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ORIGINAL ARTICLE

Griffonia simplicifolia (DC.) Baill. attenuates gentamicin and cisplatin-induced nephrotoxicty in rats

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Abstract

Nephrotoxicity is one of the most common kidney conditions. However, most conventional drugs are not adequate for treatment. This study was designed to evaluate the nephroprotective activity of 50% hydroethanolic leaf extract of *Griffonia simplicifolia* (DC.) Benth in drug-induced nephrotoxicity in Sprague–Dawley rats. Nephrotoxicity was induced in experimental animals by administering gentamicin and cisplatin after pre-treatment with hydroethanolic extract of *G. simplicifolia* (GSE). GSE at 100 and 250 mg/kg were administered for 7 and 10 days by oral gavage in the gentamicin and cisplatin models, respectively. Silymarin (120 mg/kg) was given as the standard nephroprotective drug. Nephroprotective effect was studied by assaying the activity of kidney function biomarkers such as creatinine, urea, sodium, chloride, and potassium concentrations. The effect of the treatments on kidney antioxidant enzymes (SOD, MDA, GSH, GPx, GST and NO), inflammatory cytokines (IL 17, IL 23 and COX-2) and the histology of the kidney were also examined. The activity of all the kidney function biomarkers changed significantly in gentamicin and cisplatin-treated rats; increased in urea and creatinine concentration and decreased in Na, K and Cl concentrations. Co-administration of GSE with the nephrotoxins restored these to normal levels. It also reduced NO concentration in both the gentamicin and cisplatin model and increased GPx concentration in the gentamicin model. GSE showed a higher percentage protection than silymarin, a standard nephroprotective drug, in both the gentamicin and cisplatin models. Intensity of structural alterations revealed that the GSE treatments have a protective potential against nephrotoxicity. GSE treatments improved expressions of IL17 and IL23, thus underscoring the proinflammatory and healing properties of GSE, respectively. The results generally indicate that leaves of *G. simplicifolia* possess nephroprotective activity.

Keywords *Griffonia simplicifolia* · Nephroprotective activity · Gentamicin · Cisplatin

Introduction

Nephrotoxicity is one of the most common kidney problems of humans globally. It is the poisonous effect of some toxic chemicals and medication on the kidneys (Chatterjee et al. 2012). It can result in renal conditions such as acute renal failure, chronic interstitial nephritis and nephritic syndrome (Gaikwad et al. 2012). Nephrotoxicity is often temporary but it can also cause permanent damage to the kidney if not detected and treated early. Commercial drugs that have nephrotoxicity as a dose-limiting side effect include chemotherapy drugs such as cisplatin, carmustine, carboplatin, mitomycin and methotrexate; aminoglycoside antibiotics

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such as amphotericin B, gentamicin and vancomycin; non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and diuretics such as furosemide (Chatterjee et al. 2012; Gaikwad et al. 2012; Asci et al. 2017).

Treatment and prevention of nephrotoxicity involve the use of nephroprotective agents (James et al. 2010), which possess protective activity against nephrotoxins (Gaikwad et al. 2012). Several studies have reported the nephroprotective activity of antihypertensive drugs of the angiotensin-converting enzyme inhibitor (ACEI) and angiotensin II receptor blockers (ARBs) class including captopril and losartan (Salvetti et al. 1999; Wenzel 2005 de Zeeuw and Lambers-Heerspink 2009; Hsu et al. 2017). Antibiotics such as fosfomycin and fleroxacin and calcium channel blockers such as nifedipine and amlodipine have also been reportedly used to treat nephrotoxicity (Balakumar et al. 2010). However, these conventional drugs have become expensive for the treatment of nephrotoxicity, hence the need for cheaper alternatives.

Medicinal plants have also been instrumental in the treatment of kidney diseases and still represent a huge source of new molecules to promote drug treatment (Ghayur and Janssen 2010). Currently, a substantial body of evidence suggests that some plants possess pharmacological properties that help to impede the progress of kidney diseases (Peng et al. 2005). Some of these properties are anti-inflammation, antioxidation, immunomodulation, prevention of drug-induced nephrotoxicity, reduction in proteinuria, renal ischemia, lipid peroxidation, apoptosis and renal necrosis (Ghayur and Janssen 2010). The folkloric use of some plants as nephroprotective agents has also been reported (Shirwaikar et al. 2003: Ali 2004; Badary et al. 2005; Chirino et al. 2008; Chatterjee et al. 2012; Adil et al. 2016; Verma et al. 2016; Asci et al. 2017).

Griffonia simplicifolia, locally known as 'kagya' in Ghana, is one of the many African legumes reported in African folklore to have nephroprotective activity. It is native to West Africa but it is primarily found in Ghana, Cote d'Ivoire and Togo (Esposito et al. 2012; Pathak et al. 2010). Apart from the seed which is popularly used in the treatment of depression, obesity, insomnia, fibromyalgia and migraine (Esposito et al. 2012; Wang et al. 2017), leaf extract of *G. simplicifolia* is also reportedly used for treating malaria, bladder and kidney problems, for relieving constipation, as an aphrodisiac and a remedy for cough (Pathak et al. 2010, Offoumou et al. 2018). The usage of the seed is due to its high concentration of 5-hydroxyl-L-tryptophan (5-HTP). Offoumou et al. (2018) has reported the presence of sterols, quinones, alkaloids and saponins in the aqueous and ethanolic extract of Griffonia leaf. A recent study by Nyarko et al. (2019) also reported that the ethanolic extract of the leaves of *G. simplicifolia* contains glycosides, tannins, flavonoids, alkaloids, saponins and coumarins. They also indicated that the median acute toxicity (LD_{50}) of the extract was < 5 g/kg body weight in mice whilst sub-chronic use for 28 days resulted in significant weight gain, reduction in platelet large cell ratio and platelet count and increase in low density lipoprotein (LDL) and blood glucose concentrations. Despite the increasing use, the nephroprotective activity of the species in drug-induced kidney damage has not been evaluated. This study was thus aimed at evaluating the nephroprotective activity of hydroethanolic extracts of leaves of *G. simplicifolia* against gentamicin and cisplatin-induced nephrotoxicity in Sprague-Dawley rats. Our hypothesis was that *Griffonia simplicifolia* leaves will attenuate nephrotoxicity in rats.

