Journal of Experimental Pharmacology

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ORIGINAL RESEARCH

# The Nephroprotective Activity of *Boesenbergia Rotunda* Rhizome by Reducing Creatinine, Urea Nitrogen, Glutamic Pyruvic Transaminase, and Malondialdehyde Levels in the Blood and Attenuating the Expression of *Havcr1 (KIM-1), Lcn2 (NGAL), Casp3*, and *Casp7* Genes in the Kidney Cortex of Cisplatin-Induced Sprague-Dawley Rats

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**Background:** Cisplatin chemotherapy induces nephrotoxicity by producing reactive oxygen species, hence, discovering add-on nephroprotective drugs for patients with cancer is challenging. *Boesenbergia rotunda* has been reported for its antioxidant properties.
**Purpose:** This study aims to explore the nephroprotective mechanism of the ethanol extract of *Boesenbergia rotunda* rhizome (EEBR) in cisplatin-induced rats.
**Methods:** The rats were randomly assigned into 6 groups: the normal control (treated with saline); the negative control (cisplatin-induced without any treatment); the positive control (treated with quercetin 50 mg/kg BW); and 3 treatment EEBR (125 mg/kg BW; 250 mg/kg BW; 500 mg/kg BW) groups for 10 days. The % relative organ weight, kidney histopathology, and nephrotoxicity biomarkers expression were evaluated.
**Results:** EEBR decreased creatinine, urea nitrogen, glutamic pyruvate transaminase, and malondialdehyde levels in the blood of cisplatin-induced rats. An insignificant increase in GOT was observed in rats treated with the highest dose of EEBR. EEBR did not significantly alter the BW and the % kidney relative weight. An abnormal shape of the Bowman capsule is observed in the negative control group. EEBR reduced the expression of *Havcr1 (KIM-1), Lcn2 (NGAL), Casp3*, and *Casp7* genes in rats' kidneys.
**Conclusion:** *Boesenbergia rotunda* could be considered a potential candidate for add-on therapy in cisplatin-treated patients, but further studies are needed to verify its efficacy and safety.
**Keywords:** acute kidney injury, boesenbergia rotunda, medicinal plants, nephroprotective activity, Zingiberaceae

## Introduction

Cisplatin, (CAS No. 15663–27-1, MF-Cl2H6N2Pt; NCF-119875), or cis-diamine-dichloro platinum (II), is a highly effective chemotherapeutic. This drug was the first FDA-approved platinum compound for anticancers.[1] However, in

Received: 19 February 2024
Accepted: 2 May 2024
Published: 6 May 2024

clinical trials, cisplatin which is often selected due to its strong activity has been reported to damage the DNA, produce reactive oxygen species (ROS), and eventually induce nephrotoxicity.[2] The most serious of cisplatin nephrotoxicity is acute kidney injury (AKI) which occurs in 20–30% of patients because this drug is eliminated by both glomerular filtration and tubular secretion. Fatty acids becoming the main source of energy for the proximal tubule, are also the primary site of cisplatin nephrotoxicity.[3]

The general diagnostic markers of nephrotoxicity and renal dysfunction are blood urea nitrogen (BUN) and serum creatinine (SCr), which are currently considered low sensitivity in detecting early renal damage. Kidney injury molecule-1 (Kim-1), cystatin C, and neutrophil gelatinase-associated lipocalin (NGAL) levels in the serum are more sensitive to detecting acute kidney injury (AKI) during nephrotoxicity.[4] Kim-1 has been considered potential in determining cisplatin-induced renal injury in both in vitro and in vivo studies.[5] Similarly in a previous pre-clinical study, NGAL proved as an early and quantitative urinary biomarker for cisplatin nephrotoxicity. Western analysis rapidly measured this protein in mice's urine within 3 h of cisplatin treatment.[6] Furthermore, in a clinical study of cisplatin-chemotherapy Iranian patients diagnosed with AKI (with a glomerulus filtration rate GFR > 45 mL/min), a significant increase in urine NGAL-creatinine ratio was observed.[7] More interesting studies reported that activation of caspases-3, −8, and −9 occurred approximately 12 h after cisplatin treatment on renal epithelial cells in vitro,[8] and blocking the activity of caspase had reduced cisplatin-induced apoptosis.[9,10]

A fast increase in the diagnosis of AKI was reported globally. In the UK, a total of 356 million inpatient of care between 1998 to 2020 have been analyzed. The frequency of occurrence in AKI was significantly higher than that of dementia. It was reported that 96% of AKI cases were encoded as N17.9 (Acute renal failure, unspecified).[11] In Indonesia, the incidence of AKI among Intensive Care Unit (ICU) patients is relatively high. A study carried out from September 2019 to February 2020 (n=148 ICU patients) reported that 52.5% were diagnosed with stage-3 AKI and the mortality rate was 77%.[12]

