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Therapeutic efficacy of N-Acetylcysteine Against Cisplatin Induced Acute Kidney Injury in Wistar Rat Model

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ABSTRACT

Acute kidney injury (AKI) refers to a clinical syndrome characterized by rapid loss of renal function, which may further aggrevates into chronic kidney damage (CKD) or even end-stage renal disease (ESRD). Recently, the term ARF (Acute Renal Failure) has been replaced by Acute Kidney Injury. Cisplatin is a platinum containing drug widely used as chemotherapeutic agent with dose-limited nephrotoxicity. The present study envisaged the evaluation of therapeutic potential of N-acetylcysteine (NAC) in wistar rats against acute kidney injury induced by cisplatin. This study comprises of three groups: Group I: Heathy control group, Group II: Positive control group (Cisplatin only), Group III: NAC treatment group (Cisplatin+N-Acetylcysteine group). Oxidative stress indices like glutathione (GSH) and Malondialdehyde (MDA) were estimated in tissue homogenate sample with the help of commercial available. Renal injury was assessed via estimation of serum creatinine and urea level. Kidney tissues were collected for histopathological evaluation at the end of the study on day 28th. The mean value of GSH was significantly ($P< 0.05$) higher in Group III in comparision to Group II in kidney tissue homogenate. MDA value was significantly ($P< 0.05$) higher in Group II and lower in Group III and Group I. BUN and Creatinine levels were significantly higher in Group II as compared to other two groups. Group II rats were showing severe histopatholohical changes in kidney tissue as compared to group III. Therefore, this study can conclude that NAC can alleviate AKI induced by Cisplatin and has a good therapeutic potential against cisplatin nephroprotoxicity.

HIGHLIGHTS

- Cisplatin can induce Acute kidney injury in experimental rat models after 72 hours of I/P injection of cisplatin.
- N-Acetycysteine have good therapeutic action against Cisplatin induced AKI in experimental rat models.

Keywords: Acute kidney injury (AKI), Cisplatin, Wistar rats, N-Acetylcysteine

Acute kidney injury or AKI refers to an expeditious, certainly reversible, significant deterioration in renal function, characterized by rapid fall in glomerular filtration rate (GFR) and retention of end products of nitrogen metabolism (Singh *et al.,* 2012). It is a common clinical condition causing high morbidity and mortality, and is multifactorial in origin. The term 'ARF' was introduced Homer W. Smith in 1951 (Smith, 1951). In 2004, the term ARF was replaced by AKI (Ronco *et al.,* 2019). AKI can be caused due to various factors such as volume depletion,

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hypotension, septicaemia and chemotherapeutic and antibacterial drugs. It can be classified into pre-renal, acute post-renal obstructive and intrinsic AKI (Makris and Spanou, 2016). RIFLE classification for defining AKI was proposed in 2004, based on serum creatinine and urine output level. RIFLE stand for Risk, Injury, Failure, Loss of kidney function, and End-stage kidney (Lopes and Jorge, 2013). It alters acid–base homeostasis of the body. When urine concentrating ability of kidney is lost, hyponatraemia and hypernatraemia can occur. In AKI hyperphosphataemia occurs due to impaired phosphate clearance (Kellum *et al.,* 2021).

Cisplatin is a platinum-containing chemotherapeutic drug, which is most widely used in different types of cancers. This chemotherapeutic agent has dose-limiting nephrotoxicity (Perse and Haler, 2018). Excretion of cisplatin majorly occurs through kidney and therefore, it is the main site of cisplatin accumulation (Arany and Safirstein, 2003). It has several limitations because it induces nephrotoxicity and acute kidney injury (Volarevic *et al.,* 2019). The key pathological manifestations in cisplatin nephrotoxicity are renal tubular cell injury as well as cell apoptosis. Nephrotoxicity is among major side effects due to chemotherapeutic drugs. Cisplatin induces an inflammatory reaction in kidney leading to the activation of inflammasomes, further aggravating renal injury or damage. Cisplatin induced AKI is characterized by increased oxidative stress leading to acute tubular necrosis of the proximal tubular epithelial cells (Williams *et al.,* 2022). Additionally, cisplatin also leads to renal vasoconstriction, which reduces renal blood flow, causing ischemic damage which ultimately have adverse effect on glomerular filtration rate (Alhoshani *et al.,* 2017).