Materials and methods

Collection and identification of plant materials

Leaves of *G. simplicifolia* were handpicked from the Kwame Nkrumah University of Science and Technology (KNUST) Botanic Garden, Kumasi (latitude 6° 35 N-6° 40 N and longitude 1° 30 W-1° 35 W) before 9:00 a.m. each sampling day, in November 2017. Plant identification was authenticated by a taxonomist at the Department of Theoretical and Applied Biology, KNUST, and a voucher specimen (KNUST/AB/2018/L9544) was deposited in the herbarium for reference purposes. The leaves were thoroughly washed with water and air-dried at room temperature under shade for 3 weeks. The dried samples were then milled and packaged in zip-locks for storage.

Preparation of plant extract

Hydroethanolic extract of the milled leaves was prepared by suspending the leaves in 50% ethanol (50:50, ethanol:water, *v*/*v*) as previously described by Anim et al. (2016). The extraction was done by cold maceration for 48 h at room temperature on a shaker (Rocking Laboratory Shaker, Ohaus, USA). The procedure was repeated twice. The extract was filtered through cotton wool and filtrate concentrated using a rotary evaporator (Buchi R-205, Switzerland) under reduced pressure and freeze-dried (Labconco, England) to obtain the *Griffonia simplicifolia* ethanolic leaf extract (GSE).

Animal selection and groupings

Sprague-Dawley rats of both sexes were obtained from the animal house of the School of Medical Science, University of Ghana, Legon Accra. The animals were housed in aluminium rodent cages with bedding of wood shavings. They were kept under standard conditions (25 ± 2 °C, 40–60% humidity and ~ 12 h light and dark cycle) and fed with standard animal feed (AGRICARE, Kumasi, Ghana) and distilled water ad libitum throughout the period of the study except an overnight fast prior to sacrificing.

The animals were grouped mainly based on their body weights to achieve approximately equal conditions among the groups. They were allowed to acclimatise to laboratory conditions for a week before the experiment begun. The animals were identified by tail marks made with permanent markers. Animal studies were conducted in the animal holding facility of the Department of Biochemistry and Biotechnology (KNUST, Ghana) and in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA, New Delhi, India) and the guide for the care and use of laboratory animals (Washington, USA).

Experimental design

This experiment was conducted in two experimental models of drug-induced nephrotoxicity according to the method described by Chatterjee et al. (2012) with some modification: gentamicin model and cisplatin model. In each of the models, the rats were put into five (5) groups of five (5) rats. Before the experiment commenced, the rats were fasted overnight. The gentamicin model experiment lasted for 7 days whilst the cisplatin model experiment lasted for 10 days.

In each model, group I served as the control and were orally administered with 1.0 ml of distilled water each day; group II was treated with nephrotoxin, group III with the standard drug (silymarin) and groups IV and V were treated with 100 mg/kg body weight (b.wt) and 250 mg/kg b.wt GSE, respectively. In the gentamicin model, rats in groups IV and V were pre-treated with single daily oral administration of 100 mg/kg b.wt and 250 mg/kg b.wt of GSE, respectively, from day 1 to 7. From day 2 to day 7, they received an intramuscular injection of 120 mg/kg b.wt gentamicin an hour after the extract was given. Group III animals were treated with 120 mg/kg b.wt silymarin as standard nephroprotective agent 1 h before the intramuscular injection of 120 mg/kg body weight of gentamicin each day. Group II animals were also dosed with 120 mg/kg body weight of gentamicin by intramuscular injection from day 2 to day 7.

In the cisplatin model, group III rats were treated with 120 mg/kg of silymarin as standard nephroprotective agent. Group IV and V rats were treated with single daily oral dose of 100 mg/kg and 250 mg/kg b.wt of GSE, respectively, for 10 days. Groups II, III, IV and V were given a single intraperitoneal injection of 7.0 mg/kg body weight of cisplatin on the third day of the experiment. Body weights of the animals were recorded on day 0 for both experimental models, and on days 7 and 10 for the gentamicin and cisplatin models, respectively.

Collection of blood, serum and isolation of organs

At the end of the experiment, animals were fasted overnight and sacrificed by ether anaesthetization. The neck area was quickly cleared of fur to expose the jugular vein. The vein, after being slightly displaced was sharply cut with a sterile surgical blade, and an aliquot of the blood was collected in sample bottles containing EDTA for the haematological analyses. Five millilitres of blood was then dispensed into gel-activated tubes and centrifuged at 3000 rpm for 5 min. The sera were aspirated with a Pasteur pipette into sample bottles for the various biochemical assays. The sacrificed animals were dissected, and the kidneys were excised, freed of fat, washed with normal buffered saline and blotted with clean tissue paper. They were weighed to obtain absolute organ weight (AOW). The relative organ weights (ROW) of the kidneys were calculated for each rat using the formula:

$$\mathrm{ROW} = \frac{\mathrm{AOW}}{\text{body weight on day of sacrifice}} \times 100\%$$

Biochemical and haematological analyses

Haematological analyses were performed using Sysmex Haematology System (USA). Parameters included red blood cell count, haemoglobin concentration and white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration (MCHC).

Evaluation of biochemical parameters was done using the Selectra E (Vital Scientific, Japan) and reagents from ELITECH (France). Parameters analysed were alanine aminotransferase (ALT), creatinine, urea, sodium, potassium and chlorine.

Histology and kidney antioxidant assays

One kidney of each rat was stored in 10% buffered formalin and processed for histological examination. They were stained with haematoxylin and eosin. Photomicrographs were taken and analysed. The other kidneys were stored in phosphate-buffered saline (PBS). These kidney tissues were homogenised separately in 10 ml of 100 mM KH_2PO_4 buffer containing 1 mM EDTA, pH 7.4 and centrifuged at 12,000 rpm for 30 min at 4 °C. The supernatant was collected and used for kidney antioxidant assays including SOD, MDA, GSH, GPx and GST.

