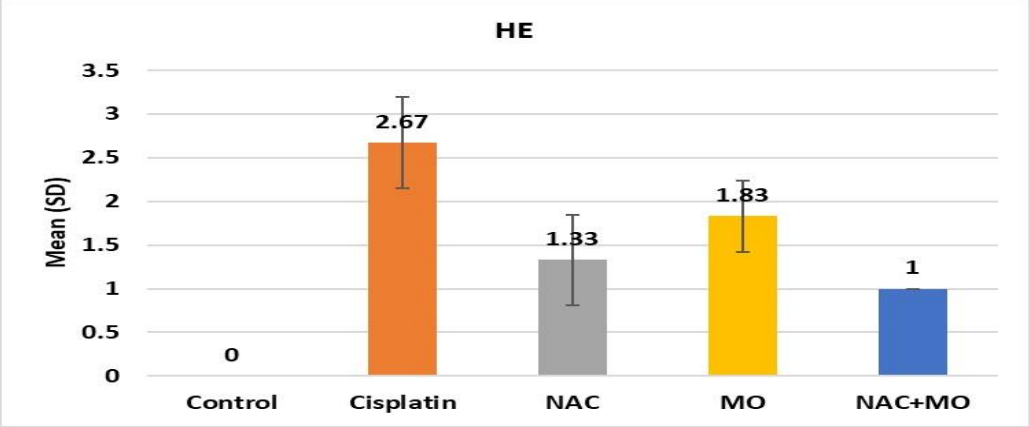
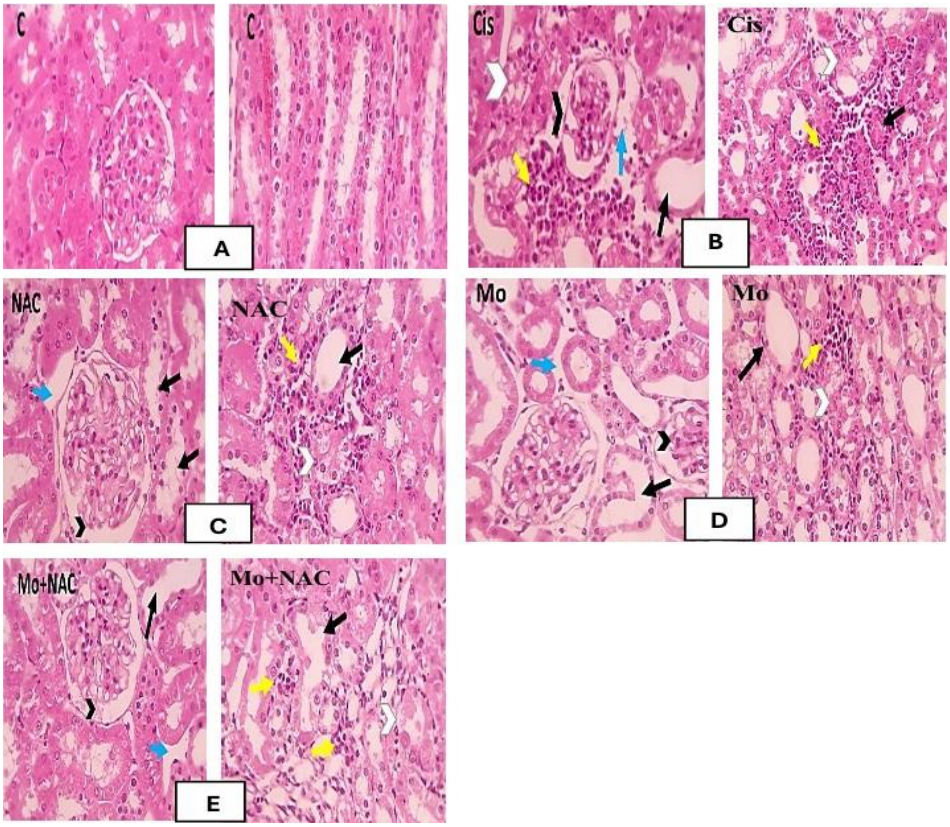


Figure (1): bar chart showing Histopathologicalscoring of the renal tissue damage by Hematoxylin & Eosin between studied groups.



