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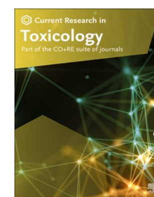

Research Paper

Early diagnostic biomarkers for acute kidney injury using cisplatin-induced nephrotoxicity in rat model

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ABSTRACT

Chronic kidney diseases (CKD) caused by acute kidney injury (AKI) results rapid and reversible loss in renal function. A real-time, highly accurate, and sensitive acute kidney injury biomarker is urgently required in order to keep these patients alive and prevent end stage renal disease and related complications that include hypertension, fluid and electrolyte retention, metabolic acidosis, anemia, stroke etc. This study was designed to develop a specific and sensitive model for the early identification of renal damage in male albino rats. Using a single intraperitoneal dose of cisplatin (10 mg/kg body weight) to the rats, the various duration-dependent nephrotoxic activities were compared using multiple physiological, biochemical, genomic, and histopathological markers. We looked into when renal dysfunction would start occurring after receiving a single high dose of cisplatin while blood urea nitrogen (BUN) and serum creatinine (sCr) remained normal. Following a single cisplatin injection, various measurements were taken in plasma, urine, and/or kidney tissues of rats euthanized on days 1, 2, 3, 5, and 7. When the urine kidney injury molecule (KIM-1), interleukine 18 (IL-18), nephrin, neutrophil gelatinase-associated lipocalin (NGAL) and serum cystatin C (Cys C) levels are greatly raised on day 3 after cisplatin treatment, BUN and sCr levels remain normal. Nephrotoxicity of cisplatin is also indicated by the upregulated mRNA expression of KIM-1, IL-18, Cys C, and NGAL and downregulated expression of nephrin in kidney tissue at very initial stage. Protein expression of KIM-1, IL-18 and NGAL level of kidney tissues was upregulated indicated confirmatory results done by western blot. Utilising an array of kidney impairment indicators has emerged as an earlier, more effective, and more reliable technique to diagnose AKI when compared to the most sophisticated signs now available.

Introduction

Acute kidney injury (AKI) is characterised by the kidneys inability to meet the body's excretory, metabolic, and endocrine needs. Sepsis and other organ failure are frequent co-morbidities of AKI. It is characterised by a slow decline in vital functions over time, which results in renal failure and necessitates dialysis or a kidney transplant to sustain life. Recent research has also shown that AKI is positively associated with the chance of developing chronic kidney disease (CKD), and that people who have CKD that is worsened by AKI have a higher death rate (Chen et al., 2018). The prevalence of AKI has steadily increased recently despite medical technology advancements, and it is linked to a higher mortality risk (Singbartl & Kellum, 2012). AKI may result from a variety of risk factors, such as specific medications, nutritional supplements, infections, ischemia, sepsis, and intravenous contrast. The majority of AKI cases in hospitalised patients, or about 60 % of cases, are caused by drug-induced nephrotoxicity. No effective therapy candidates have been found in the clinic despite efforts to discover medications for the therapy of AKI. Early awareness of the onset of AKI and prompt care can lower morbidity and death because kidney failure detection time and mortality are directly correlated (Faria, et al., 2019). Various biomarkers that assist in decision-making for moving a chemical to the following non - clinical or diagnostic stages of development must be found, validated, and assessed. Studies should be conducted to confirm the efficacy of the

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early kidney damage markers Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule (KIM-1), interleukine 18 (IL-18), cystatin C (Cys C), and nephrin (Benoit et al., 2020). A glycoprotein known as NGAL is generated by neutrophils and epithelial cells in a variety of organs. Following ischemia and nephrotoxic injury, the proximal tubular cells release this protein into the bloodstream (Tsigou et al., 2013). KIM-1 is a type 1 *trans*-membrane protein, and ischemic kidneys express it at much higher levels in the proximal tubule. With a predictive value for individuals following cardiac surgery, KIM-1 is a potential factor in the identification of AKI. The proximal renal tubular injury is notably reflected by this initial biomarker (Sabbisetti et al., 2014). Inflammatory events cause the pro-inflammatory cytokine IL-18 to become more active. It mediates the effects of hypoxia on tissue. An early, quick, and affordable marker called urine IL-18 allows for the early diagnosis of kidney injury brought on by ischemia or nephrotoxicities (Rizvi and Kashani, 2017). All nucleated cells consistently produce Cys C, a cysteine proteinase inhibitor protein. It is a glomerular filtration biomarker and is freely filtered by the kidney with almost complete reabsorption and catabolism in the proximal tubule and no substantial urine excretion (Benoit et al., 2020). Nephrin is a transmembrane protein that has a highly specialised extracellular domain and a brief intracellular tail. It is synthesised by glomerular podocytes and is present near the slit diaphragm. The foot processes of the podocytes have intercellular contact and the extracellular domain is heavily glycosylated (Wang et al., 2018). The diagnosis of kidney damage is typically made using serum indicators like blood urea nitrogen (BUN) and serum creatinine (sCr) levels, which become elevated only after significant (roughly 30 %) kidney damage. AKI is characterised by a sudden decrease in kidney function or urine output, whereas chronic kidney damage is characterised by sustained functional and structural alterations (Udupa & Prakash, 2018). The emerging renal biomarkers have lately been employed as a tool to assess early kidney impairment in both preclinical species and humans due to their insensitivity and lack of specificity. In order to evaluate the difficulty in preclinical toxicological investigations and to help reduce attrition of led to a significant in drug development, early kidney injury must be detected utilising urinary biomarkers.

Cisplatin is a cytotoxic chemotherapeutic drug based on inorganic platinum that is frequently used to treat different malignancies that increasing the risk of renal damage (Qu et al., 2018). In patients who took the chemotherapeutic drug cisplatin, the incidence of acute and chronic renal injury can reach 40 %. Additionally, the use of cisplatin results in roughly 19 % of AKI-related fatalities (Hucke et al., 2019). Cisplatin causes nephrotoxicity through a variety of cytotoxic mechanisms. In addition to causing DNA damage, and also activates apoptotic pathways, damages cells through oxidative stress and inflammation, and causes cytoplasmic organelle malfunction, especially with in mitochondria and endoplasmic reticulum (Pabla and Dong, 2008). The large volume of cisplatin dispersion and long-term buildup of cisplatin inside the kidney are the primary causes of cisplatin-induced nephrotoxicity (Ibrahim et al., 2019). In general, cisplatin-induced nephrotoxicity is caused by pathogenic mechanisms that primarily show up as reductions in renal blood flow, glomerular filtration rate, and ischemia or necrosis of proximate renal tubular epithelial cells (Fang et al., 2021).

A set of biomarkers, including KIM-1, IL-18, Cys C, NGAL and nephrin levels were analysed in urine and/or kidney tissue samples and was especially in comparison with the traditional end points. The present research work designed to develop an in vivo model of AKI that could be used for early detection of drug induced nephrotoxicity by non invasive urinary biomarkers of both glomerular and tubular injury.