Immunohistochemistry

The immunohistochemical staining was performed using the Streptavidin-biotin peroxidase complex kit (M IHC Select Detection System, HRP/DAB, Merck, Germany LOT: 2775482) with slight modification of the procedure as previously described by Jarikre and Emikpe (2017). Thin sections (4 μm) of each kidney sample were cut in triplicates, floated and mounted on APES charged glass slides. Each section was deparaffinised before being placed in citrate buffer solution (10 mM citric acid, pH 6.0) for antigen retrieval in microwave for 8 min. The sections were incubated in 70% methanol with 3% H_2O_2 for 10 min to inhibit endogenous peroxidase activity, and were then washed three times in phosphate buffered saline (PBS). The sections were then treated with blocking solution for 10 min. After draining of blocking serum, each of the sections was individually incubated with primary antibodies: monoclonal mouse Cyclooxygenase COX antibody (sc-19999, Lot # L1113), interleukin IL 17 antibody (SAB3701439, Lot R127357) and interleukin IL 23 antibody (Cat. # 06-1079, Lot # 2914943), respectively, at a dilution of 1:100 in PBS, and incubated at 4 °C overnight in a humidified chamber. After washing three times with PBS, the sections were treated with biotinylated anti-mouse polyvalent secondary antibody for 10 min. The sections were washed three times in PBS and treated with the peroxidase-conjugated streptavidin for another 10 min. After another PBS bath, the sections were incubated with 3,3 diaminobenzidine (DAB), washed in tap water following colour change and counterstained with Mayer's haematoxylin. Tissue sections from the control rats served as negative controls. The slides were mounted with cover slips and DPX for examination.

Grading technique: The photomicrographs were taken with the aid of digital camera (Amscope MU900) attached to the microscope. The images were quantified for staining intensity using reciprocal intensity of the stained markers (COX-2, IL17, IL24) on the open source Fiji (ImageJ) software. The optical density of the staining intensities was calculated using the formula:

$$\mathrm{OD} = \log_{10}\left(\frac{\text{maximum reciprocal intensity}}{\text{mean reciprocal intensity}}\right)$$

Cut off for the OD values was set at 50. Immuno-positive staining was then graded as weak (50–100), moderate (101–200) and strong (> 200).

Percentage protection was calculated using the formula below:

$$\%\text{Protection} = \frac{\text{Values of toxin control} - \text{Values of test sample}}{\text{Values of toxin control} - \text{Values of normal control}}$$

Data analyses

Data were analysed with GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA). The experimental results (body weights, haematological parameters, serum biochemistry and antioxidants) were expressed as mean ± standard error of the mean (SEM). Data were assessed by one-way ANOVA followed by Tukey's multiple comparison test. All analyses were conducted assuming a significance value of 5%.

Results

Effects of treatments on relative kidney weight

In the gentamicin model, the nephrotoxin significantly increased the relative kidney weights (Fig. 1; $p < 0.05$). However, co-administration with silymarin and GSE decreased relative kidney weight compared to the gentamicin control. No significant changes were observed in the cisplatin model.

Effects of treatments on haematological parameters

None of the haematological parameters showed any significant changes except for platelet count (Table 1). In both models, platelet (PLT) count decreased significantly ($p < 0.05$) in all treatment groups relative to the control. In the gentamicin model, the 250-mg/kg GSE group had a higher concentration than both silymarin and 100-mg/kg group. On the contrary, in the cisplatin model, the 100-mg/kg group had the highest PLT ($1233.33 \times 10^3/\mu L$), second only to the control group whereas the silymarin had the lowest count ($701.33 \times 10^3/\mu L$).

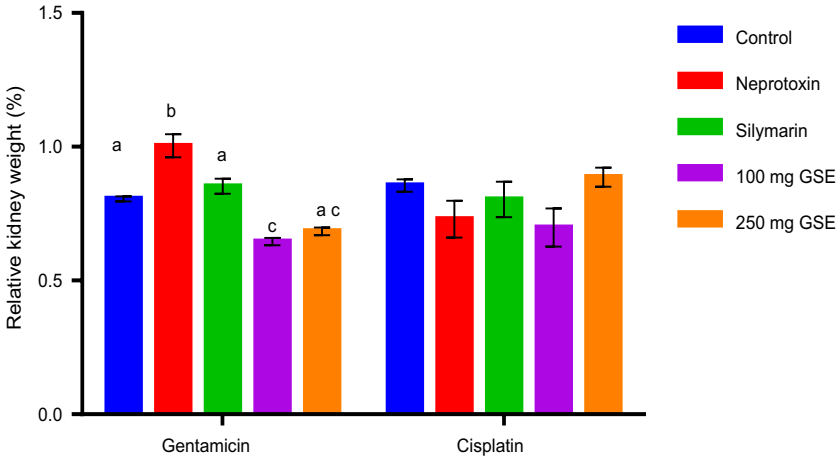

Fig. 1 Relative kidney weights of animals in nephrotoxicity study. Each point represents a mean of five animals. Mean ± SEM; different letters indicate significant difference at $p \leq 0.05$

Effects of treatments on serum biochemical parameters

Administration of nephrotoxins resulted in significant increases in kidney function biomarkers (i.e., creatinine and urea). Furthermore, gentamicin caused significant reduction in the levels of electrolytes (i.e., Na, K and Cl). Administration of silymarin and GSE at all doses resulted in restoration of these parameters to normal levels (Table 2). Silymarin, however, could not restore creatinine to normal levels in the gentamicin model.

Protection of *G. simplicifolia* against gentamicin and cisplatin

Percentage protection was based on major indicators of kidney function: creatinine, urea and relative kidney weight (Fig. 2). GSE at 100 mg/kg recorded the best protection in both the gentamicin and cisplatin models (100% and 98.10%, respectively). The 250-mg/kg GSE group followed with 57.23% and 95.50% for gentamicin and cisplatin, respectively. Silymarin had the least protection in both models (31.30% for gentamicin and 82.01% for cisplatin).

Effects of treatments on the kidney antioxidant defence system

Treatments showed no significant effects on the oxidants and antioxidants assessed except nitric oxide (NO) and glutathione peroxidase (GPx) (Table 3). Gentamicin increased the concentration of NO, though not significantly. GSE treatments at 100 mg/kg and 250 mg/kg significantly reduced the concentration of NO when compared to both the control and gentamicin only groups. GSE also significantly increased the concentration of GPx compared to all other treatment groups. The 250 mg/kg treatment recorded the highest GPx of 372.48 U/mg prot whilst the gentamicin only group recorded the least (231.84 U/mg prot). Silymarin recorded a higher GPx concentration than gentamicin group but was not significantly different compared to the control group.

Treatment with the cisplatin control, 100-mg/kg and 250-mg/kg GSE groups significantly reduced the concentration of GPx, with the 100-mg/kg GSE group recording the least concentration of 221.40 U/mg prot. Silymarin recorded the highest concentration of 379.35 U/mg prot among all the treatment groups.