Figure(2): Representative pictures**(A,B,C,D,E)** of the H&E-stained renal sections examined at highmagnifications (x400 Scale bar 50 µm)in different groups: (A) control group with normal renal structure and intact glomeruli and tubules.(B) cisplatin group with marked tubular necrosis desquamated epithelial cells, hyaline cast and vacuolation. (C) cisplatin+ n-acetylcysteine group showed normal structure of most of tubules with few scattered hyaline casts.(C) cisplatin+ moringa oleifera group showed tubular necrosis with minimal hyaline cast. (E)) cisplatin +n-acetylcysteine +moringa oleifera group restored normal renal structure with very few hyaline casts and without tubular vacuolation.

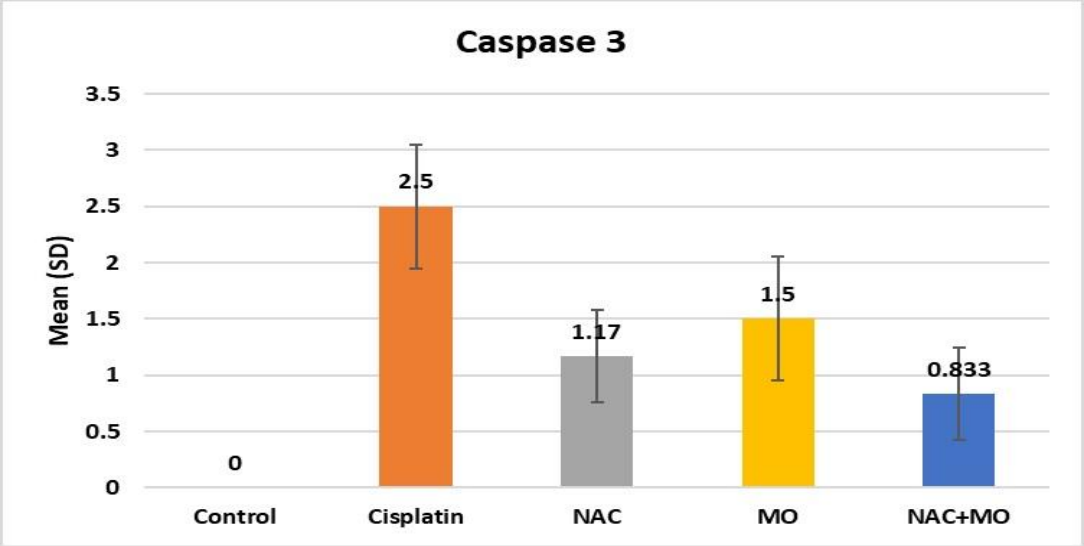
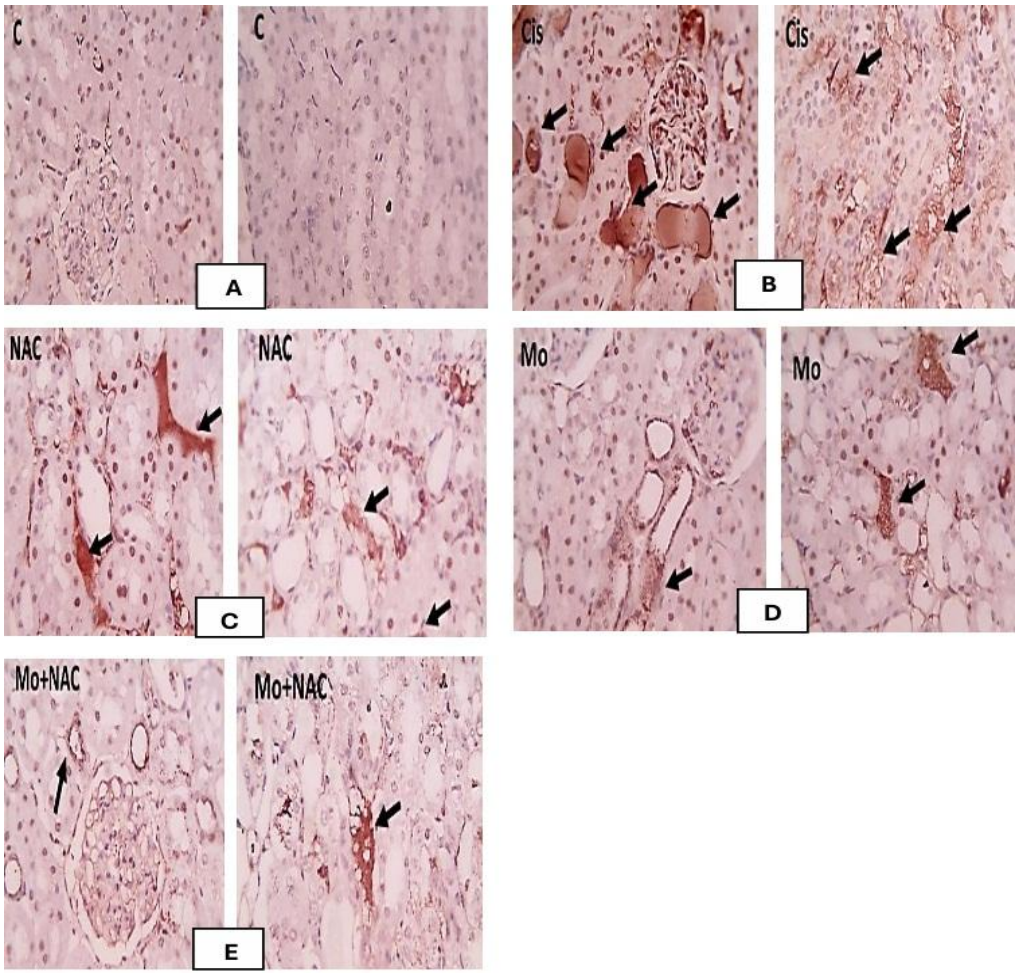