AKI was also observed in COVID-19 patients. In Saudi Arabia, the incidence of AKI in hospitalized patients with COVID-19 was 36% related to higher 30-day mortality.[13] It was recently reported that about 20% of hospitalized COVID-19 patients developed AKI within 2 days of post their admission to the intensive care unit.[14,15]

Likewise, AKI was observed in cancer patients treated with cisplatin chemotherapy. A prospective observational study of 50 patients (aged 54.8±10.3 years) receiving cisplatin chemotherapy in North India reported that 38% of the patients developed AKI.[16] The incidence of cisplatin-induced AKI was also reported in 527 patients at a general hospital in Beijing, China.[17] Similarly, a single-center pilot study carried out between April 2014 and June 2016 in 28 patients treated with first-line cisplatin therapy revealed the development of AKI in 28.6% of the patients.[18] Moreover, a case of stage 3C re-current ovarian cancer patient in Baltimore treated with cisplatin confirmed the development of AKI.[19]

Therefore, discovering nephroprotective agents, particularly for cisplatin-treated patients, is challenging. Several studies have confirmed the strong antioxidant properties of *Boesenbergia rotunda*.[20–22] Chemically, it contains unprenylated flavonoids (chalcones eg, cardamonin, pinocembrin), prenylated flavonoids (eg, boesenbergin A), essential oils, and other miscellaneous compounds.[23]

Boesenbergin A in *Boesenbergia rotunda* was described to reveal considerable antioxidant and anti-inflammatory activity.[24] Moreover, a recent study reported that *B. rotunda* could induce osteoblast cell proliferation[25] and significantly increase pancreatic antioxidant enzyme activities (glutathione, superoxide dismutase, and catalase).[26] Until recently, the nephroprotective activity of *B. rotunda* has been explored very limitedly, however, EEBR was confirmed for its protective effect on cisplatin-exposed human embryonic kidney-293 cells,[27] thus, this present study aims to investigate the nephroprotective mechanism of EEBR in cisplatin-induced male Sprague-Dawley rats by measuring the general diagnostic markers of nephrotoxicity (SCr and BUN) and the liver function parameters (glutamic oxaloacetic transaminase or GOT and glutamic pyruvic transaminase or GPT), the % relative weight of the kidney, malondialdehyde (MDA) level in the kidney, kidney histopathology, the expression of AKI biomarkers (*KIM*-1 and *NGAL*) and apoptotic pathway proteins (*Casp3* and *Casp7*) in the kidney cortex of the rats were also evaluated using the reverse transcription-polymerase chain reaction (RT-PCR).

AKI biomarkers are chosen as the study interest because preliminary identification of nephrotoxicity will improve the current point of care before dysfunction of the kidney emerges.[5] Caspases are a group of cysteine-aspartate enzymes

divided into initiator caspases (caspase-2, −8, −9, and −10) and effector caspases (caspase-3, −6, and −7). Cell death is attributable to the activation of initiator caspases and subsequently effector caspases.[28,29] Nephroprotective agents, which are expected to maintain the viability of kidney cells, should reduce the activation of caspases.

# Materials and Methods

## Plant Materials

The rhizomes of *B. rotunda* were collected from the Indonesian Medicinal and Aromatic Crops Research Institute (BALITTRO) via the IP2TP Manoko, West Java, Indonesia (https://goo.gl/maps/s9AwwZzVm24JARkt6). The rhizomes were taxonomically authenticated in the Indonesian Institute of Sciences (LIPI), Research Center for Plant Conservation and Botanic Garden, Bogor, West Java, Indonesia (https://goo.gl/maps/smjtMww4szh5tbzC9) (document No. B-774/III/KS.01.03/2/2021).

## Preparation of the Ethanol Extract of B. Rotunda (EEBR)

The extraction procedure was carried out by following a previous report.[30] The viscous extract of EEBR yielded 15.12% w/w. Water content in the viscous EEBR was determined using azeotrope distillation (toluene distillation) as described in the Indonesian Herbal Pharmacopoeia[31] and yielded 6.0%.

## Animals and Ethical Considerations

The in vivo experiment was carried out at the iRATco Veterinary Laboratory Services, Bogor, Indonesia (https://www.iratco.co.id/). Thirty male Sprague-Dawley (SD) rats, 5–6 weeks, 160–170 g, were randomly kept in 6 animal cages (dimension: 55 cm length, 45 cm width, 45 cm height; 5 rats/cage) at 22–25 °C under a 12 h light, 12 h dark cycle, 55% RH. The animals were provided with standard pellet food (containing crude protein 18%, crude fat 3.5%, and crude fiber 3.5% manufactured by the Indofeed, Bogor, Indonesia) and water freely for 5 days. Animal handling and euthanasia procedures were performed by following the 3R and 5F principles of animal welfare, as approved by the Research Ethics Committee, Padjadjaran University, Indonesia (approval document No. 768/UN6.KEP/EC/2021).