Effects of treatments on kidney histology

Microscopic examination of kidney tissue on gentamicin administration showed membrano-proliferative glomerulonephritis and patchy tubular necrosis and cellular infiltrates as compared to normal kidney tissue (Fig. 3a, b). There were no observable lesions in histopathological slides on co-treatment with silymarin compared with normal kidney tissue (Fig. 3c). Co-treatment of gentamicin with hydroethanolic leaf extract of *G. simplicifolia* revealed a mild presence of patchy tubular epithelial degeneration and necrosis and focal glomerular atrophy (Fig. 3d). Intensity of structural alterations in microphotographs revealed that the GSE treatments had a protective potential against gentamicin-induced nephrotoxicity.

Cisplatin treatment exhibited severe histological changes such as tubular epithelial necrosis and cast in tubular lumen with a few peritubular inflammatory cells as well as tubular degeneration and ectasia (Fig. 3e). However, the protective effect of silymarin was further supported by the lack of observable lesions in the kidney tissue (Fig. 3f). Co-treatment of cisplatin with GSE reduced the severity of the histopathological changes

Table 1 Effects of treatments on haematological parameters of animals in nephrotoxicity study

Parameter	Mean concentration ± SEM				
	Control	Nephrotoxin	Silymarin	100 mg GSE	250 mg GSE
Gentamicin					
WBC (10^3/μL)	7.08 ± 0.73	6.44 ± 0.94	7.58 ± 0.45	7.72 ± 0.51	5.94 ± 0.94
RBC (10^6/μL)	6.91 ± 0.06	6.62 ± 0.30	6.47 ± 0.17	7.52 ± 0.21	6.55 ± 0.24
HGB (g/dL)	12.88 ± 0.17	12.48 ± 0.62	12.10 ± 0.23	13.62 ± 0.37	12.18 ± 0.53
LYM (%)	77.56 ± 3.38	55.44 ± 7.10	63.62 ± 3.64	63.54 ± 2.42	58.82 ± 6.18
HCT (%)	47.68 ± 0.52	44.68 ± 2.40	43.66 ± 1.01	51.06 ± 1.43	43.52 ± 1.76
MCV (fL)	69.02 ± 0.43	67.36 ± 0.70	67.54 ± 0.85	67.90 ± 0.28	66.44 ± 0.82
RDW-SD (fL)	40.16 ± 0.60	33.42 ± 1.96	33.72 ± 0.88	37.16 ± 0.70	31.74 ± 0.51
MCH (pg)	18.64 ± 0.24	18.82 ± 0.21	18.70 ± 0.28	18.12 ± 0.09	18.56 ± 0.16
MCHC (g/dL)	27.04 ± 0.41	27.98 ± 0.42	27.72 ± 0.25	26.68 ± 0.11	27.98 ± 0.27
NEUT (%)	22.44 ± 3.38	44.56 ± 7.10	36.38 ± 3.64	36.46 ± 2.42	41.18 ± 6.18
RDW-CV (%)	14.90 ± 0.50	11.54 ± 0.81	11.80 ± 0.40	13.34 ± 0.31	10.96 ± 0.25
PDW (fL)	8.64 ± 0.14	8.98 ± 0.20	9.00 ± 0.23	8.44 ± 0.16	8.86 ± 0.11
MPV (fL)	7.48 ± 0.09	7.58 ± 0.12	7.64 ± 0.15	7.30 ± 0.09	7.54 ± 0.07
P-LCR (%)	8.02 ± 0.51	8.86 ± 0.87	9.18 ± 0.93	7.22 ± 0.37	8.80 ± 0.31
LYM (10^3/μL)	5.58 ± 0.78	3.50 ± 0.50	4.78 ± 0.19	4.90 ± 0.39	3.66 ± 0.91
NEUT	1.50 ± 0.16	2.94 ± 0.74	2.80 ± 0.42	2.82 ± 0.23	2.28 ± 0.23
PCT (%)	1.22 ± 0.10	0.90 ± 0.08	0.96 ± 0.09	0.85 ± 0.08	1.08 ± 0.12
PLT (10^3/μL)	1625.60 ± 133.97[a]	1186.20 ± 115.75[b]	1258.40 ± 105.05[b]	1160.00 ± 108.98[b]	1431.40 ± 165.12[c]
Cisplatin					
WBC (10^3/μL)	7.08 ± 0.73	8.20 ± 0.12	5.40 ± 0.49	8.70 ± 0.00	9.47 ± 1.55
RBC (10^6/μL)	6.91 ± 0.06	7.69 ± 0.01	7.57 ± 0.12	7.44 ± 0.41	5.75 ± 0.32
HGB (g/dL)	12.88 ± 0.17	14.13 ± 0.03	13.53 ± 0.18	13.73 ± 0.24	11.07 ± 0.37
LYM (%)	77.56 ± 3.38	64.23 ± 2.31	67.10 ± 3.54	74.77 ± 1.37	52.50 ± 5.56
HCT (%)	47.68 ± 0.52	46.63 ± 0.70	45.97 ± 0.41	46.57 ± 0.64	36.63 ± 1.75
MCV (fL)	69.02 ± 0.43	61.10 ± 0.93	60.30 ± 0.38	65.03 ± 0.92	61.40 ± 0.67
RDW-SD (fL)	40.16 ± 0.60	32.60 ± 1.22	32.37 ± 0.90	26.63 ± 0.91	26.47 ± 0.45
MCH (pg)	18.64 ± 0.24	18.30 ± 0.06	17.73 ± 0.03	18.63 ± 0.83	19.20 ± 0.40
MCHC (g/dL)	27.04 ± 0.41	29.97 ± 0.47	29.40 ± 0.12	28.40 ± 1.14	30.90 ± 0.49
NEUT (%)	22.44 ± 3.38	35.40 ± 2.34	32.43 ± 3.64	26.07 ± 1.19	46.53 ± 5.79
RDW-SD (fL)	14.90 ± 0.50	13.20 ± 0.35	13.03 ± 0.67	10.00 ± 0.15	8.90 ± 0.42
PDW (fL)	8.64 ± 0.14	7.43 ± 0.38	7.97 ± 0.35	7.53 ± 0.47	6.80 ± 0.23
MPV (fL)	7.48 ± 0.09	6.57 ± 0.33	6.93 ± 0.20	7.03 ± 0.27	6.37 ± 0.20
P-LCR (%)	8.02 ± 0.51	4.97 ± 0.88	6.47 ± 0.98	7.67 ± 0.61	4.33 ± 0.58
LYM (10^3/μL)	5.58 ± 0.78	5.30 ± 0.12	3.53 ± 0.15	6.47 ± 0.41	4.53 ± 0.27
NEUT	1.50 ± 0.16	2.87 ± 0.23	1.90 ± 0.36	2.23 ± 0.18	4.93 ± 1.27
PCT (%)	1.22 ± 0.10	0.38 ± 0.10	0.49 ± 0.03	1.10 ± 0.07	0.63 ± 0.12
PLT (10^3/μL)	1625.60 ± 133.97[a]	723.33 ± 6.12[b]	701.33 ± 16.60[b]	1233.33 ± 53.42[c]	905.00 ± 173.28[d]