Methods and materials

Drugs and chemicals

Cisplatin was purchased from Sigma Aldrich, ELISA kits for KIM-1, IL-18, Cys C, NGAL and nephrin was purchased from ELK Biotechnology, other chemicals for biochemical and genomic analysis were purchased from HiMedia and Merck. Primers for qPCR analysed were obtained from Edison Life Science (India).

Animal selection and care

Male albino Wistar strain rats weighing 120–130 g were bought from Saha Enterprise in Kolkata. ~~T~~ Prior to the experiment, the rats were kept in cleaned polypropylene cages with unlimited access to food and drink in controlled humidity, temperature (25 ± 5^0c), and ventilation systems with a 12-hour light/dark cycle for 7 days for prior to experimentation. The maintenance protocol of animals was approved by the CPCSEA registered Institutional Animal Ethics Committee (Registration number – 1905/PO/Re/S/2016/CPCSEA (Alhoshani et al., 2017).

Cisplatin induced AKI

Thirty-six healthy male rats were divided into six groups (n = 6). Control (C) group is provided with normal food and water *ad libitum* for 7 days with a single intraperitoneal injection of saline water at day 1. AKI was achieved by single intraperitoneal injection of cisplatin dissolved in saline water (2 mg/ml) @ 10 mg/kg body weight to all the other group of animals. Cisplatin was given as previously reported dose and duration dependent studies done by researchers (Sahu et al., 2019; Mi et al., 2018). Other group of animals were sacrificed at day 1, day 2, day 3, day 5 and day 7 respectively after single injection of cisplatin. Before scarification urine were collected from all the animals for ELISA tests. After rat scarification blood was collected for biochemical tests, kidney tissue was collected for histopathological analysis, ~~and~~ related gene expression and protein expression studies.

Measurement of relative kidney weight and renal somatic index in cisplatin treated rats

According to Zaaba et al., the relative kidney weight was determined after the rats were sacrificed by performing the following methodology (Zaaba et al., 2022). Body weight was determined in this experiment by subtracting initial body weight from final body weight. In order to calculate the renal somatic index (RSI), both kidneys were weighed after being cleaned with an ice-cold 0.9 % NaCl solution and then kidney weight (g) was divided by body weight (g) multiplied by 100 (Nosrati et al., 2021).

Biochemical tests

Serum was collected from blood samples and used for analysis of BUN and sCr as kidney function tests, reduced glutathione (GSH) and super oxide dismutase (SOD) was measured in kidney tissue for antioxidant activities (Jollow et al., 1974).

Detection of kidney injury markers

Early detection of kidney injury was done by measuring urinary KIM-1, IL-18, nephrin NGAL and serum Cys C level by ELISA machine (Robonik) as per manufacturers instruction (ELK biotechnology, Wuhan) (Zdziechowska et al., 2020). Briefly, 100 μl of assay diluent was added into 96-well microplate, and 100 μl of standard, control, or sample were then added to each well. After incubation at 37 °C for 80 min, removed the liquid and each well was washed 3 times by using wash buffer and then detection antibody was added and incubate at 37 °C for 50 min. After washed with wash buffer, HRP-conjugated antibody was added into each well and further incubated for 50 min at 37 °C. After washing, 90 μl of TMB substrates were added to each well and incubate for 20 min at 37 °C and stop solution was added to stop the reaction. Then, run the microplate reader and conduct measurement to

absorbance at 450 nm immediately.

Western blot analysis for protein expression

Kidney tissues were lysed with RIPA buffer, and their protein content was determined using the Lowry technique. The loading buffer was added to the 50 μg of protein sample, which was then be boiled for 5 min before being electrophoresed by SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad). The membrane was then blocked with 4 % bovine serum albumin (BSA) and incubated with primary antibody overnight at 4 °C. The primary antibody was used in this study included β-actin, KIM-1, IL-18 and NGAL at 1:1000 ratio (Affinity). The membrane was then washed three times with phosphate buffer saline with tween-20 (PBST) and incubated with HRP labelled secondary antibody 1:7500 (Abgenex) for 1 h at 4 °C. Specific protein bands were detected using 3,3′,4,4′-Tetraaminobiphenyl tetrahydrochloride (DAB). To measure protein immunoreactivity, ImageJ software was used here (Chakrabarti et al., 2018).

Gene expression studies by quantitative polymerase chain reaction (qPCR)

Total RNA from rat kidney tissues was extracted with TRIzol (Life Technologies). RNA concentration was detected by NanoDrop 2000c Bio spectrophotometer. For each RNA sample, 1 μg of total RNA was reversely transCribed into cDNA using a High-capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA). Target gene expression for KIM-1, IL-18, nephrin, NGAL and Cys C was analyzed by semi qPCR analysis. Primer for the GAPDH was forward 5′-CGCTGGTGCTGAGTATGTCG-3′ reverse 5′–CTGTGGTCATGAGCCCTTCC-3′, Kim-1 forward 5′-GTCTGTATTGTTGCCGAGTG-3′ reverse 5′-GGTCTTGTTGGAGGACTT GT-3′, IL-18 forward 5′-GACTGGCTGTGACCCTATC-3′ reverse 5′-TG TCCTGGCACACGTTTCTG-3′, nephrin forward 5′-GCTGTGTCTGACG CTTTTGG-3′ reverse 5′-TAGGAGACACAAGCTCGGGA-3′, NGAL forward 5′-GACAACCAATTCCAGGGGAAG-3′ reverse 5′- GCATACATCTTTTGCG GGTCT-3′, Cys C forward: 5′ GCCTGTGCCTATCACCTCTTAT-3′ Reverse: 5′-CCTTCTCTGTCTGTCTCCTGGT-3′, PCR was performed as follows: initial denaturation of DNA at 95 °C for 5 min, denaturation at 95^0c for 30sec, DNA annealing at 62 °C for 45sec, and extension of DNA at 72 °C for 1 min, final extension of DNA at 72 °C for 5 min, second and third steps were repeated for a total of 42 thermal cycles. Lastly, the PCR tube with the sample was held at 4 °C. After that the GeNeiTMmini-submarine gel system was used to investigate the gene expression of KIM-1 and IL-18 using Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a standard. Five microliter of PCR product was mixed with 1 μl sample loading dye and 5 μl of DNA size standard 3000 and then run using GeNeiTMmini-submarine gel system. The amplified fragments were separated according to their respective size. Results were analysed using the Gel Documentation Imaging System (Jiménez-Castilla et al., 2021).

Densitometric analysis

The DNA bands on the gel was quantified by densitometric analysis using a software. Here ImageJ software was used to compare the density of the bands on the agarose gel. Rectangular area of the specific bands was drawn and the mean area value was shown for the bands. Area of each bands were selected same to compare the density of the bands on an agarose gel. All the mean density value of bands was plotted in excel, and the gene of interest (KIM-1, IL-18, NGAL and nephrin) was divided by housekeeping gene (GAPDH) (Stael et al., 2022).