Figure (3): bar chart showing caspase 3 density between studied groups



Figure(4): Representative pictures**(A,B,C,D,E)** of Immunohistochemical staining of caspase 3 in renal sections examined at highmagnifications (x400 Scale bar 50 µm)in different groups: (A) control group showed no expression of caspase 3.(B)cisplatin group without treatment showed a significant increase in caspase 3 immunoreactivity.(C) cisplatin+ n-acetylcysteine group showed moderate reduction in caspase 3 immunoreactivity. (D) cisplatin+ moringa oleifera group demonstrated mild reduction in immunoreactivity. (E)combined group demonstrated marked reduction in caspase 3 immunoreactivity. Brown colour indicates caspase 3 positivity.

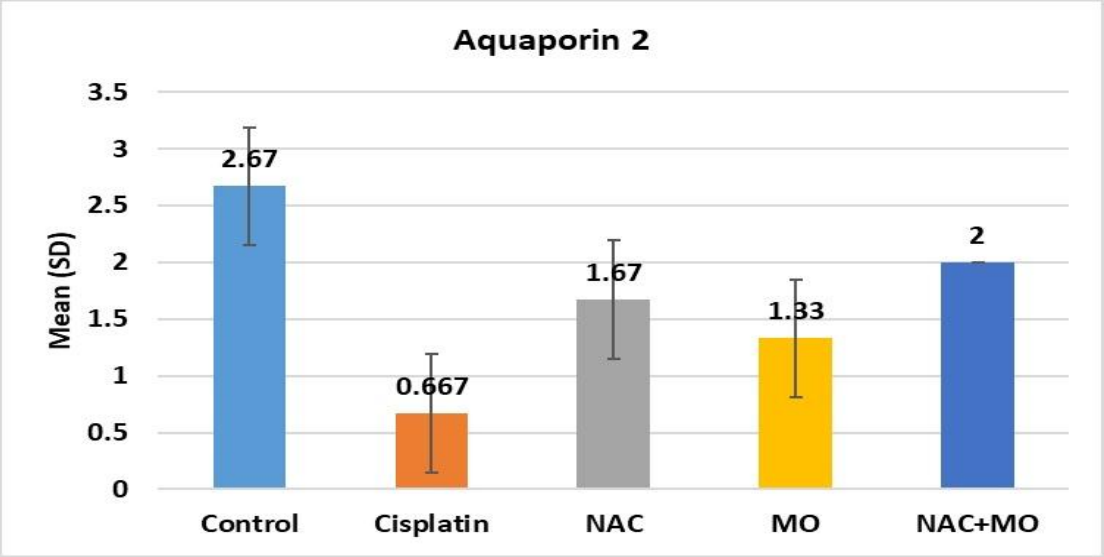
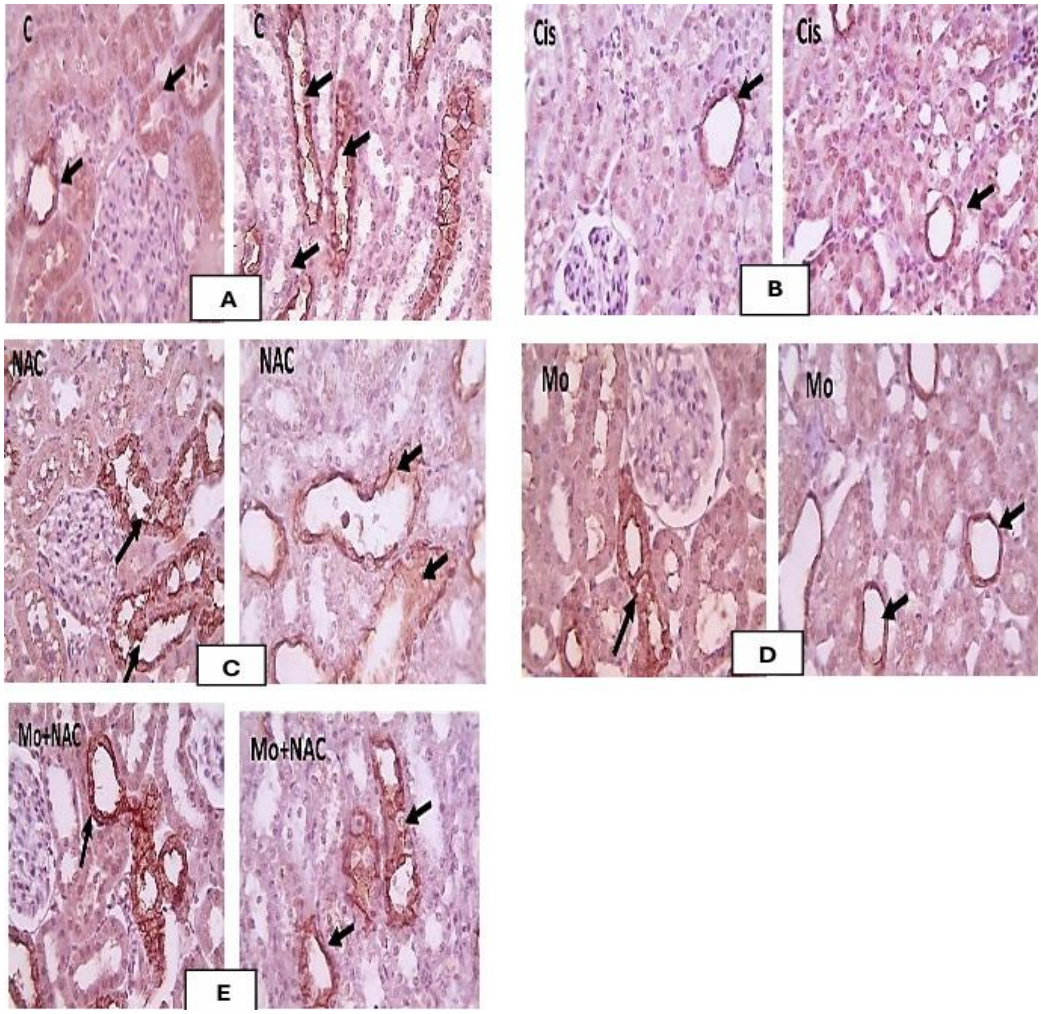


Figure (5): bar chart showing aquaporin 2 density between studied groups



Figure(6): Representative pictures**(A,B,C,D,E)** of Immunohistochemical staining of Aquaporin-2 in renal sections examined at highmagnifications (x400 Scale bar 50 µm)in different groups: (A) control group showed marked positive brown tubular expression.(B)cisplatin group without treatment showed a few positively stained tubules .(C) cisplatin+ n-acetylcysteine group showed moderate increase in positively stained tubules.(D) cisplatin+ moringa oleifera group showed mild increase in positively stained tubules.(E)combined group showed marked increase in positively stained tubules.