Mean ± SEM ($n = 5$); different letters indicate significant difference at $p \leq 0.05$. *WBC* white blood cell, *RBC* red blood cell, *HGB* haemoglobin, *LYM* lymphocyte, *HCT* haematocrit, *MCV* mean corpuscular volume, *NEUT* neutrophil, *RDW* red cell distribution width, *MCH* mean corpuscular haemoglobin, *MCHC* mean corpuscular haemoglobin concentration, *PDW* plate volume distribution width, *MPV* mean platelet volume, *P-LCR* platelet large cell volume, *PCT* plateletcrit, *PLT* platelet

associated with nephrotoxicity from cisplatin treatment alone. There was diffuse congestion of glomerular and interstitial capillaries and patchy vacuolar degeneration of epithelial cells and proliferative glomerulo-nephritis. Also, there were hyperplasia of mesangial cells and thickening of glomerular tufts as well as marked tubular epithelial necrosis and inflammatory cellular infiltrates (Fig. 3g).

Table 2 Effects of treatments on serum biochemical parameters of animals in nephrotoxicity study

Parameter	Mean concentration ± SEM				
	Control	Nephrotoxin	Silymarin	100 mg GSE	250 mg GSE
Gentamicin					
ALT (U/L)	48.88 ± 4.31	50.55 ± 3.21	46.88 ± 3.41	55.54 ± 5.62	50.00 ± 6.87
Creat (mmol/L)	48.98 ± 4.25^{a}	124.77 ± 2.73^{b}	107.04 ± 12.84^{bc}	48.78 ± 4.53^{a}	85.00 ± 9.32 ^{ac}
Urea (mmol/L)	7.20 ± 0.81^{a}	19.09 ± 2.37^{b}	9.53 ± 0.84^{a}	7.01 ± 0.57^{a}	8.90 ± 0.28^{a}
Na (mmol/L)	143.26 ± 2.29^{a}	126.67 ± 5.37^{b}	139.16 ± 1.04^{a}	141.98 ± 1.53^{a}	142.06 ± 1.07^{a}
Cl (mmol/L)	109.24 ± 2.08^{a}	91.81 ± 3.60^{b}	103.60 ± 1.38^{a}	106.26 ± 1.82^{a}	106.24 ± 1.17^{a}
K (mmol/L)	8.50 ± 0.65^{a}	3.99 ± 0.76^{b}	8.03 ± 0.67^{a}	7.18 ± 0.08^{a}	7.53 ± 0.35^{a}
Cisplatin					
ALT (U/L)	48.88 ± 4.31	52.84 ± 3.39	49.53 ± 1.20	35.84 ± 3.79	43.55 ± 2.76
Creat (mmol/L)	48.98 ± 4.25^{a}	165.73 ± 6.51^{b}	50.13 ± 1.45^{a}	55.35 ± 3.35^{a}	71.14 ± 10.45^{a}
Urea (mmol/L)	7.20 ± 0.81^{a}	17.08 ± 0.19^{b}	8.56 ± 0.61^{a}	6.45 ± 0.10^{a}	7.80 ± 0.21^{a}
Na (mmol/L)	143.26 ± 2.29^{a}	141.70 ± 2.10 ^{ac}	141.00 ± 0.58 ^{ac}	134.23 ± 1.47 ^{ac}	141.47 ± 0.68^{bc}
Cl (mmol/L)	109.24 ± 2.08	107.43 ± 0.70	106.10 ± 0.59	104.27 ± 1.37	108.10 ± 0.82
K (mmol/L)	8.50 ± 0.65	7.46 ± 0.02	6.32 ± 0.34	7.04 ± 0.04	7.49 ± 0.25

Mean ± SEM ($n = 5$); different letters indicate significant difference at $p \leq 0.05$. *ALT* alanine aminotransferase, *Creat* creatinine, *Na* sodium, *Cl* chloride, *K* potassium

Effects of treatment on inflammatory cytokines in kidney tissues

There was strong expression of COX-2 in cisplatin control group. However, the COX-2 expression was reduced remarkably with 250-mg/kg GSE treatment but not as silymarin treatment. The expressions of IL 17 and 23 were weak in cisplatin control but strong to moderate with GSE treatments (Figs. 4 and 5).

Discussion

Kidney toxicity induced by gentamicin and cisplatin treatment is mediated by excess production of ROS and RNS, reduction in antioxidant defence mechanisms, acute tubular necrosis, inflammation and glomerular congestion (Balakumar et al. 2010; Miller et al. 2010; Adil et al. 2016). Thus, to ameliorate the damage caused by these nephrotoxic drugs, therapeutic approaches that can attenuate oxidative stress, apoptosis, necrosis and inflammation are required.

Organ toxicity is the result of changes in cellular structure or function that persists beyond the period of administration and elimination of a substance (Kharasch 2007). Changes in organ weight often precede morphological changes (Piao et al. 2013). Thus, organ weight is one of the most sensitive drug toxicity indicators (Bailey et al. 2004; Piao et al. 2013). The increase in relative kidney weight with gentamicin administration observed in this study gives a strong indication of gentamicin nephrotoxicity as stated by Adil et al. (2016).