Quantitative real time reverse transcription polymerase chain reaction (qRT-PCR) for mRNA expression

Target gene expression for KIM-1, IL-18 and NGAL was further analyzed by real-time PCR using a G8830A AriaMx Real-Time PCR system (Agilent Technologies) with SYBR Green Gene Expression detection assays. Expression levels of target gene was normalized to the housekeeping gene GAPDH. Gene expression was measured using total 1 μl of gene-specific primers. Brilliant III ultra-fast SYBR Green QPCR Master Mix (Agilent, USA) was added in a volume of 10 μl to each 1 μl of cDNA sample, and total volume was adjusted to 20 μl using molecular biology grade water. The PCR reactions were performed using thermal profile RT-PCR as follows: initial denaturation of DNA at 95 °C for 1 min, denaturation at 95 °C for 30 sec, DNA annealing at 59 °C for 45 sec, and extension of DNA at 72 °C for 2 min, final extension of DNA at 72 °C for 10 min. The second and third steps were repeated for a total of 37 thermal cycles. Lastly, the PCR tube with the sample was held at 4 °C. The fold change in the expression of the target gene was calculated by the $^{2-}\Delta\Delta$CT threshold method and normalized to controls (Kuang et al., 2018; Livak & Schmittgen, 2001).

Histopathological analysis

Rats from each experimental group had their kidneys taken, fixed, processed, and sectioned using paraffin. The tissue sections were stained using the hematoxylene and eosine (H&E) staining procedure and examined under a light microscope (Chowdhury et al., 2019). These sections were examined in a blinded fashion by a renal pathologist and nephrologist. The percentage of histological changes in the cortex and medulla were scored using a semiquantitative scale designed to evaluate the degree of necrosis, cell loss, and necrotic casts on a five-point scale based on extent of involvement as follows: 0, normal kidney; 0.5, < 10 %; 1, 10–25 %; 2, 25–50 %; 3, 50–75 %; and 4, 75–100 % (Ascon et al., 2009).

Statistical analysis

All values were expressed as means ± SE, n = 6. Student's *t*-test was used between two groups and one way ANOVA analysis was used for comparing multiple groups, with statistical significance set at $p < 0.05$. This analysis were performed by using GraphPad Prism 6 software.

Results

Effect of body weight and kidney somatic index on cisplatin induced nephrotoxicity

For each of the experimental animals, the initial and end body weights were recorded. Table 1 showed that there was significant ($p < 0.05$) decrease in body weight for cisplatin treated animals sacrificed at day 3 and the body weight was further reduced severely on day 5 and 7 when compared to control group. In case of other cisplatin treated animals sacrificed at day 1 and 2 there was no significant reduction in body weight was observed compared to control. When cisplatin treated rats were euthanized at days 3, 5, 7 Fig. 1 indicated a substantial ($p < 0.05$)

Table 1
Effect of cisplatin on the body weight of experimental rats at different duration dependent study.

Groups	Initial body weight (g)	Final body weight (g)	Difference (Initial-Final)
Control	182.36 ± 0.86*	191.35 ± 1.08*	+ 8.99 ± 0.79
Day 1	180.56 ± 0.94*	181.79 ± 1.24*	+ 1.23 ± 0.31
Day 2	182.17 ± 0.99*	178.26 ± 1.09**	- 2.91 ± 0.31
Day 3	184.3 ± 0.98*	176.26 ± 1.32**	- 8.04 ± 0.67
Day 5	183.39 ± 1.06*	163.25 ± 1.42***	- 20.14 ± 0.93
Day 7	181.18 ± 0.96*	158.36 ± 1.25***	- 22.82 ± 1.17

+ Increased
- Decreased
Information presented as Mean SE (n = 6). Multiple two tail t tests were conducted after an Anova (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

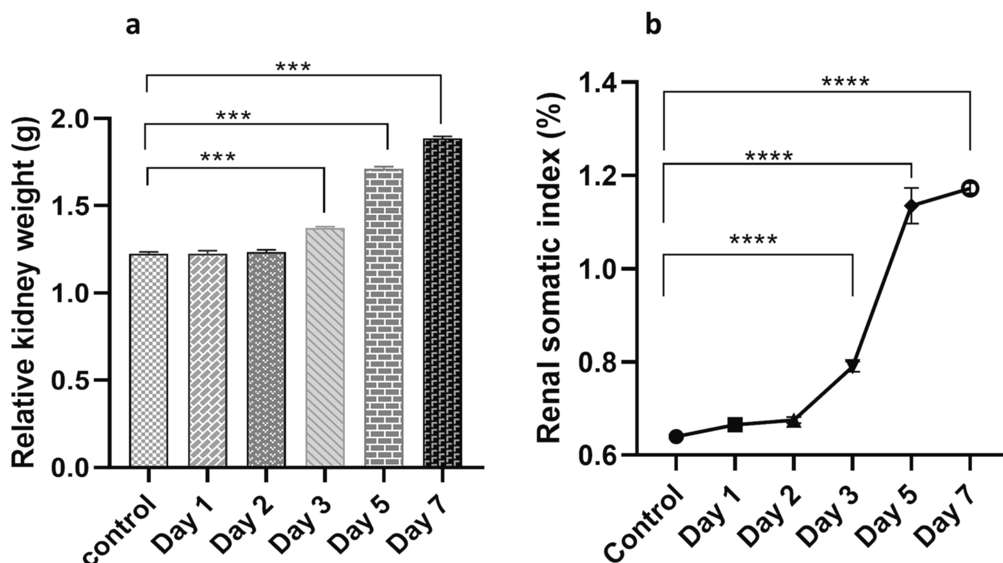


Fig. 1. Effect of cisplatin on the kidney weight (a) and renal somatic index (b) of experimental rats at different duration dependent study. Information presented as Mean ± SE (n = 6). Multiple two tail t tests were conducted after an Anova (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

elevation in the kidney weight and renal somatic index as compared to control group.

Alterations in the uremic profile due to cisplatin induced nephrotoxicity

A significant ($p < 0.05$) elevation in BUN and (sCr) level was observed to the cisplatin treated rats sacrificed at day 5 and 7 when compared to the control group which indicates decreased renal functionality. These conventional markers of kidney injury were not significantly elevated at day 1, 2 and 3 after single intraperitoneal injection of cisplatin when compared to control group of rats (Fig. 2).

Changes in the antioxidant enzymes

The results in Fig. 3 showed that single intraperitoneal injection of cisplatin significantly ($p < 0.05$) decreased both GSH and SOD activities in kidney homogenate to the rats sacrificed at day 5 and 7. The antioxidant activities was not significantly altered to the cisplatin treated rats sacrificed at day 1, 2 and 3 when compared to control. This is due to oxidative stress produced due to cisplatin induced nephrotoxicity.

Changes in the kidney injury markers due to cisplatin induced nephrotoxicity

Level of urinary KIM-1, IL-18, nephrin, NGAL and serum Cys C were measured using enzyme-linked immunosorbent assay. Fig. 4 (a, b, c, & d) suggested that the urinary KIM-1, IL-18, nephrin and NGAL was significantly ($p < 0.05$) elevated at day 3, 5 and 7 after intraperitoneal injection of cisplatin when compared to control group of rats. At that time SCr and BUN remains at the normal level and there was no significant different between control and cisplatin treated animals sacrificed at day 1, 2 and 3. In case of serum Cys C the level only elevated at 5 days after cisplatin treatment when other convention markers like BUN and SCr levels were also increased (Fig. 4 (e)).