## Experimental Design

After 1 week of acclimatization, the rats were randomly assigned into 6 groups (n=5):

Group 1 (the normal control) was treated with a saline solution orally for 10 consecutive days; Group 2 (the negative control) was treated with a saline solution orally for 10 consecutive days; Group 3 (the positive control) was treated with quercetin 50 mg/kg BW for 10 consecutive days following the previous work of Ilić and co-workers;[32]

Groups 4–6 were treated with EEBR doses of 125 mg/kg BW; 250 mg/kg BW; and 500 mg/kg BW, respectively, for 10 consecutive days. These doses were chosen because, in our previous study, a higher dose of EEBR (dose of 1000 mg/kg BW) elicited ulceration in both the stomach and intestine.[30]

On day 5, the rats in groups 2–6 were nephrotoxicity-induced using a single dose of cisplatin 7.5 mg/kg BW intraperitoneally.[33] The body weight of the rats in all groups was monitored on day 5 and day 10.

In this study, quercetin was chosen as the positive control drug because it prevents the nephrotoxic effect of cisplatin without affecting the drug's chemotherapeutic activity. Quercetin significantly reduces MDA levels in cisplatin-induced nephrotoxicity Wistar albino rats and restores the GSH/GSSG ratio in cisplatin-induced male Fischer F344 rats to normal values.[32,33] Quercetin also improved the rats' kidney histology architecture by reducing the formation of hyaline casts and tubular epithelial cell sloughs.[33]

## Biochemical Assay of the Kidney and Liver Function

On day 11, the rats were euthanized with ketamine hydrochloride (44 mg/kg BW, intramuscularly), and the orbital sinus blood was drawn using glass micro-hematocrit capillary tubes. The serum was separated by centrifugation (ScanSpeed 406G) at 3000 rpm for 5 minutes for the assessments of kidney function (SCr and BUN) and liver function (GPT and GOT) of the rats as markers of toxicity,[34] which were carried out using Glory Diagnostics kit (Linear Chemicals,

Barcelona, Spain). The absorbance was measured according to the manufacturer's protocol using a spectrophotometer (Genesys 10 UV-visible).

## Calculation of the Kidney Relative Weight

On day 11, the kidneys were collected immediately, washed with water followed by cold phosphate buffer saline, and dried using filter paper. Both left and right kidneys were weighed and calculated for their relative weights.

## Determination of Malondialdehyde (MDA) Level in Kidney Lysate

The left and right kidneys of rats from each group were sliced and homogenized in cold lysis buffer (0.1 M) of pH 7.4 and centrifugated at 13,000 g for 10 minutes at 4°C. The supernatant was used for the determination of MDA as described in a previous method[35] using the Abbkine CheKine™ Lipid Peroxidation (MDA) Assay kit (Cat. KTB1050) size 48/96T.

## Histopathological Examination of the Kidney

Two kidney tissues of rats from each group preserved in 10% neutral formalin, were processed with paraffin wax. For histopathological examination, 5 µm of the tissue slices were hematoxylin and eosin (H&E) stained and observed under a light microscope to count the total number of normal cells.[36] The H&E staining is the fundament of anatomical pathology diagnosis as per routine protocol in the Histopathology Laboratory that provides a detailed view of the tissue, including the cytoplasm, nucleus, organelles, and extra-cellular components in contrasting colors.[37]

## Reverse Transcription Polymerase Chain Reaction (RT-PCR) Analysis of KIM-1, NGAL, Casp3, and Casp7 Expression

The mRNA was isolated from renal cortex samples with RiboZol™ RNA Extraction Reagent (US & Canada) and purified using the ethanol precipitation method according to the manufacturer's protocol. The rat primers used:[37]

- for the *KIM-1/Havcr1* gene:
  forward primer GGTCACCCTGTCACAATTCC;
  reverse primer: CTCGG-CAACAATACAGACCA;
- for the *NGAL/Lcn2* gene:
  forward primer CACCACGGACTACAACCAG-TTCGC;
  reverse primer TCAGTTGTCAATGCATTGGTCGGTG;
- for the Caspase 3/*Casp3* gene:
  forward primer AAAGGATGACTGGGAG-TGG;
  reverse primer ATGACGACCTGGAACATCG;
- for the Caspase 7/*Casp7* gene:
  forward primer TCATCTCATCCCTTCTCTGGA;
  reverse primer TACATTTGCCCATCTTCTCG;
- for β-actin:
  forward primer GGAAATCGTGCGTGACATTAAA;
  reverse primer GCGGCAGTGGCCATCTC;