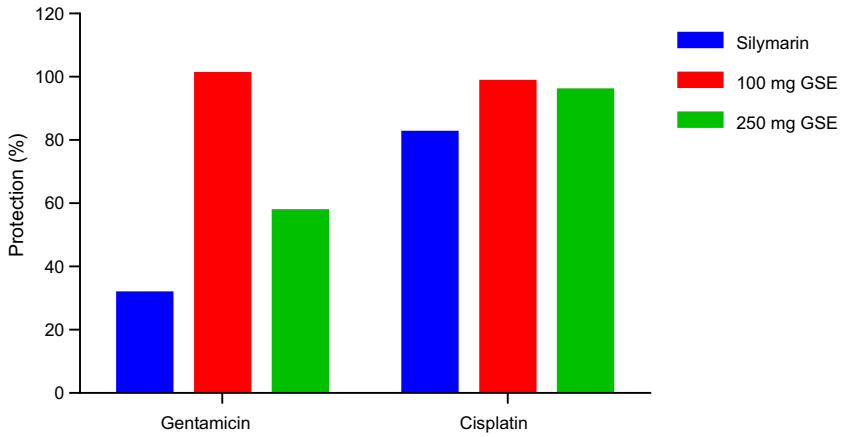

Fig. 2 Percentage protection based on kidney function biomarkers (creatinine, urea and relative kidney weight)

Table 3 Effect of treatments on kidney antioxidant defence system of animals in nephrotoxicity study

Parameter	Mean concentration ± SEM				
	Control	Nephrotoxin	100 mg GSE	250 mg GSE	Silymarin
Gentamicin					
NO (U/mg prot)	15.14 ± 0.61^{ab}	29.40 ± 3.00^{b}	6.93 ± 0.33^{a}	7.05 ± 0.81^{a}	12.16 ± 1.28^{ab}
GPx (U/mg prot)	323.43 ± 10.09^{a}	231.84 ± 9.57^{b}	353.06 ± 15.93^{c}	372.48 ± 9.04^{d}	339.96 ± 8.05 ^{ac}
SOD (U/mg prot)	11.16 ± 0.30	8.00 ± 0.32	12.36 ± 0.56	13.08 ± 0.26	11.74 ± 0.27
H_2O_2 (mM/mg prot)	32.55 ± 0.64	33.88 ± 0.91	33.20 ± 2.10	33.33 ± 2.16	31.28 ± 0.79
GST (mM/mg prot)	9.00 ± 0.69	4.78 ± 0.38	8.06 ± 0.60	8.68 ± 0.44	8.70 ± 0.59
GSH (mM/g tissue)	95.00 ± 5.55	90.68 ± 5.23	90.38 ± 5.22	87.77 ± 4.79	91.89 ± 5.30
MDA (mM/mg prot)	6.24 ± 0.30	6.38 ± 0.31	6.78 ± 0.44	5.19 ± 0.16	7.55 ± 1.63
Cisplatin					
NO (U/mg prot)	15.14 ± 0.61	7.75 ± 1.14	12.17 ± 0.99	9.09 ± 1.02	10.78 ± 0.81
GPx (U/mg prot)	323.43 ± 10.09^{a}	284.77 ± 41.77^{b}	221.40 ± 19.45^{c}	261.72 ± 53.73^{bc}	379.35 ± 14.98^{d}
SOD (U/mg prot)	11.16 ± 0.30	10.44 ± 1.56	8.07 ± 0.79	10.29 ± 2.31	13.25 ± 0.49
H_2O_2 (mM/mg prot)	32.55 ± 0.64	41.17 ± 2.44	36.10 ± 1.13	38.85 ± 0.48	33.10 ± 0.84
GST (mM/mg prot)	9.00 ± 0.69	5.63 ± 1.02	4.63 ± 0.23	6.57 ± 1.85	9.46 ± 0.80
GSH (mM/g tissue)	95.00 ± 5.55	81.55 ± 2.34	97.51 ± 17.13	114.96 ± 3.82	118.79 ± 4.67
MDA (mM/mg prot)	6.24 ± 0.30	3.51 ± 0.51	2.93 ± 0.06	3.39 ± 0.71	4.98 ± 0.45

Mean ± SEM ($n = 5$); different letters indicate significant difference at $p \leq 0.05$. *TP* total protein, *NO* nitric oxide, *GPx* glutathione peroxidase, *SOD* superoxide dismutase, *H_2O_2* hydrogen peroxide, *GST* glutathione-S-transferase, *GSH* glutathione, *MDA* melondialdehyde

On the other hand, the significant reduction in kidney weight following co-administration of gentamicin with GSE gives a clear indication of nephroprotection. However, in the cisplatin model, the treatments showed no significant effect on kidney weight.

In the haematological study, there was marked decrease in platelet count as a result of the activity of the nephrotoxins. This is consistent with the results of several other studies which have reported low platelet count as a frequent manifestation of acute and chonic kidney failure (Gafter et al. 1987; Angiolillo et al. 2010; van Bladel et al. 2012; Dorgalaleh et al. 2013). However, co-adiministration with GSE was not able to successfully reverse this effect. This could be due to the fact that the extract itself causes marked reduction in platelet count (Nyarko et al. 2019). Thus, a combination of the extract and nephrotoxin worsened the effect of the latter with respect to platelet count.

Furthermore, in the clinical setting, serum creatinine and urea are important renal functional biochemical indices (Shirwaikar et al. 2003; Yaman and Balikci 2010). In this study, gentamicin and cisplatin both caused significant increase in serum creatinine and urea levels. Co-administration of the nephrotoxins with silymarin or GSE restored the concentrations back to normal levels. Additionally, gentamicin caused alterations to the level of electrolytes (Na, K and Cl) but treatment with silymarin and the GSE extracts ameliorated the effect. This observation is consistent with previous studies on other nephroprotective plant extracts (Shirwaikar et al. 2003; Ali 2004; Badary et al. 2005; Yaman and Balikci 2010 Chatterjee et al. 2012; Adil et al. 2016). GSE treatment proved to protect the kidney from injury better than even silymarin especially at the least dose (100 mg/kg) as indicated by its high percentage protection in both the cisplatin and gentamicin models. Also, the histological damage in *G. simplicifolia*-treated groups were minimal in contrast to the control rats, whilst the expressions of COX-2 further buttress the necrotic and inflammatory reactions in the cisplastin and gentamin nephrotoxic models. There was remarkable reversal of nephrotoxicity with GSE and silymarin interventions.