Changes in protein expression level of KIM-1, IL-18 and NGAL in cisplatin induced nephrotoxicity

As shown in Fig. 5 relative protein expression of KIM-1, IL-18 and NGAL in the kidney tissues showed a significant ($p < 0.05$) upregulation in all the kidney injury markers like KIM-1, IL-18 and NGAL with a concomitant increment observed at day 3 after single intraperitoneal

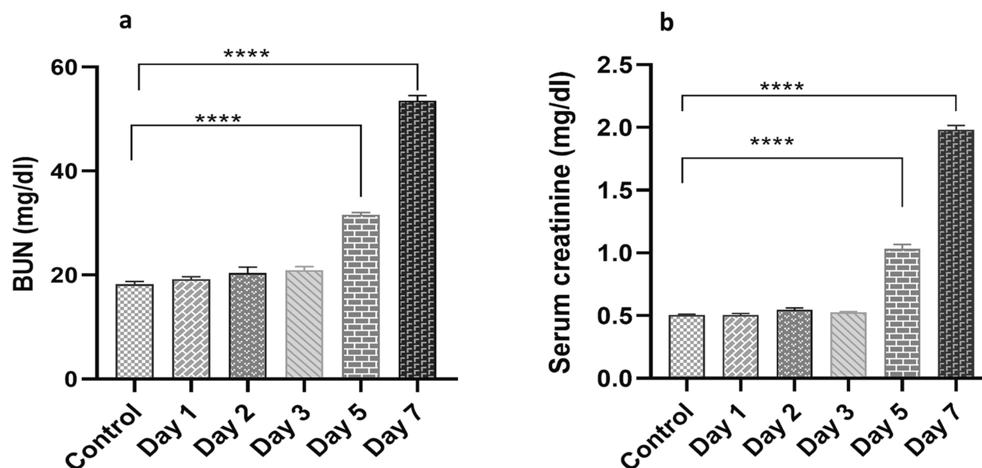


Fig. 2. Alteration of BUN (a) and SCr (b) level on the cisplatin treated acute kidney injury in rats at different duration dependent study. Information presented as Mean ± SE (n = 6). Multiple two tail t tests were conducted after an Anova (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

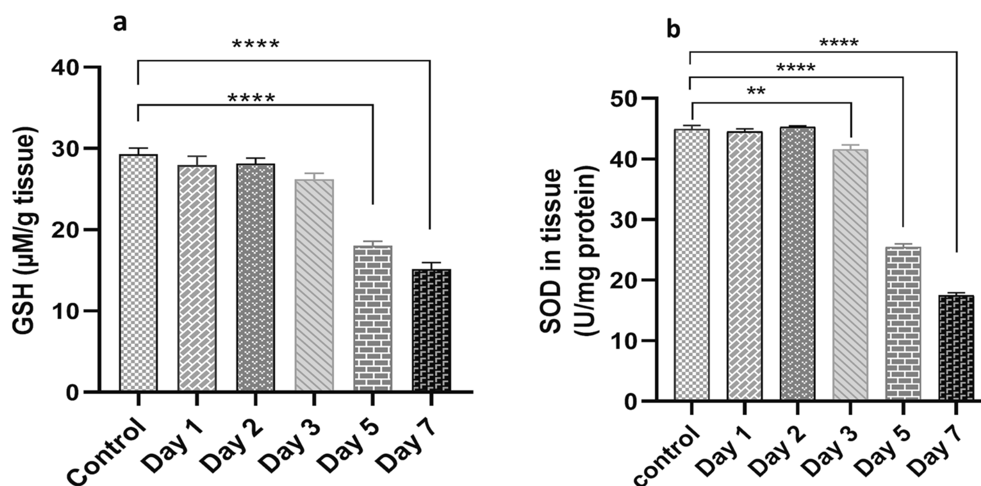


Fig. 3. Changes in the GSH (a) and SOD (b) level on the kidney tissues of cisplatin treated acute kidney injury in rats at different duration dependent study. Information presented as Mean ± SE (n = 6). Multiple two tail t tests were conducted after an Anova (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

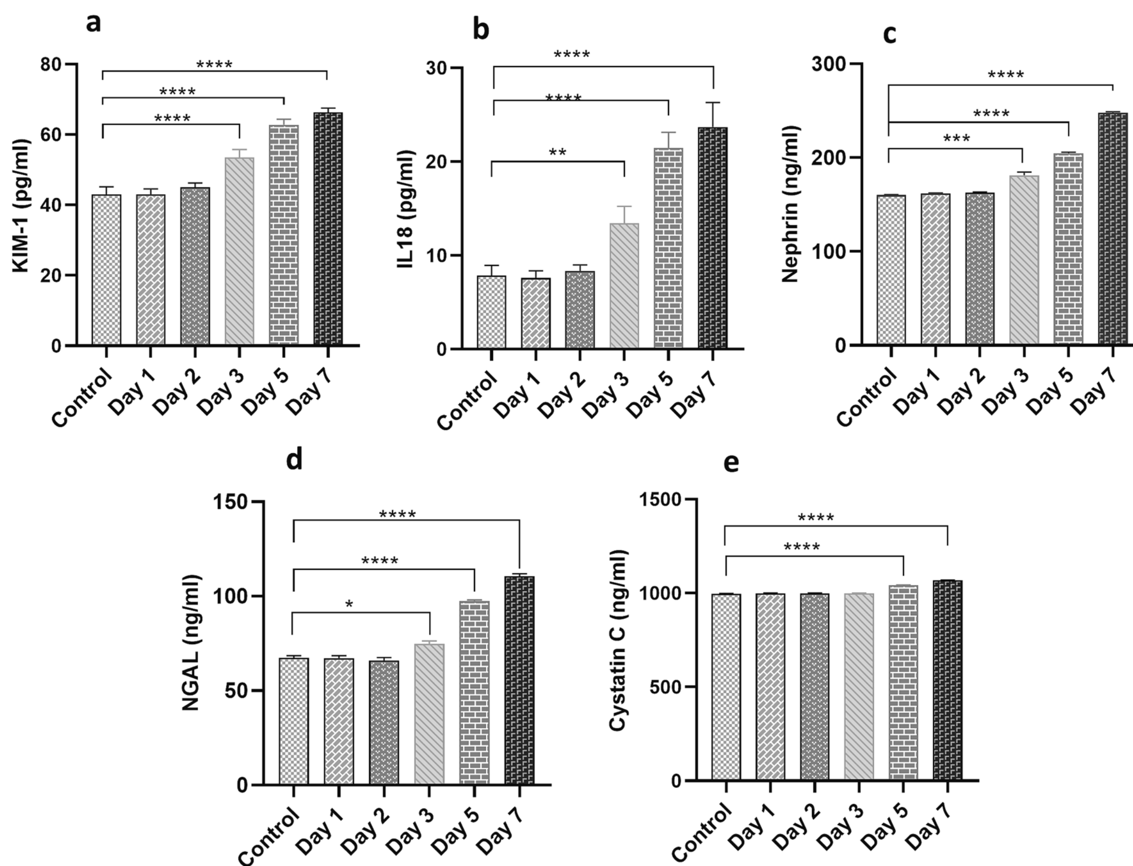


Fig. 4. Changes in the urinary KIM 1 (a), IL 18 (b), Nephrin (c), NGAL (d) and serum Cys C (e) level of cisplatin treated acute kidney injury in rats at different duration dependent study. Information presented as Mean ± SE (n = 6). Multiple two tail t tests were conducted after an Anova (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

injection of cisplatin to the rats and the protein expression further increased to day 5 and 7 as compared to control group of rats.