Cisplatin induces the generation of reactive oxygen species (ROS) in plenty of cells via the mitochondria, xanthine-xanthine oxidase system, and nicotinamide adenine dinucleotide phosphate oxidase (Shinde *et al.,* 2021). The key pathological findings in cisplatin generated nephrotoxicity are renal tubular cell injury and death. In 1971 the first report of experimental cisplatin-induced nephrotoxicity was given. In experimental animal models nephrotoxicity of cisplatin can be induced either by single or multiple dose. Severity of nephrotoxicity depends on the dosage and frequency of cisplatin. It can ranges from AKI to chronic kidney damage. In experimental rodents model of cisplatin induced nephrotoxicity, it is usually injected via intraperitoneal route and less frequently via intravenous or subcutaneous routes. According to studies significant quantities of cisplatin were observed in the kidney tissue for upto one month (Esteban-Fernández *et al.,* 2008).

For prevention, amelioration and treatment of AKI various strategies can be followed such as inhibition of inflammation, enhancement of renal perfusion via vasoconstriction or vasodilation, reducing the oxidative stress via inhibition of reactive oxygen species (ROS), by accelerating renal recovery through growth factors. N-Acetylcysteine (NAC) is commonly used to treat paracetamol toxicity (Shimizu *et al.,* 2017). It is a precursor of glutathione having anti-inflammatory and antioxidant action. It contains thiol group, which hinders cisplatin from removing glutathione or accumulating the peroxide. It directly reduces the amount of reactive oxygen species or ROS and protects against kidney damage (Moreira *et al.,* 2016). NAC treatment has enhanced the renal function, reduced pathological deterioration, alleviated inflammation, and decreased renal oxidative damage. The protective benefits might be achieved via reducing renal inflammation and the complement system (Huang *et al.,* 2019). NAC can ameliorates AKI induced via cisplatin by deactivating the P53 protein and by reducing oxidative stress in kidney tissue (Zhang *et al.,* 2014). NAC constitutes its antioxidant property due to its powerful free radical scavenger role and therefore, it can be a beneficial and effective therapeutic drug in cisplatin-induced acute renal damage. NAC has a free sulfhydryl group which has radical scavenger action because it may react directly with free radicals, reducing oxidative stress. Via GSH synthesis, it has the ability to enhance the endogenous antioxidant activity (Elsayed *et al.,* 2021).

MATERIALS AND METHODS

Study location and experimental animals

The present study was conducted in the Departmment of Veterinary Medicine in G.B.P.U.A.T, Pantnagar, India in 2023. The experimental animals were housed in laboratory animal house of the department according to CPCSEA guidelines. Adult healthy wistar female and male rats of 12-13 weeks of age and weighing not less than 150-250 gms were procured from Laboratory Animal

Resource Section, ICAR-IVRI, Izatnagar, Bareilly, India. The animals were housed in division animal sheds, under ideal conditions of management and provided feed and water ad libitum. Experiments were conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India approval No. IAEC/CVASc/VMD/490. The animals were acclimatized for 10 days before starting the experimental study. For this study rats were divided into three groups comprising six animals in each group.

Experimental design

After completion of the acclimatization period of rats. The animals were divided into three groups comprising six animals in each group. Group I: Heathy control group in which AKI was not induced in this group, neither they received any treatment, Group II: Positive control group in which cisplatin was given @10 mg/ kg as a single high nephrotoxic dose to induce AKI but no treatment protocol was followed in this group. Group III: NAC treatment group - After 4 hr of induction of AKI with cisplatin @10 mg/kg single dose, N-Acetylcysteine was given @200mg/ kg I/P for seven consecutive days.

Collection of blood, urine and tissue samples for analysis

Blood sample was collected from each rat by puncturing the retrobulbar venous plexus through the inner eye canthus using heparinised microcapillary tubes taking care not to injure the ocular structure. Blood was collected in tubes without any anticoagulant for separation of serum. Urine samples were collected in sterile vials for evaluation.

Both right and left Kidney tissue samples were dissected, decapsulated and collected in 10% Neutral Buffered Formalin in sterile containers for histopathological evaluation. Kidneys were divided into halves with central transverse section in this study.