The RT-PCR for all the mentioned genes was performed using Promega (GoTaq® 1-Step RT-qPCR System). β-actin is used to normalize all these genes. Gene expression was read by a qPCR Thermal Cycler. The fluorescent detection system employed was Cxr Dye as a DNA binder. The thermal cycles used:

- for reverse transcription: 1 cycle at ≥37 °C for 900 seconds
- for reverse transcriptase inactivation and GoTaq® DNA Polymerase activation: 1 cycle at 90 °C for 600 seconds
- for denaturation: at 95 °C for 10 seconds
- for annealing and data collection: 40 cycles at 60 °C for 30 seconds
- for extension: at 72 °C for 30 seconds

**Table 1** Effect of EEBR on the BW of the Rats Before and After Cisplatin-Induced Nephrotoxicity

| Animal Groups | Body Weight (g) + SD | | |
|---|---|---|---|
| | Day 0 (Initial Body Weight) | Day 5 (Before Cisplatin Induction) | Day 10 (After Cisplatin induction) |
| Normal Control | 161.02 ± 27.81 | 164.31 ± 30.77# | 168.45 ± 30.27# |
| Negative Control | 144.94 ± 12.86 | 146.29 ± 13.09 | 130.45 ± 17.35 |
| Positive Control | 176.19 ± 18.95 | 177.32 ± 19.01* | 167.42 ± 25.20* |
| EEBR 125 mg/kg BW | 174.66 ± 20.58 | 176.43 ± 20.90 | 160.96 ± 21.66 |
| EEBR 250 mg/kg BW | 142.39 ± 14.15 | 143.98 ± 14.06 | 135.21 ± 15.19 |
| EEBR 500 mg/kg BW | 143.48 ± 8.56 | 146.14 ± 10.10 | 135.10 ± 12.84 |

**Notes**: Statistical analysis was done using IBM SPSS Statistics version 25.0 for Windows. *indicates a significant difference ($p<0.05$) compared to the negative control. #indicates a significant difference ($p<0.05$) compared to day 0 of the normal control.

## Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 25.0 and graphs were generated using GraphPad Prism version 8.4.2 for Windows. Clinical biochemistry parameters and histopathological data were analyzed using Non-Parametric Kruskal–Wallis and post hoc Dunn's Multiple Comparison Test. Other data were analyzed using the Kolmogorov–Smirnov test (for normality) and the Levene Test (for homogeneity/equality of variance). p-value <0.05 indicates a statistical significance. The experiments were performed in triplicates.

# Results

## Effect of EEBR on the Body Weight and the Kidney Relative Weight of Cisplatin-Induced Nephrotoxicity in Rats

Cisplatin produced remarkable alterations in the body weight (BW) (tabulated in Table 1) and likewise, the biomarkers, eg serum creatinine (SCr) and blood urea nitrogen (BUN) (portrayed in Figure 1) when compared to the normal control group, indicating the occurrence of cisplatin-induced nephrotoxicity.