Relative mRNA expression levels of kidney tissue on cisplatin induced kidney injury

The results were further confirmed by related gene expression studies of the kidney tissues as shown in Fig. 6. It was showed that mRNA expression of KIM-1, IL-18, NGAL and Cys C was significantly upregulated at day 3 after intraperitoneal injection of cisplatin when compared to control and the level of expression further upregulated to day 5 and 7 respectively. Another related biomarker of acute kidney injury is nephrin and the expression of nephrin was started to down-regulated significantly from day 3 after cisplatin treatment when compared to control group. There was significant decreased expression of nephrin in cisplatin treated rats sacrificed at day 3, 5 and 7 when compared to control rats. The mRNA expression was further studied by qRT PCR (Fig. 7) as confirmatory analysis for KIM-1, IL-18 and NGAL,

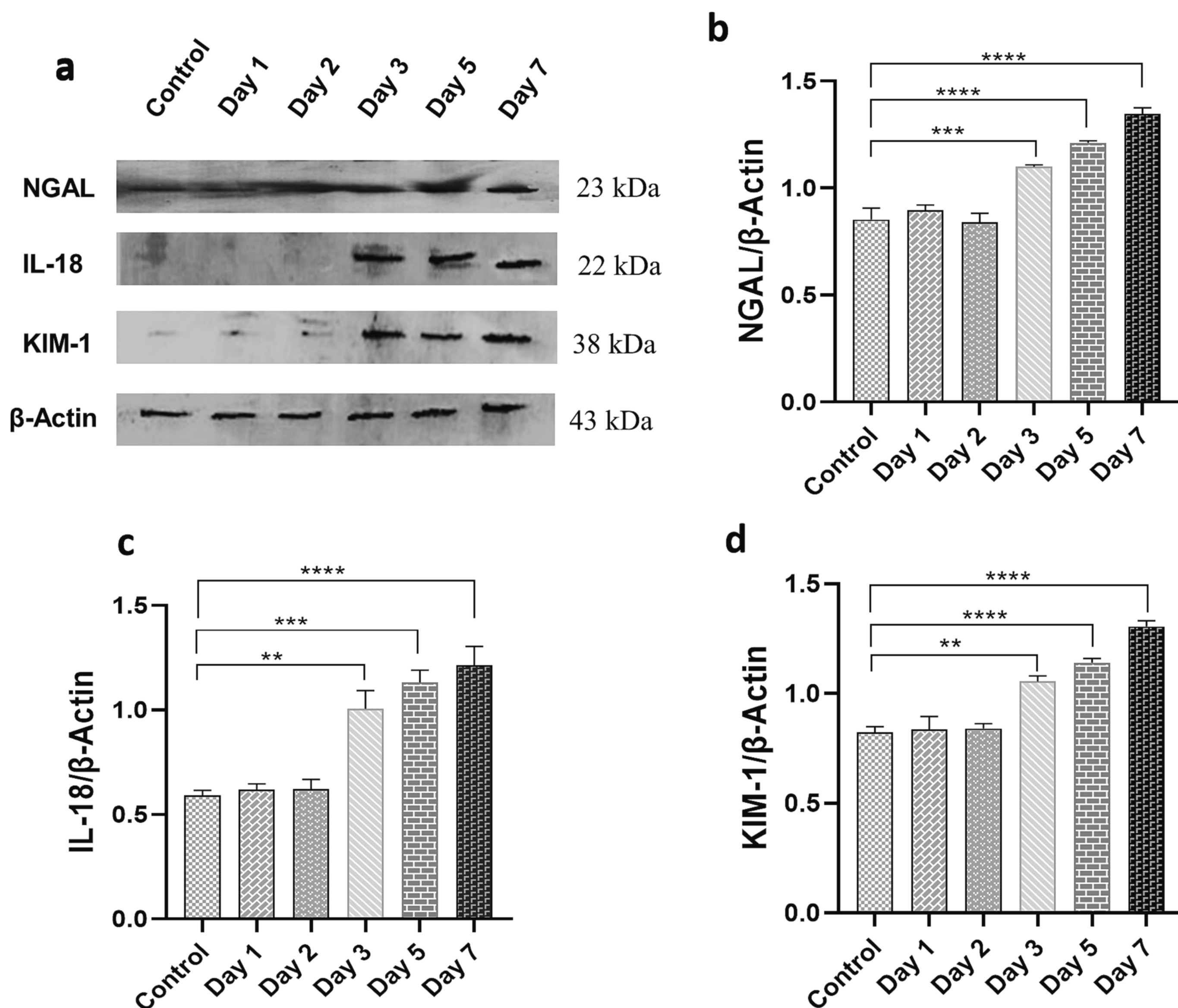


Fig. 5. The protein expression (a-d) of NGAL, IL-18 and KIM-1 was measured in kidney tissues against cisplatin induced acute kidney injury in rats at different duration dependent study were determined by western blot. Information presented as Mean ± SE (n = 6). Multiple two tail t tests were conducted after an Anova (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

and the results indicate there was a similar observation found as of qPCR results.

Histopathological alterations due to cisplatin induced nephrotoxicity

The biochemical analysis were further confirmed by the histopathological analysis of kidney tissues using H&E staining. The control group showed that glomerulus and renal tubules of kidney tissues are present with well organized histoarchitecture. Kidney tissue section of cisplatin treated rats sacrificed at day 1 and 2 showed similar histological structures with control and no evident lesion (Fig. 8 a-c). Rats sacrificed at day 3 after cisplatin treatment showed intact glomerulus but slightly widening in the tubular part and dilation was observed, presence of hyaline cast was prominent in some part (Fig. 8 d). Kidney tissue section of rats sacrificed at day 5 and 7 after cisplatin treatment showed loss of glomerular basement membrane, widening and more dilated tubules, tubular necrosis and formation of many hyaline casts specially found in day 7 (Fig. 8 e and f). Tubular injury was not seen in the control group of rats. Tubular injury scores were significantly higher in the single intraperitoneal cisplatin treated rats sacrificed at day 3, 5 and 7 than in control group (Fig. 8g). The changes in the histological architecture may be due to the adverse reaction of cisplatin injection and results renal fibrosis (Fig. 8 a and b).