Biochemical analysis, Urine volume evaluation and estimation of tissue antioxidant indices

Analysis of creatinine, blood urea nitrogen (BUN), Serum sodium, serum potassium, total protein and albumin was done for all the rats in all the three groups with the help of commercial kits as per the instructions on day 0, 3rd, 10th, 18th and 28th. GSH and MDA activities were evaluated in tissue homogenate samples of all experimental animals by using commercially available kits (Real Gene) as per the instructions on 28th day. 24 hr Urine volume of different groups was estimated in different time intervals.

Histopathological evaluation

Kidney tissue samples, previously stored in 10% neutral formalin were used to make histopathology slides. Paraffin blocks containing kidney tissues were cut into sections of approximately 5 µm size with the help of microtome and were processed for histopathological examination by H&E staining as per the standard method. Histopathological evaluation was done under light microscopy.

Statistical analysis

The data of this study was analyzed using two way ANOVA and Tukey's test. Data analysis was done by using Statistical package for the social sciences (SPSS) software. GraphPad Prism 6.0 was used for making graphs. $P<0.05$ was considered statistically significant, with results expressed as means ± standard error (SE).

RESULTS AND DISCUSSION

Serum biochemistry and Urine Volume

Byproduct of protein metabolism in blood is known as Blood urea nitrogen or BUN. It is classified as an NPN (non-protein nitrogenous) waste product. Ammonia is produced by deaminating amino acids generated from protein breakdown. Through the action of liver enzymes, ammonia is transformed to urea. As clearance of NPN products is basically depends on the Kidney tissue, it is a helpful analyte for assessing renal function. Breakdown of phosphocreatine and creatine leads to formation of creatinine and which can be used to evaluate kidney functionality. Creatinine, as opposed to BUN, is less impacted by diet and so more suited as an indication of renal function. Creatinine clearance can be used a measure for evaluating glomerular filtration rate, which will reveal the renal function status (Salazar, 2014). It was observed that in Cisplatin induced AKI BUN and creatinine were

elevated markedly on day 3rd after the IP injection of cisplatin in rat models. In this study Serum creatinine and BUN values differs significantly in group II in comparison to the other two groups. Serum creatinine and BUN values were significantly (P<0.05) higher in Group II on day 3, 10th, 18th and 28th in comparison to Group I. Significant (P<0.05) reduction in BUN and creatinine level was noticed in Group III. Therefore, it can be concluded that NAC treatment had shown a considerable improvement in Group III. NAC had improved the renal clearance activity in Group III and reduced the oxidative damage in kidney parenchyma due to cisplatin.

AKI can alter the glomerular structure and function and, hence increases albumin filtrion rate. Proximal tubular injury alters the tubular reabsoption capacity reduces albumin reabsorption (Ware, 2011). In kidney diseases, hypoproteinemia and hypoalbuminemia are usually noticed as a result of renal tubular damage expediting the loss of protein in the urine. Serum total protein and albumin levels were significantly (P<0.05) lower in Group II on day 10th, 18th and 28th as compared to group I levels. While for Group III significant (P<0.05) increase of serum albumin levels were recorded on day 28th in comparison to day 3rd value.

A reduction in kidney function results in an electrolyte problem since the kidneys are crucial for maintaining Na and K homeostasis (Gao *et al.,* 2019). In acute kidney injury alterations in serum sodium and potassium level occurs. On day 3rd a significant (P<0.05) decrease in serum sodium level was recorded in group II and III as compared to healthy control group values. In Group II significant decrease was recorded in all the days while in group III a highly significant (P<0.05) increase in sodium level was recorded on day 18th and 28th. Serum potassium level was significantly (P<0.05) low in Group II on day 10th, 18th and 28th as compared to healthy control group. While for group III significant (P<0.05) increase in serum potassium level was recorded.