In the antioxidant study, only nitric oxide and glutathione peroxidase were significantly affected by the leaf extract of *G. simplicifolia.* The extract was able to reduce the concentration of nitric oxide, a highly reactive free radical. At high concentrations, nitric oxide causes the accumulation of ROS, reduces the activity of antioxidant enzymes and accelerates peroxidation (Zhu et al. 2008). Some studies have, however, established that reduced bioavailability of NO precipitates hypertension. This is because nitric oxide produced from the vascular endothelium helps to maintain a continuous vasodilator tone that is essential for the regulation of blood flow, blood pressure, platelet aggregation and vasodilation. Thus, reduced NO bioavailability has been

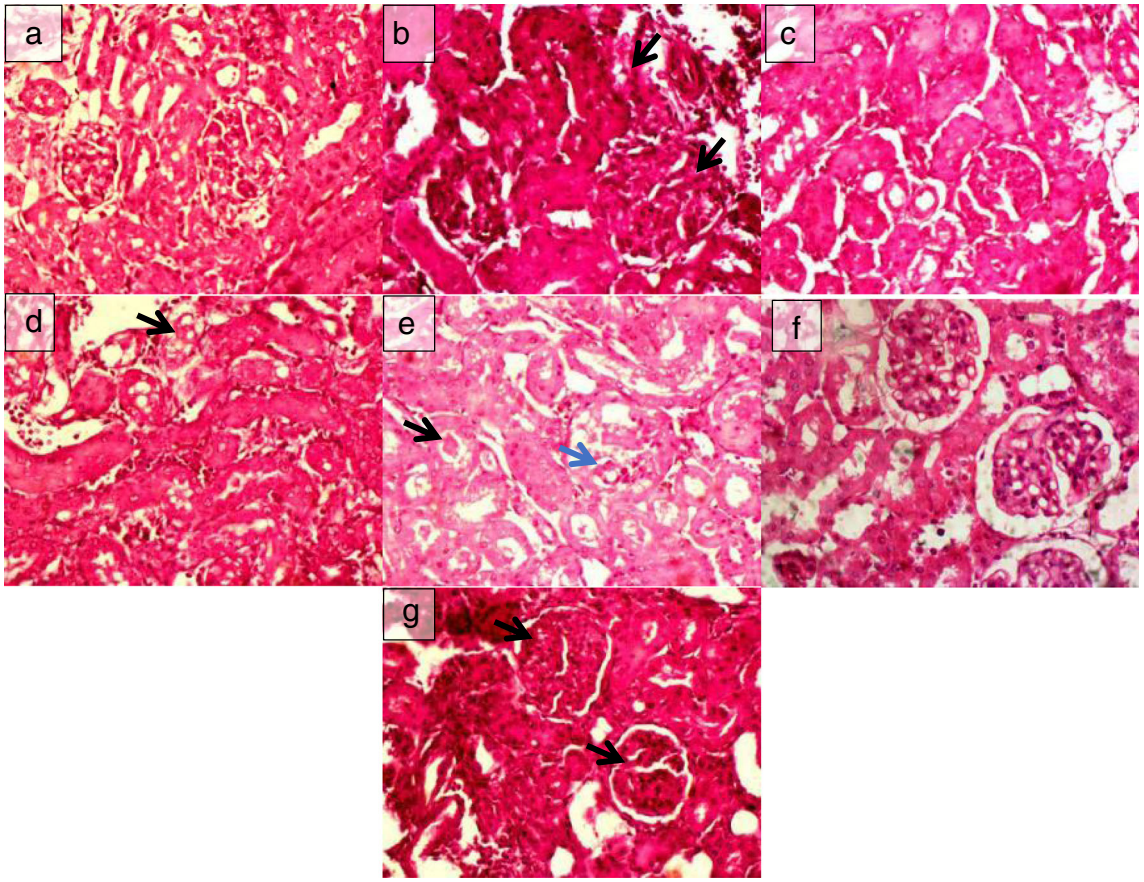

Fig. 3 The photomicrographs (H&E stained, × 400) of kidney sections taken from rats. **a** Normal kidney (1 ml/kg distilled water) with no lesion. **b** Kidney treated with 120 mg/kg gentamicin showing glomerulonephritis (arrows). **c** Kidney treated with 120 mg/kg gentamicin and 120 mg/kg silymarin with no lesion. **d** Kidney treated with 250 mg/kg GSE + 120 mg/kg gentamicin showing patchy tubular epithelial degeneration and necrosis (arrow). **e** Kidney treated with 7.0 mg/kg cisplatin showing tubular epithelial necrosis and casts (black arrow) with a few peritubular inflammatory cells (blue arrow) as well as tubular ectasia. **f** Kidney treated with 7.0 mg/kg cisplatin and 120 mg/kg silymarin with no lesion. **g** Kidney treated with 250 mg/kg GSE + 7.0 mg/kg cisplatin showing thickened glomerular tufts (arrow). H&E × 400

associated with endothelial dysfunction and hypertension (Oyagbemi et al. 2016). Additionally, hyperglycaemia is reported to cause changes in vascular structure and function by decreasing NO bioavailability in vascular endothelium either by reduction in the activity of NO synthase or by depletion of NO by superoxide anion radicals (Fasola et al. 2016). Considering the ability of *G. simplicifolia* to significantly increase blood glucose level (Nyarko et al. (2019), this significant reduction in NO may potentiate the development of hypertensive state. Nevertheless, NO also offers protection against cellular damage at low concentrations. It has been reported to decrease the concentration of melondialdehyde and reactive oxygen species, and increase the activity of antioxidant enzymes such as superoxide dismutase and catalase (Wink et al. 1993; Zhu et al. 2008).

The GSE was also able to increase the concentration of GPx in the gentamicin model. In the cisplatin model, however, the extracts were not able to restore the GPx concentration. Only silymarin was able to significantly increase the GPx concentration after the toxin had significantly reduced it. GPx is a selenium-containing cytosolic antioxidant enzyme that catalyses the reduction of H_2O_2 to water and oxygen, and peroxide radicals to alcohol and oxygen (Tabet and Touyz 2007; Fanucchi 2014). It is a key enzyme in the maintenance of glutathione homeostasis in tissues. Similar trends were observed with COX-2 expression. However, GSE treatments improved expressions of IL17 and IL 23 than even silymarin treatments, underscoring the proinflammatory and healing properties of this extract.