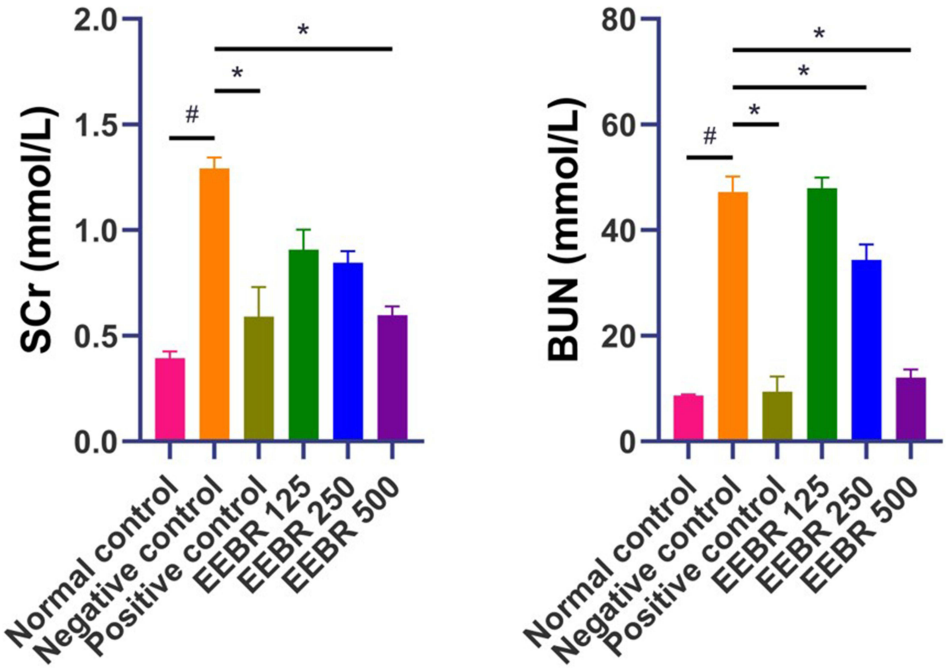


**Figure 1** Effect of EEBR on kidney function (left: SCr; right: BUN) of cisplatin-induced nephrotoxicity rats. Only the highest dose of EEBR (500 mg/kg BW) shows protection against nephrotoxicity as portrayed by a significant difference in SCr and BUN levels compared to the negative control. The # denotes the significant difference with the normal control ($p<0.05$). The * indicates a remarkable difference with the negative control ($p<0.05$).

The rat group treated with cisplatin (the negative control group or placebo) showed a noticeable decrease in their BWs. The effect of various doses of EEBR on the BW of rats before (day 5) and after (day 10) cisplatin induction is tabulated in Table 1. On day 5 the rats in all groups indicated an increase in BW although not significant compared with the negative control group ($p>0.05$). Interestingly, on day 10 all groups of rats (except the normal control group) suffered a decrease in their BW, although not significant compared to the negative control and normal control groups. In the normal control, there is a slight increase in BW of the rats between day 0 and day 10.

The effect of various doses of EEBR on the left and right kidney relative weight of cisplatin-induced rats (Table 2) indicated no significant differences compared to that of the negative control group. All doses of EEBR slightly increase, although not significant, the relative weight of the cisplatin-induced rats' left and right kidneys. Only rats treated with quercetin and the normal control showed a significant difference compared to the negative control group.

## Effect of EEBR on the Serum Kidney Biomarkers (SCr and BUN)

Determination of serum kidney biomarkers (Figure 1) revealed a sharp rise of BUN by 4.95-fold (48.384 mg/dL) and SCr by 1.43-fold (1.2921 mg/dL) in the cisplatin-induced rats (the negative control group) compared with the normal control group (BUN=9.776 mg/dL; SCr=0.4397 mg/dL). This elevation indicates the intraperitoneal single dose of cisplatin 7.5 mg/kg BW prompted significant (#in Figure 1) alteration onto these biomarkers. Plainly evident that only the highest dose of EEBR (500 mg/kg BW) shows protection against nephrotoxicity as portrayed by a significant difference in both SCr and BUN levels (BUN = 11.476 mg/dL; SCr = 0.5979 mg/dL) compared to those of the negative control, while EEBR dose of 250 mg/kg BW significantly reduces BUN, but not SCr (Figure 1).

## Effect of EEBR on the Serum Liver Biomarkers (GPT and GOT)

Determination of serum liver biomarkers revealed a significant steep increase of both GPT (69.1894 μ/L) and GOT (148.41 μ/L) in the cisplatin-induced rats (the negative control group) compared with the normal control group (marked with #) (Figure 2). The effect of EEBR on the levels of serum GPT and GOT confirmed that all doses of EEBR improved GPT levels (Figure 2A), but not GOT levels (Figure 2B), of the cisplatin-induced nephrotoxicity rats. In fact, the highest dose of EEBR elevated the GOT levels of the cisplatin-induced nephrotoxicity rats.

## EEBR Reduced Malondialdehyde Levels in Kidney Lysate and Increased Cell Viability in Kidney Tissue

The effect of EEBR on the level of MDA in kidney lysate is presented in Figure 3 (upper left). Cisplatin notably elevates MDA levels in cisplatin-induced rats (the negative control group, MDA=11.455 nmol/g) compared with the normal control group (MDA=3.982 nmol/g, denoted by #). EEBR, however, reduces MDA levels in kidney lysate of cisplatin-induced rats as follows: doses of 125 mg/kg BW (MDA=7.285 nmol/g), 250 mg/kg BW (MDA=7.018 nmol/g), and 500 mg/kg BW (MDA=5.634 nmol/g), thus confirming that EEBR inhibits lipid peroxidation process in cisplatin-induced nephrotoxicity rats.