The kidneys are essential for the elimination of NPN waste products as urea and creatinine. Collection, testing of a 24-hour urine sample and determination of creatinine clearance are one of the standard tests for determination of kidney function tests. RAS is a hormonal system that regulates blood pressure and fluid and electrolyte homeostasis by acting in concert with the heart, arteries, and kidneys. In cispaltin induced nephrtoxicity a decrease in glomerular filtration rate, polyuria due to reduced reabsorption in tubules due to decreased expression of aquaporins water channels in the nephron, and a significant defect in urine concentrating ability are all consequences of injury to the proximal and distal tubules. On day 3rd significant (P<0.05) increase in urine volume was recorded in Group II and Group III as compared to Group I (Healthy control group) In Group III (NAC) urine volume decreased significantly (P < 0.05) on day 18th and 28th as compared to day 3rd value and the urine volume level in Group III was equal to the values of healthy control group. The results indicated that NAC reduced the damage to renal parenchyma and increases the aquaporin gene expressions and thereby, decreases the urine volume to normal.

Oxidative Stress Indices

The common pathophysiological mechanism of cisplatin toxicity is the production of a large number of reactive oxygen species i.e., ROS and the activation of oxidative stress (Yu *et al.,* 2018). In cisplatin-induced cute kidney injury, tubular cell injury and death occurs mainly due to mitochondrial damage and oxidative damage (Mao *et al.,* 2022). Reduced glutathione or GSH is a non-enzymatic antioxidant that is required in mammalian cells. GSH may operate as an antioxidant directly to protect cells from free radicals as well as also acts as cofactor for various antioxidants and enzymes which help in detoxification such as glyoxalase, glutathione peroxidases and glutathione S-transferases (Averill-Bates, 2023). Malondialdehyde also referred as MDA is a main byproduct formed during oxidative reaction, therefore it has been proved as an effective biomarker of oxidative stress in any tissue injury or damage (Bencivenga *et al.,* 2023). Oxidative stress indices were estimated in all the animals after their sacrifice on day 28th. In this study it was found that kidney tissue homogenate was showing significantly (P <0.05) reduced mean GSH values in Group II as compared to Group I and Group III while Group III i.e., NAC treatment group was showing significantly (P <0.05) higher GSH level in tissue homogenate sample. The mean values of MDA level were significantly higher in Group II (Positive control group) while in Group I and III, MDA levels were significantly low. As NAC has pharmacological antioxidant properties. Therefore, it can be concluded from this study that NAC

Table 1: Different serum biochemical parameters and 24 hr urine volume in rats (Values with same superscript does not differ significantly)