Correlation between KIM-1andAquaporin2:

Table(3)strong, significant and negative correlation between KIM-1 and aquaporin2, this correlation is in harmony with bio-chemical and histo-pathological results.

Table(3):correlation between KIM-1andAquaporin2 among studied groups

correlation between	Control	Cisplatin	NAC	Mo	NAC+Mo
KIM1& Aquaporine2	r= -0.906 p=<0.002*	r= -0.964 p=<0.0001*	r= -0.962 p= 0.0001*	r= -0.873 p= 0.005*	r= -0.933 p= 0.001*

r: Correlation coefficient, *statistically significant (p<0.05ineachcorrelationindicates significance)

Discussion

This study is considered a step forward in identifying an effective defense against cisplatin-induced AKI. It explored the effect of combining N-acetylcysteine (NAC) and Moringa oleifera (MO) to determine whether the combination offers greater protection against cisplatin-induced AKI compared to their individual effects. To the best of our knowledge, this is one of the earliest studies to evaluate this combined approach.

The current study confirmed the nephrotoxic effect of cisplatin, as the cisplatin group showed impaired renal function, indicated by significant increases in serum creatinine and BUN levels compared to the control and treated groups. These findings can be attributed to cisplatin's direct toxic effects on renal tubular cells and glomeruli, including thickening of the basement membrane and foot process effacement, along with indirect effects such as inflammation in the renal interstitium, all contributing to acute renal damage**[13].**

NAC significantly decreased serum creatinine, BUN, and potassium levels and increased creatinine clearance, although these values remained significantly different from the control group. These results align with earlier studies reporting NAC's antioxidant, anti-inflammatory, and anti-apoptotic effects**[14].**

MO also significantly improved serum creatinine, BUN, and potassium levels and increased creatinine clearance, although values remained significantly altered compared to controls. These improvements are consistent with previous findings attributing MO's nephroprotective effects to its antioxidant and anti-inflammatory properties**[15]**.However, MO alone did not restore renal function to control levels.

The combination group showed a significant reduction in serum creatinine, BUN, and potassium and a significant increase in creatinine clearance compared to the cisplatin group. The combination was more effective than MO alone and not significantly different from the control group for most parameters except creatinine clearance. This finding supports the view that creatinine clearance is a more accurate indicator of renal function.

Fractional Excretion of Sodium (FENa) is a valuable diagnostic tool for differentiating between prerenal and intrinsic Acute Kidney Injury (AKI). A FENa <1% typically indicates prerenal AKI, suggesting decreased blood flow to the kidneys, whereas a FENa >2% suggests intrinsic AKI, indicating damage to the renal

parenchyma. In the context of cisplatin-induced nephrotoxicity, a FENa >2% is consistent with intrinsic renal injury, highlighting the importance of FENa in guiding diagnosis and management strategies for AKI**[16].** Although FENa can be influenced by multiple factors including volume status, diuretic use,underlying kidney disease and other diagnostic tests, such as serum creatinine, urine output**[17].**

KIM-1 is a transmembrane protein located in the proximal tubule's apical membrane, has a higher sensitivity and specificity than blood creatinine when it comes for detecting and tracking early proximal tubule failure brought on by nephrotoxic drugs like cisplatin. According to some research, increased KIM-1 levels may be predictive that they may occur before histological alterations in AKI. Its level is highly correlated with the minimal degree of renal tissue damage found in healthy tubules. Through the NF-κB pathway's activation, elevated KIM-1 levels are linked to inflammation**[18].**

Ourresults showed that cisplatincausedasignificantelevationofKIM-1in the cisplatingroup comparedto the controlgroupand the othertreatedgroups. KIM-1 elevation attributed to shedding of extracellular domain of KIM-1 due to cisplatin induced inflammation**[19].**

KIM-1 levels significantly decreased after NAC comparedto the cisplatin group, thisdeclinemightbeattributedto decrease in ROS production with subsequent decrease in the expression of matrix metalloproteinase(MMP)-3. That in turn decreased Kim-1 expression**[20].**

The use of MO significantly reduced KIM-1 level compared to the cisplatin group. However, KIM-1 level is still significantly elevated compared to control group, this finding is also revealed byHussein et al.(2024)**[21]**.