**Table 2** Effect of EEBR on the Kidney Relative Weight of the Cisplatin-Induced Rats

| Animal Groups | Relative Weight of the Left Kidney (Mean + SD) | p | Relative Weight of the Right Kidney (Mean + SD) | p |
|---|---|---|---|---|
| Normal Control | 0.372 ± 0.028 | 0.001* | 0.372 ± 0.016 | 0.002* |
| Negative Control | 0.556 ± 0.021 | – | 0.540 ± 0.055 | – |
| Positive Control | 0.429 ± 0.093 | 0.005* | 0.42 7± 0.068 | 0.004* |
| EEBR 125 mg/kg BW | 0.446 ± 0.039 | 0.221 | 0.462 ± 0.043 | 0.194 |
| EEBR 250 mg/kg BW | 0.480 ± 0.054 | 0.146 | 0.459 ± 0.062 | 0.169 |
| EEBR 500 mg/kg BW | 0.435 ± 0.063 | 0.108 | 0.435 ± 0.033 | 0.108 |

**Notes**: Statistical analysis was done using IBM SPSS Statistics version 25.0 for Windows. *indicated a significant difference ($p<0.05$) compared to the negative control.

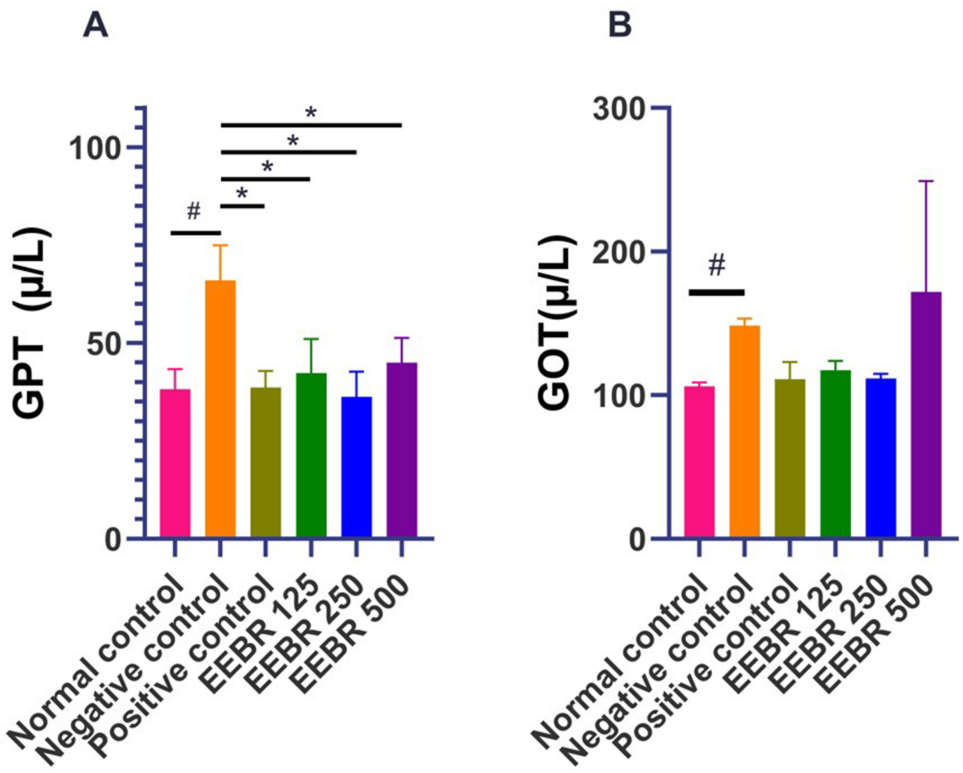


**Figure 2** Effect of EEBR on liver function ((**A**). GPT; (**B**) GOT) of cisplatin-induced nephrotoxicity rats. All doses of EEBR improved GPT levels but not GOT levels of the cisplatin-induced nephrotoxicity rats. The highest dose of EEBR elevated the GOT levels of the cisplatin-induced nephrotoxicity rats. The # denotes the significant difference with the normal control ($p<0.05$). The * indicates a remarkable difference with the negative control ($p<0.05$).

The effect of EEBR on the total number of normal cells in kidney tissue is depicted in Figure 3 (lower left). Cisplatin inhibits the growth of cells in cisplatin-induced rats (the negative control group, total number of normal cells=87.84 +28.74) compared with the normal group (total number of normal cells=87.84+28.74, denoted by #). EEBR, however, increases the viability of cells in kidney lysate of cisplatin-induced rats as follows: doses of 125 mg/kg BW (total number of normal cells=105.04+23.71), 250 mg/kg BW (total number of normal cells=126.8+37.28), and 500 mg/kg BW (total number of normal cells=157.72+46.59).

Histology examination of kidney tissue shows the invasion of inflammatory cells in cisplatin-induced rats (negative control, Figure 3b) and rats administered with EEBR 500 mg/kg BW (Figure 3f). An abnormal shape of the Bowman capsule is observed in the negative control group. On the contrary, rats treated with either EEBR or positive control drugs reveal a regular Bowman capsule.