Parameters	Day 0	Day 3	Day 10	Day 18	Day 28
Blood urea nitrogen (mg/dl)					
Group I	17.5^{ax} **±0.66**	17.9^{ax} **±0.72**	17.9^{ax} **±0.75**	18.2^{ax} **±0.75**	18.3^{ax} **±0.77**
Group II	19^{ax} **±0.70**	46.8^{cz} **±3.93**	52.1^{cz} **±3.50**	50.1^{cz} **±3.24**	49.8^{cz} **±3.48**
Group III	17.5^{ax} **±0.66**	41.3^{cz} **±3.14**	32.8^{bcy} **±3.01**	28.4^{aby} **±2.88**	23.0^{az} **±1.81**
Serum creatinine (mg/dl)					
Group I	0.7^{ax} **±0.04**	0.7^{ax} **±0.05**	0.6^{ax} **±0.04**	0.6^{ax} **±0.04**	0.7^{ax} **±0.04**
Group II	0.7^{ax} **±0.04**	2.6^{by} **±0.47**	3.5^{cz} **±0.37**	4.3^{cz} **±0.32**	4.0^{cz} **±0.32**
Group III	0.6^{ax} **±0.04**	2.8^{by} **±0.42**	1.6^{aby} **±0.28**	1.2^{aby} **±0.25**	0.9^{ax} **±0.12**
Serum albumin (g/dl)					
Group I	4.4^{ax} **±0.30**	4.4^{ax} **±0.30**	4.4^{ax} **±0.29**	4.5^{ax} **±0.29**	4.4^{ax} **±0.28**
Group II	4.5^{ax} **±4.48**	3.5^{aby} **±0.35**	2.9^{bz} **±0.46**	2.9^{bz} **±0.25**	2.7^{bz} **±0.12**
Group III	4.4^{ax} **±0.30**	3.2^{aby} **±0.28**	3.5^{aby} **±0.20**	3.9^{aby} **±0.17**	4.2^{ax} **±0.15**
Total protein (g/dl)					
Group I	6.4^{ax} **±0.23**	6.4^{ax} **±0.23**	6.4^{ax} **±0.18**	6.3^{ax} **±0.19**	6.3^{ax} **±0.22**
Group II	6.4^{ax} **±0.18**	5.6^{aby} **±0.29**	4.9^{by} **±0.24**	4.8^{by} **±0.17**	4.5^{by} **±0.18**
Group III	6.6^{ax} **±0.16**	4.8^{by} **±0.34**	5.7^{bcx} **±0.14**	6.3^{ax} **±0.19**	6.4^{ax} **±0.22**
Serum Potassium (mEq/l)					
Group I	3.9^{ax}±0.07	3.9^{ax}±0.2	4.1^{ax}±0.2	4.0^{ax}±0.2	4.0^{ax}±0.2
Group II	3.9^{ax}±0.07	3.3^{aby}±0.2	2.8^{bz}±0.2	2.3^{bz}±0.2	2.1^{bz}±0.2
Group III	3.9^{ax}±0.07	3.1^{aby}±0.2	3.6^{aby}±0.2	3.6^{aby}±0.2	3.9^{ax}±0.2
Serum Sodium (mEq/l)					
Group I	140.9^{ax}±0.89	140.6^{ax}±1.02	140.7^{ax}±0.88	140.7^{ax}±1.07	140.9^{ax}±0.86
Group II	140.9^{ax}±0.89	118.9^{bz}±6.68	110.2^{bz}±5.6	114.8^{bz}±6.87	115.8^{bz}±6.59
Group III	140.9^{ax}±0.89	114.7^{bz}±5.86	131.8^{ay}±4.3	140.7^{ax}±6.8	140.9^{ax}±6.5
Urine volume (ml)					
Group I	2.0^{ax}±0.10	1.9^{ax}±0.22	2.0^{ax}±0.10	2.0^{ax}±0.10	2.1^{ax}±0.12
Group II	2.0^{ax}±0.10	3.0^{by}±0.12	3.25^{by}±0.15	3.5^{by}±0.15	3.25^{by}±0.12
Group III	2.0^{ax}±0.10	3.0^{by}±0.12	2.25^{ax}±0.10	2.0^{ax}±0.10	2.0^{ax}±0.10

Table 2: Alteration in oxidative stress indices in rats after sacrifice on day 28 of the study

Oxidative Stress Indices in kidney tissues after sacrifice of animal		
On 28th day	**GSH (mmol/g)**	**MDA (Nm MDA/g)**
Group I	0.181±0.001	0.765±0.007
	0.181±0.001	0.765±0.007
Group II	0.168±0.005*	1.110±0.028*
	0.112±0.000***	1.500±0.014***
Group III	0.168±0.005	1.110±0.028*

The values have been expressed as Mean±SEM. ***indicates highly significant difference (P<0.05) .

helps in combating the oxidative damage in cisplatin induced AKI.

Histopathology

The histopathological changes in light microscopy revealed that in Group II there was severe congestion of the glomerulus, interstitial haemorrhage and congestion was present, few of the tubules has shown epithelial cast. Kidney tubular epithelial cells were necrosed. In cortex tubules were showing necrosis of the tubular epithelial cells. In medulla also there was interstitial haemorrhages and necrosis of Kidney tubular epithelial cell in majority of the tubules. Inflammatory cells infilterated in the renal tubule interstitium.

Group I animal were showing normal structure of kidney, with no injury. While in Group III histopathological changes were minimum and tubules were showing less damage. Majority of the kidney tubular epithelial cells were intact. Almost nill congestion of glomerulus and mild shrinkage of glomerulus was noticed in group III. According to the results of histopathological evaluation we can say that NAC has shown good effect against cisplatin induced acute kidney injury.