Discussion

Early and differential diagnosis and treatment of AKI remain challenging due to the fact that the international consensus criteria for the classification and staging of AKI are only focused on changes in sCr, urine output (oliguria), and estimated glomerular filtration rate. Due to their lack of sensitivity and specificity as well as their delayed reaction to renal damage, these currently used indicators have some limitations. Better biomarkers are urgently required for AKI in order to diagnose it quickly, predict its severity and outcome, and monitor proximal tubule injury in both AKI and CKD (Han et al., 2009). Due to the time needed for its accumulation and equilibration, there is a delay in the detectable rise in sCr. Creatinine fluctuations can be non-specific since they might be caused by a variety of non-renal variables, including muscle mass and

Fig. 6. The mRNA expression of kidney injury related genes by qPCR (a) for KIM-1 (b), IL-18 (c), nephrin (d), NGAL (e) and Cys C (f) in kidney tissues against cisplatin induced AKI in rats at different duration dependent study. Information presented as Mean ± SE (n = 6). Multiple two tail t tests were conducted after an Anova (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

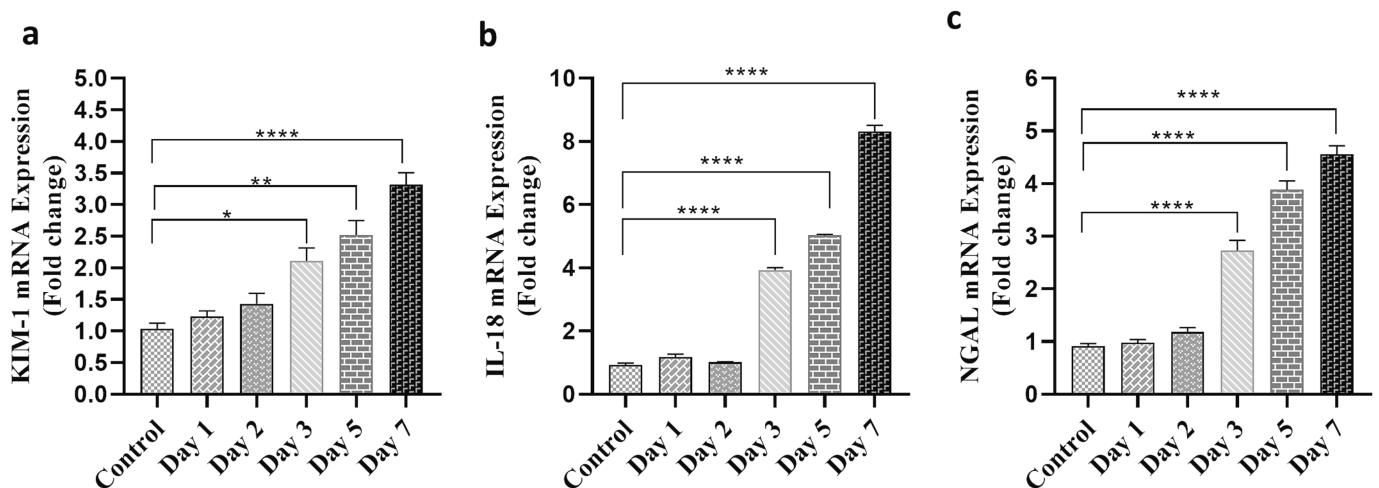
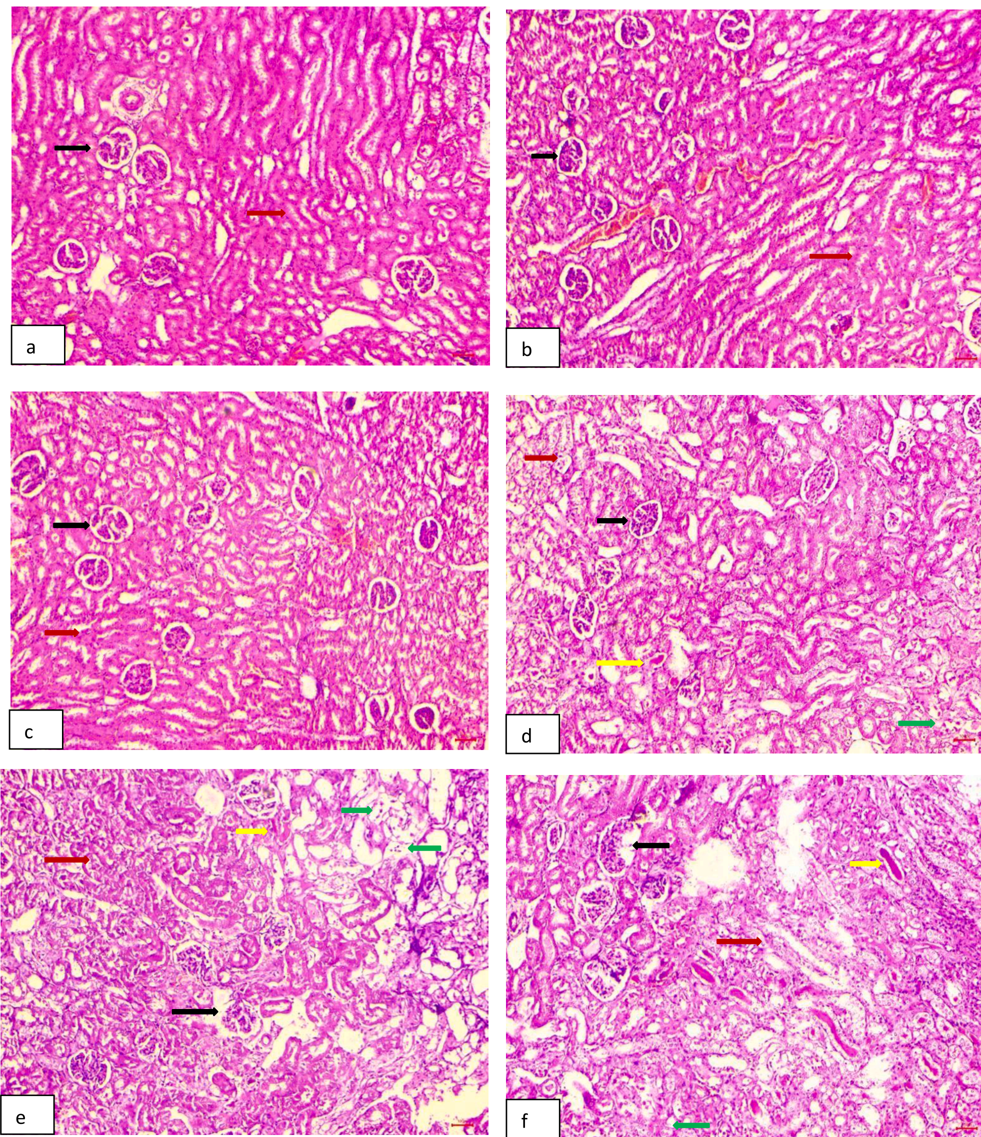


Fig. 7. The mRNA expression of KIM-1 (a), IL-18 (b) and NGAL (c) in kidney tissues against cisplatin induced acute kidney injury in rats at different duration dependent study determined by qRT-PCR. The relative gene expression was normalized to that of GAPDH. Fold changes in the related mRNA expression were presented as Mean ± SE (n = 6). Multiple two tail t tests were conducted after an Anova (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

diet (Devarajan, 2011).