## EEBR Reduced the Expression of KIM −1, NGAL, Casp3, and Casp7 Genes

The effect of EEBR on the expression of AKI biomarkers and apoptotic pathway proteins is presented in Table 3 and the overall statistical analysis is in Table 4. Cisplatin stimulates the expression of *KIM −1, NGAL, Casp3*, and *Casp7* as shown in the negative control. All doses of EEBR significantly alter the expression of *Kim-1, NGAL*, and *Casp7* genes. Only EEBR doses of 250 mg/kg BW and 500 mg/kg BW, not the dose of 125 mg/kg BW, reduce the expression of *Casp3* genes. Quercetin (the positive control or standard drug) significantly suppresses the expression of *Kim-1, NGAL*, and *Casp3*, but not *Casp7*, genes. The expression of the *Casp3* gene is significantly reduced by quercetin and higher doses of EEBR (250 mg/kg BW and 500 mg/kg BW). Interestingly, all doses of EEBR, not quercetin, significantly inhibit the expression of the *Casp7* gene.

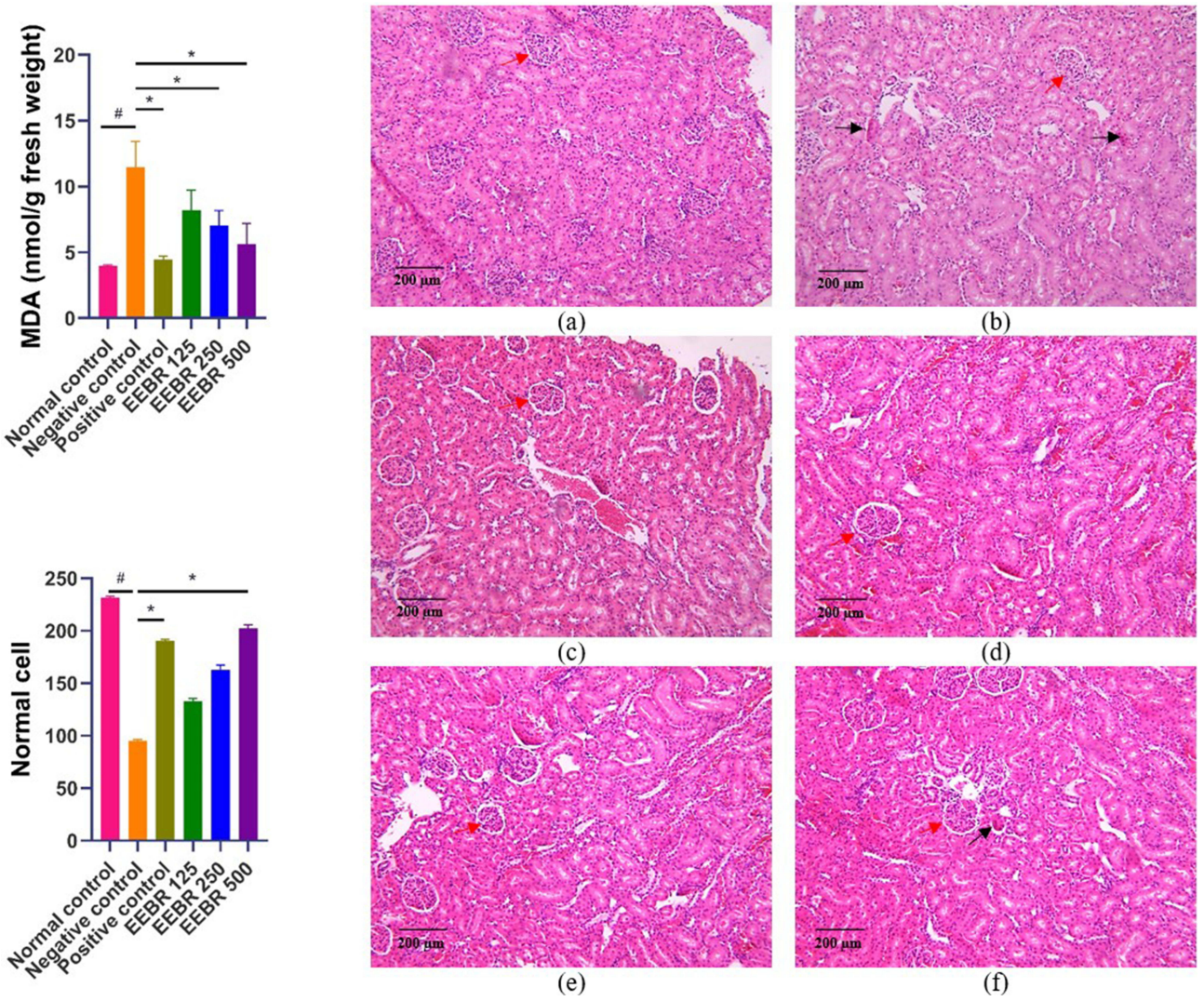


**Figure 3** Effect of EEBR on MDA level and viability of kidney cells in cisplatin-induced nephrotoxicity rats. The * indicates a remarkable difference with the negative control ($p<0.05$). The # indicates a remarkable difference from the normal control group ($p<0.05$). Histology of the kidney tissue of rats in (**a**) normal control; (**b**) negative control; (**c**) positive control; (**d**) EEBR 125 mg/kg BW; (**e**) EEBR 250 mg/kg BW; (**f**) EEBR 500 mg/kg BW. Red arrows show the Bowman capsule. Inflammatory cells are shown by black arrows.

## Discussion

*B. rotunda* has been very limitedly explored for its nephroprotective activity, despite the fact that it possesses strong antioxidant activity. The rhizome of this plant has been proven for its polyphenolic compounds and both prenylated and unprenylated flavonoid contents.[23] These polyphenols and flavonoids are key roles in the plant's biological activity. It was reported that plant metabolites with antioxidant and anti-inflammatory activity, such as flavonoids and polyphenols, can be utilized for the treatment of AKI.[38] Flavonoids have revealed kidney protective effects against various nephrotoxic agents causing AKI or chronic kidney diseases, eg, alcohol. Flavonoids also improved cisplatin-induced kidney impairment by downregulating the expression of NF-kappaB p65 and its downstream effectors (eg, inducible nitric oxide synthase and tumor necrosis factor-α), with the restoration of IL-10. Flavonoids also suppressed the expression of the apoptotic protein caspase-3, thus, reducing cisplatin-induced kidney cell death.