Conclusion

This study confirms the folkloric use of *G. simplicifolia* as a treatment for kidney ailments because it was able to counteract the effect of the toxins by restoring urea and creatinine concentrations back to normal levels, as well as electrolyte balance in the rats. It also decreased the concentration of nitric

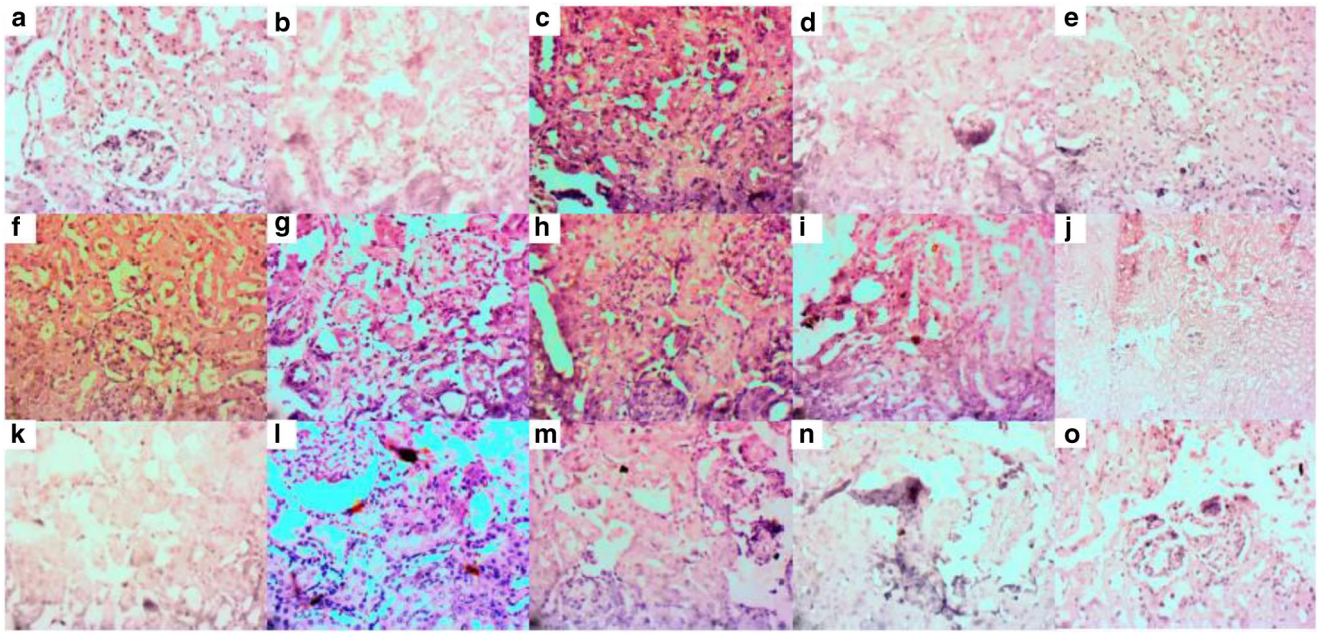

Fig. 4 Representative examples of immunohistochemistry of inflammatory cytokines in the kidney tissue specimen for the gentamicin model experiment. Plates A to E represent Cox-2 immunohistochemistry for treatment groups I–V, respectively. Plates F to J represent IL 17 immunohistochemistry for treatment groups I–V, respectively and plates K to O represent IL 23 immunohistochemistry for treatment groups I–V, respectively

oxide and increased the concentration of glutathione peroxidase in the gentamicin model. The protective effect of this extract was also confirmed by histological and immunohistochemistry observations. However, the extract showed better protection against gentamicin-induced nephrotoxicity than cisplatin-induced nephrotoxicity. Thus, 50% hydroethanolic extract *G. simplicifolia* is an effective nephroprotective agent which brings about functional improvement of kidney function and can be exploited as a therapeutic agent against kidney disease.

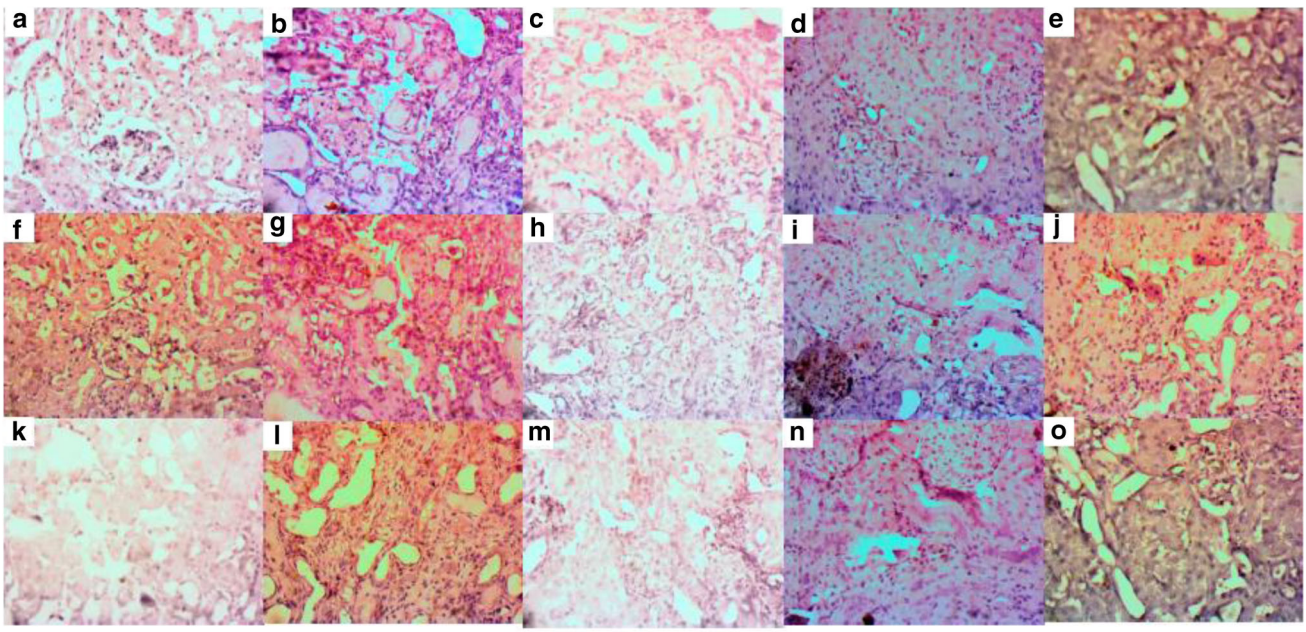

Fig. 5 Representative examples of immunohistochemistry of inflammatory cytokines in the kidney tissue specimen for the cisplatin model experiment. Plates A to E represent Cox-2 immunohistochemistry for treatment groups I–V, respectively. Plates F to J represent IL 17 immunohistochemistry for treatment groups I–V, respectively and plates K to O represent IL 23 immunohistochemistry for treatment groups I–V, respectively. ABC HRP/DAB counterstained with haematoxylin, × 400

Compliance with ethical standards

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

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