The findings of this experiment demonstrated that a high dose of single intraperitoneal administration of cisplatin (10 mg/kg body weight) caused a duration-dependent decline in the functional capacity of kidney and its histoarchiecture. The majority of these impacts pronounced when cisplatin was administered at a very high dose (10 mg/kg body weight). This result strengthens the case for using an animal model of CKD called cisplatin-induced nephrotoxicity. In order to diagnose



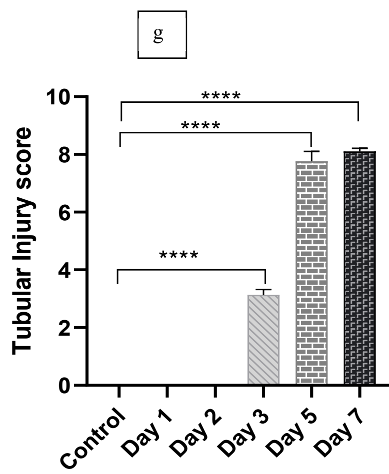


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Fig. 8. HE staining of rats kidney tissue sections in control and cisplatin treated groups under 10X. (a- control, b to f- single intraperitoneal injection of cisplatin @ 10 mg/kg body weight and sacrificed at day 1, 2, 3, 5 and 7 respectively). Control group (a) showed normal and organized histoarchitecture of kidney tissue with intact glomerulus (black arrow) and renal tubules with brush borders (red arrow) finely arranged. Kidney tissue section of cisplatin treated rats sacrificed at day 1 (b) and 2 (c) showed similar histological structures with control and no evident lesion. Rats sacrificed at day 3 (d) after cisplatin treatment showed normal glomerulus but slightly widening in the tubular part and dilation was observed, presence of hyaline cast (yellow arrow) was prominent in some part. Kidney tissue section of rats sacrificed at day 5 (e) and 7 (f) after cisplatin treatment showed loss of glomerular basement membrane (black arrow), widening and more dilated tubules (red arrow), tubular necrosis (green arrow) and formation of many hyaline casts (yellow arrow) specially found in day 7. The changes in the histological architecture may be due to the adverse reaction of cisplatin injection and results renal damage. Cisplatin causes increased in the tubular injury score (g) at day 3, 5 and 7 in kidney tissues where control group and cisplatin treated rats sacrificed at day 1 and 2 does not showed evident tubular injury score. Data were expressed as Mean ± SE ($n = 6$). Anova followed by multiple two tail t test were done (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

mild kidney dysfunction early and prevent end stage renal disease consequences, several new biomarkers were analysed.

In the present study, single intraperitoneal injection of cisplatin to the rats sacrificed at day 5 and 7 may caused a significant reduction in body weight, a significant elevation in the renal somatic index and in the weight of kidney compared to control rats. According to Ali et al. (2003), this weight loss may be the result of increased catabolism, which causes acidosis, anorexia, and decreased food intake, or direct injury to the renal tubules that prevents the tubular cells from reabsorbing water. Additionally, renal tubule injury affects the ability of the tubules to reabsorb water, causing polyuria, which is followed by dehydration and loss in body weight. According to past study, inflammation and oedema may be the causes of the rise in kidney weight following cisplatin injection (Abdelrahman, 2018).

The typical pattern of nephrotoxicity brought on by cisplatin was accompanied by a marked rise in sCr and BUN levels. The kidney's capacity to filter creatinine is reduced during renal failure as a result of decreased glomerular filtration rate, and the nonprotein waste material is created (Al Za'abi et al., 2021). The findings of this study demonstrate that intraperitoneal injections of cisplatin caused nephrotoxicity in rats, which was exhibited by an increase in serum urea and sCr levels, which indicate glomerular injury. Cisplatin actively travels from the glomerular filtrate to the renal epithelial cells, where it undergoes metabolic activation to become a more potent nephrotoxin. This disruption of cellular antioxidant defence lowers the body's antioxidant status (Ojha et al., 2016). After cisplatin reaches renal tubular cells, it induces the production of reactive oxygen spices (ROS), and because the amount of ROS produced is greater than what the body can neutralise, the redox system becomes unbalanced (Kim et al., 2019). Our findings support earlier research by demonstrating that intraperitoneal injection of cisplatin caused oxidative stress in kidney tissue, as shown by the marked decline in SOD and GSH levels. These results point to oxidative pathways as a significant factor in the cell membrane damage caused by cisplatin, and they may be related to a decline in the activities of antioxidant enzymes (Abo-Elmaaty et al., 2020).

KIM-1 is a membrane-spanning protein that is found in the renal tubules. Its expression is dramatically increased in cases of acute nephrotoxic kidney damage, and its levels change earlier than those of any other common renal injury markers (McDuffie et al., 2013; Khreba et al., 2019). According to earlier research, urine KIM-1 excretion levels have a good biomarker for detecting renal injury. According to Bonventre, (2009), it was reported that KIM-1 protein in tissue specimens from 102 patients who underwent a kidney biopsy for a variety of kidney diseases and showed that positive KIM-1 staining in dedifferentiated proximal tubular cells correlated with tubulo-interstitial fibrosis and inflammation. Another pro-inflammatory cytokine increased and implicated in acute tubular necrosis in AKI is IL-18. It has been shown via studies on both humans and animals that this interleukin can cause acute tubular necrosis of the kidney. Numerous clinical investigations have concentrated on the IL-18 level's ability to accurately predict AKI in terms of diagnosis. IL-18 disruption could therefore be a risk factor for AKI. NGAL which may be detected in plasma and urine, is a biomarker that is specific for AKI. Kidney epithelial cells under stress mostly release monomeric NGAL, which they are unable to dimerize. NGAL levels in the urine are typically very low because filtered NGAL is almost entirely reabsorbed by the proximal tubules through megalin-cubulin receptor-mediated endocytosis. Monomeric NGAL levels rise either as a result of enhanced tubular cell production or as a result of poorer reabsorption of the extrarenal tissues' filtered burden. Neutrophil infiltration in the kidney has also been seen in animal models of AKI. Experimental ischaemic kidney injury causes upregulation of NGAL gene expression and protein synthesis, which predominantly seems to occur in renal distal tubular epithelial cells (Kovacevic et al., 2021; DU et al., 2014; Jana et al., 2022; Li et al., 2019). Similar to the previous studies, this one revealed that after receiving a single intraperitoneal injection of cisplatin (10 mg/kg body weight), the level of KIM-1, nephrin, IL-18, and NGAL in the urine was considerably higher at day 3. These values were further increased to a very high level at day 5 and 7 that confirms the slow progression of renal failure. And also the protein expression level of KIM-1, IL- 18 and NGAL on kidney tissue were confirmed by western blotting, indicated that cisplatin causes nephrotoxicity after single intraperitoneal injection and these expressions were significantly increased on day 3 of cisplatin treatment and further increased to a very high level at day 5 and 7. Current research indicate that cisplatin accumulation in the kidney released more kidney injury markers like KIM-1, IL-18 and NGAL which contributed to development of nephrotoxicity.