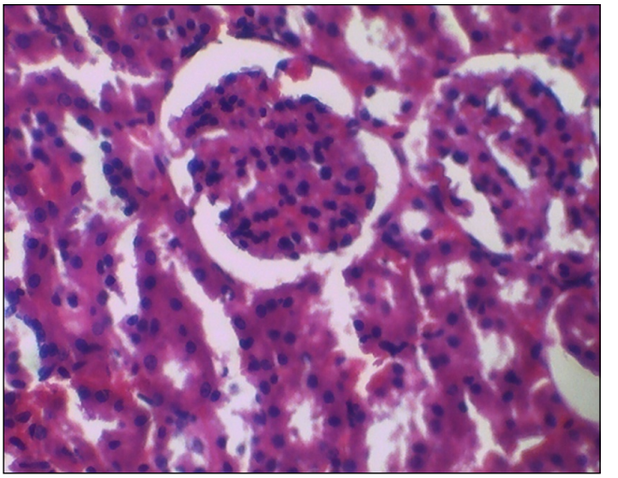

Fig. 1: Group III (NAC treatment Group)

Figure: Histopathology of kidney tissue of rats

(Fig. 1): Histopathological section of kidney tissue of Group III rats showing the therapeutic effect of NAC in cisplatin induced AKI, less inflammatory cells were noticed, tubules were showing very less damage. Majority of the kidney tubular epithelial cells were intact. Glomeruli was showing very less or no congestion.

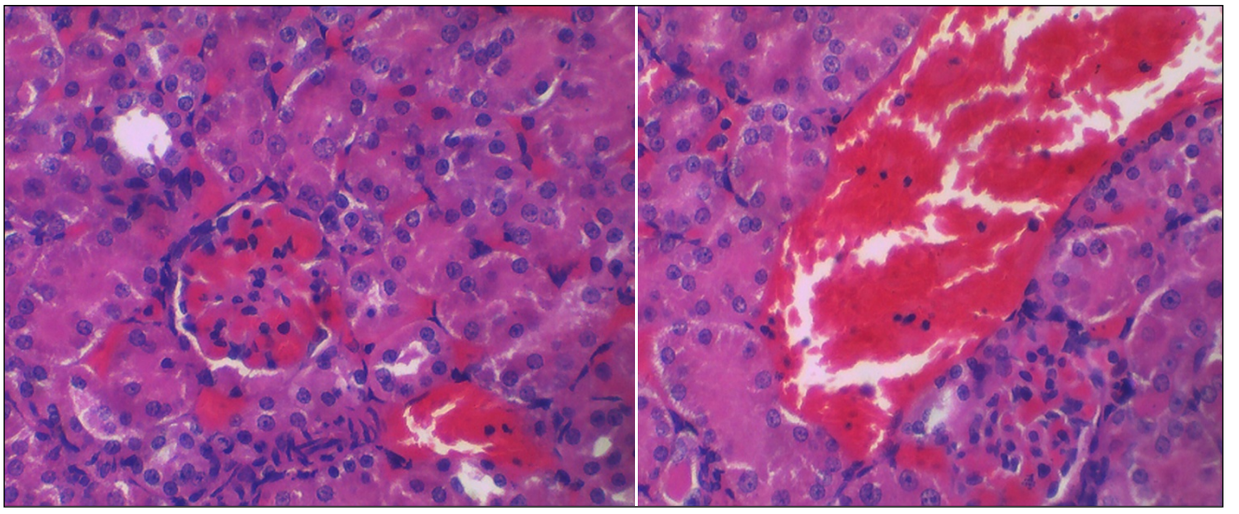

Fig. 2: Group II (Posistive Control Group)

(Fig. 2): Histopathological section of kidney tissue of Group II showing severe congestion of the glomerulus, interstitial haemorrhage and congestion was present, few of the tubules has shown epithelial cast, inflammatory cells were infilterated throughout the tissue section in histopathology.

CONCLUSION

NAC is a thiol-containing antioxidant, which hinders cisplatin from removing glutathione or accumulating the peroxide. It directly reduces the amount of reactive oxygen species (ROS) and protects against kidney damage. Our work showed similar results that cisplatin can cause a significant decrease in the contents of SOD and GSH and an increase in the content of MDA in the kidneys. These data suggested that NAC ameliorated oxidative damage in kidney tissues by increasing antioxidant enzyme activity. Therefore, it can be concluded from this study that NAC can be an effective measure to reverse the oxidative damage caused by cisplatin. It can be a useful therapeutic agent against cisplatin induced nephrotoxicity.

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