NGAL is a protein that is secreted by activated neutrophils which is a very sensitive marker of AKI. It can be detected in plasma within two hours of AKI, with a concentration peak after 6 h. AKI also resulted in the elevation of urine NGAL levels. Elevated serum and urine NGAL due to AKI were observed 24 h earlier than the increase in serum creatinine**[22].**

Urine NGAL was found to be a more reliable biomarker for AKI compared to serum NGAL with higher sensitivity and specificity and its urine concentrations were associated with the severity and duration of AKI**[23].**

Cisplatincausedasignificantelevationof serum and urine NGALin the cisplatin group comparedto thecontrolgroupand the othertreatedgroups**[24]**. Serum and urine NGAL levels significantly decreased after NAC treatment comparedto the cisplatin group, NAC may be more effective in enhancing antioxidant defense and decrease mitochondrial damage leading to decreased NGAL levels**[25].**Also, the use of MO has significantly reduced NGAL level compared to the cisplatin group**[26].**

In our study, the combination of MO and NAC showed a significant reduction in serum KIM-1,serum NGAL and urine MGAL levels compared to the cisplatin group,the MO group and theNAC group but as regard the control group, combination group is not significantly different in KIM-1 levels but significantly different as regard serum and urine NGAL. This discrepancy may be due to differences in the sensitivity and of KIM-1 and NGAL as biomarkers. NGAL might be much more sensitive to changes in kidney function. Or

may be due to distinct pathophysiological mechanisms, as MO and NAC might be targeting different pathways to exert their protective effects**[27].**

Mitochondrial dysfunction is considered one of the possible mechanisms of cisplatin induced AKI. Mitochondrial damage leads to increased generation of ROS. ROS damage cell membranes through peroxidation of its phospholipid fatty acids, producing lipid peroxides that initiate cell oxidative damage. The lipid peroxidation degree can be quantified through the release of MDA as ROS make chemical bonds with the proteins and disrupt the function of receptors and enzymes bound to the cell membrane**[28].**

NAC treatment before and after cisplatin injection has partially regained disorganized oxidative stress markers by cisplatin and that is evidenced by a significant decrease in MDA and a significant elevation in GSH levels compared to the cisplatin group. Our results confirm the antioxidant effect of MO against cisplatininducedAKIbysignificantdecreaseofMDA andsignificant increase of GSH compared to cisplatin group.

MDA is significantly reduced and GSH is significantly elevated in the NAC group when comparedto the MO group and that may indicate more potency of NAC as an antioxidant. The combination ofNAC and MO revealed a significant increase in serum GSH levels and improved lipid peroxidation by significant decrease in MDA levels compared to the cisplatin group. Moreover, pretreatmentofNAC and MO in combined group was more efficient than NAC or MO alone in ameliorating oxidative stress.

Histopathological pictures are in harmony with biochemical results as the cisplatin group demonstrated marked tubular necrosis with desquamated epithelial cells, vacuolation, tubular dilatation, and hyaline cast compared to the control group

The possible mechanism of this finding may be due to formation of ROS and activating transcription factor (NF-κB) which trigger transcription of genes responsible for encoding proinflammatory cytokines**[29].**

MO alleviated histopathological picture of renal injury induced by cisplatin as demonstrated by minimal hyaline cast **[21, 30].**

NAC has improved the histopathological picture of renal injury induced by cisplatin demonstrated by nearly normal structure of most of renal tubules with few scattered hyaline casts. These results are coincident with previous studies **[8, 11].**

Enhanced better effect of NAC may be attributed to that NAC is generally considered more powerful due to its specific mechanism of action, potent antioxidant properties, and extensive research supporting its use**[31].**

The combination of MO and NAC has demonstrated nearly normal structure of glomeruli and tubules, no hyaline cast and absence of epithelial degeneration. And these results indicated that combination may afford greater protection than when used separately.