Nephrin contributes to maintaining the glomerular basement membrane's integrity as it is structural protein of the podocyte. The early phases of kidney damage include podocyte injury. Nephrin sheds off from the podocytes and is discharged into the urine when the binding of skeletal protein to the nephrin protein on the surface of podocytes is compromised (Xu et al., 2020). As a result, a key player in the aetiology of renal failure is podocyte dysfunction. In support to that, this experiment showed that the urinary nephrin level was significantly elevated at day 3 after cisplatin treatment and continue to increase at day 5 and 7, whereas the kidney tissue expression of nephrin was significantly decreased at day 3 and the decreased expression of this protein may aid in the pathophysiology of AKI and the development of glomerular disease (Senouthai et al., 2019). Nephrin displacement from the slit area disturbs the renal barrier. Nephrin, for instance, has been discovered to be redistributed to the surface of podocytes in glomerular proteinuric disorders such minimal change disease. Massive proteinuria is caused by the complete lack of nephrin from slit diaphragms in Finnish type congenital nephrotic syndrome. Extreme proteinuria and structural alterations to the podocytes that result in diminished expression of nephrin are caused by podocin gene mutations (Agrawal et al., 2013), which may be the outcome of cisplatin-induced nephrotoxicity.

All nucleated cells continuously produce and release the protein Cys C, which is entirely reabsorbed and destroyed by renal tubules but is not secreted. The glomerulus may freely filter Cys C because of its small size and positive charge. In light of this, serum Cys C is also thought to serve as an early diagnostic marker of AKI that can indicate the beginning of abnormalities in renal function (Lagos-Arevalo et al., 2015). In this experiment serum Cys C level was significantly elevated only at day 5 and continue to increase at day 7 when other conventional biomarker levels were also increased.

These biomarkers are released from the kidneys tissues during very minimal injury, there was alterations in the expression of these markers and the findings were further confirmed by the mRNA expressions done

by qPCR & qRT PCR analysis. Genetic deletion of caspase-1, which cleaved IL-18 to make it active (Meng et al., 2017), is crucial in cisplatin mediated toxicity. A recent study indicated that the expression of KIM-1, IL-18 and NGAL was significantly increased in unilateral ureteric obstruction in mice. The mRNA expression of Kim-1, IL-18 and NGAL was significantly increased after cisplatin treatment are considered to be important markers for evaluating renal tubular damage. A study showed that in hyperuricemic nephropathy condition the increased mRNA expression of KIM-1, IL-18, NGAL and Cys C indicated inflammation, damage of renal tubular cells (Xu et al., 2021). In this study, mRNA and protein expression levels of KIM-1, IL-18 and NGAL were upregulated in the cisplatin treated group that indicated damages occurred in the tubular cells and inflammation of kidney tissues. The mechanisms associated with cisplatin-induced AKI are complex, cisplatin accumulation triggers increased production of reactive oxygen compounds, stimulating inflammation, oxidative stress that increased release of various biomarker like KIM-1, IL-18, NGAL and Cys C that promote renal tissue damage leading to the key clinical manifestation of nephrotoxicity (Griffin et al., 2019).

An examination of the kidneys' histology revealed that cisplatin caused some tubular dilatation and localized epithelial cell death throughout constrained regions of the cortex. On the other hand, it caused pyknotic nuclei and chronic, significant cytolysis of epithelial cells in the outer medulla. The inner medulla showed dilatation of tubules as well as capillaries that linked with tubules. Animals given cisplatin demonstrated renal tissue damage in the form of lesions and tubular dilatation. Which is in line with a previous study conducted by Saifi et al. (2019). Current study found that intraperitonial injection of cisplatin at high doses results damage to the glomerulus and renal tubules that leading to fibrosis started after 3 days of cisplatin injection and a severe disorientation were observed after 7 days due to the nephrotoxicity of this drug.

Moreover the results suggest that urinary KIM-1, IL-18, nephrin and NGAL was significantly upregulated 48 h prior to the increase of sCr and BUN level. This investigations have demonstrated that KIM-1, IL-18, nephrin and NGAL are far more sensitive kidney damage markers than BUN and creatinine.

Nearly 30 % of patients experience nephrotoxicity, the most frequent side effect of cisplatin. The proximal tubular interior medulla and outer cortices of the glomerular basal membrane are where cisplatin builds up because of its low molecular weight (Farooqui et al., 2017). The pathophysiological action of cisplatin on the kidneys is exerted by oxidative stress, inflammation, and vascular constriction of the renal vascular structure, which results in necrosis and apoptosis in proximal tubule cells. In this experiment, cisplatin-induced nephrotoxicity results in kidney damage starting on day 3 after a single injection of cisplatin and the cellular damage in the tubules and glomerular parts keeps escalating on days 5 and 7 as shown by the histological analysis. This suggests that the kidney cellular damage may be caused by a comparatively long term/ high dose usage of cisplatin.

Conclusions

Current study found that KIM-1, IL-18, nephrin and NGAL levels in the urine are highly specific biomarkers for cisplatin-induced nephrotoxicity in male albino rats. This shows the sensitivity of employing these biomarkers in assessing drug-induced nephrotoxicity at very initial stage when the symptoms of kidney dysfunction were not observable. It is interesting that all of these kidney biomarkers had earlier-onset alterations than either blood BUN and sCr. According to relative gene and protein expression of these biomarkers (KIM-1, IL-18 and NGAL), nephrotoxicity can be detected prior to an increase in the common biomarkers. Before BUN and sCr levels raise and pathological tubular cell injury happened, we tried to build a new system to early quantify drug-induced nephrotoxicity. This method may be used to study kidney injury and as a means of early detection for drug-induced nephrotoxicity.

Future prospectus

Although KIM-1, IL-18 and NGAL have a promising future as biomarkers, further research into its function in acute and chronic renal diseases will be especially fascinating because it could eventually be useful not only as a biomarker but also as a therapeutic target.

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CRediT authorship contribution statement

Sahadeb Jana: Methodology, Investigation, Project administration. **Palash Mitra:** Software, Resources. **Ananya Dutta:** Software, Resources. **Amina Khatun:** Software, Resources. **Tridip Kumar Das:** Software, Resources. **Shrabani Pradhan:** Validation, Visualization, Supervision. **Dilip Kumar Nandi:** Validation, Visualization, Supervision. **Suchismita Roy:** Conceptualization, Writing – review & editing, Funding acquisition, Formal analysis, Writing – original draft, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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