Cisplatin induces DNA damage, triggering the formation of reactive oxygen species (ROS), which in turn activates caspase-3, a key marker of apoptosis, ultimately leading to cell death. This process highlights the role of caspase-3 as a critical

indicator of apoptotic cell death in the context of cisplatin-induced toxicity**[32].**

cisplatin induces tubular cell apoptosis through activation of the pro-apoptotic proteins as BAX, which form pores in the mitochondrial outer membrane, with cytochrome c release into cytosol and activation of executioner caspases (caspase-3) **[33].**

Our results show thatlevel of caspase 3 in the cisplatin group showed significant increase compared to the control group and the other treated groups.Thisresult is in agreement with previous results ofUsefzay et al. (2022)**[34].**

MO treatment notably decreased caspase-3 expression compared to the cisplatin group, suggesting its anti-apoptotic properties**[35]**. Similarly, NAC treatment significantly reduced caspase-3 levels, confirming its anti-apoptotic effect**[36].**

Caspase 3 level in the combination group showed non-significant increase compared to control group but showed a significant decrease compared to the cisplatin group, the cisplatin &MO group and the cisplatin & NAC group. That established thatcombination ofMO andNAC givebetter outcomeinreduction of apoptosis.

Cisplatin's effect on aquaporin 2 (AQP2) expression is quite interesting. Research has shown that cisplatin can significantly decrease AQP2 expression in the kidney cortex, particularly at 72 hours after treatment. However, surprisingly, cisplatin up-regulates AQP2 expression at 168 hours, suggesting a potential compensatory mechanism. Overall, the impact of cisplatin on AQP2 expression appears to be complex and time-dependent, highlighting the need for further research to fully elucidate the effects of this chemotherapy agent on kidney function **[6, 37]**.

Level of aquaporin2 in cisplatin group showed a significant decrease compared to control group and other treated groups**[38].**

The level of aquaporin2 in the NAC group illustrated significant increase compared to the cisplatin group and treatmentwithMO caused a significantincrease in aquaporin2 expression when compared to cisplatin group, but there is no significant difference between the combination group and NAC group.

NAC may have a protective effect on cisplatin-induced nephrotoxicity by increasing AQP2 expression and translocation to the apical membrane, NAC reduced oxidative stress and inflammation, and activated the PI3K/Akt signaling pathway, which is involved in AQP2 regulation**[39].**

MO extract may exert Renoprotective effects by increasing AQP2 expression, reducing oxidative stress and inflammation, and improving renal function, supposing that the extract reduced oxidative stress markers and inflammatory cytokines (TNF-α, IL-1β) in the kidneys, also the extract may facilitate AQP2 translocation to the apical membrane, enhancing water reabsorption **[35].**

Our study confirmed that effect of these drugs on Aquaporin 2 but it has shown that there is non-significant difference between the combination group and NAC group that may suggest that NAC alone is effective as NAC was able to increase AQP2 expression and improve renal function, indicating its potential role as a Renoprotective agent and MO may not add any significant benefit in terms of AQP2 expression or renal function.

There could be reasons for this finding as NAC's potent antioxidant effects might have masked any potential benefits of MO and MO's effects might be modest on AQP2 expression compared to NAC's more pronounced effects.

In our study, serum KIM-1 showed strong, significant and negative correlation with aquaporin2.This correlation is in harmony with bio-chemical and histo-pathological results and may be attributed to inflammation, damage of tubular cells and oxidative stress in the kidneys, leading to decreased AQP2 expression. So, the study suggests that serum KIM-1 levels may be a useful biomarker for monitoring kidney function and AQP2 expression.

Overall, our study suggests that MO and NAC combination is more potent in ameliorating nephrotoxicity than when used separately. Thus, our results are in line with previous studies which declared that combinations of antioxidants may afford greater protection against cisplatin-inducednephrotoxicity than singleagents**[40].**

Conclusion: Co-administration of n-acetylcysteine and/or moringa oleiferawith cisplatin is advisable because they attenuate nephrotoxic effect of cisplatin and they have anti-cancer property potentiating the efficacy of cisplatin as chemotherapeutic agent. Combined administration ofn-acetylcysteine and moringa oleifera has the best outcome regarding histopathologicaland biochemical changes than separate administration ofn-acetylcysteine or moringa oleiferawith cisplatin.

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Limitations of study:

1. Financial constraints: Limited funding might have restricted the scope of the study, such as the number of rats used, the duration of the study, or the range of tests conducted.

2. Rat mortality: High mortality rates among rats could impact on the study's outcomes and limit the generalizability of the findings**.**

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