[39] Moreover, two flavonoids, namely apigenin and myricetin, have significantly decreased BUN, SCr, caspase-3, TNF-α, IL-6, cyclooxygenases, MDA levels, and elevated GSH level and catalase activity in female Wistar rats.[40]

Meanwhile, the polyphenols and flavonoid content of *B. rotunda* function in its antioxidant activity, have challenged for further explorations. A very recent study reported that the ethanol extract of *B. rotunda* decreased the serum urea and

**Table 3** RT-PCR Analysis of Kim-1, NGAL, Casp-3, and Casp-7 Expression in the RNA Isolated from the Kidney Cortex of Cisplatin-Induced Sprague-Dawley Rats

| Name of the Gene Expressed | Expression of Genes in RT-PCR Analysis (p-value Compared to the Negative Control) | | | | |
|---|---|---|---|---|---|
| | Normal Control | Positive Control | EEBR 125 mg/kg BW | EEBR 250 mg/kg BW | EEBR 500 mg/kg BW |
| *Kim-1* | 0.558 (p = 0.000*) | 0.624 (p = 0.002*) | 0.729 (p = 0.040*) | 0.647 (p = 0.003*) | 0.602 (p = 0.001*) |
| *NGAL* | 0.463 (p = 0.044*) | 0.491 (p = 0.022*) | 0.448 (p = 0.034*) | 0.277 (p = 0.002*) | 0.170 (p = 0.000*) |
| *Casp-3* | 0.521 (p = 0.003*) | 0.530 (p = 0.02*) | 0.641 (p = 0.067) | 0.561 (p = 0.022*) | 0.510 (p = 0.001*) |
| *Casp-7* | 0.486 (p = 0.004*) | 1.560 (p = 0.971) | 0.651 (p = 0.037*) | 0.608 (p = 0.021*) | 0.411 (p = 0.000*) |

**Notes**: All values have been normalized against the housekeeping gene (β-actin). Statistical analysis was done using IBM SPSS Statistics version 25.0 for Windows. *Indicated a significant difference (p<0.05) compared to the negative control.

**Table 4** Statistical Analysis of All Parameters

| Group | Kidney Function | | Liver Function | | MDA in the Kidney Tissue | Total Number of Normal Cells |
|---|---|---|---|---|---|---|
| | BUN | sCr | GPT | GOT | | |
| Normal Control | 0.000* | 0.001* | 0.003* | 0.020* | 0.000* | 0.001* |
| Positive Control | 0.000* | 0.020* | 0.004* | 0.051 | 0.000* | 0.039* |
| EEBR 125 mg/kg BW | 0.998 | 0.400 | 0.011* | 0.284 | 0.080 | 0.491 |
| EEBR 250 mg/kg BW | 0.000* | 0.221 | 0.002* | 0.126 | 0.013* | 0.168 |
| EEBR 500 mg/kg BW | 0.000* | 0.013* | 0.025* | 0.491 | 0.002* | 0.006* |
| | ANOVA and post hoc Tukey's test (p<0.05) | Kruskal–Wallis and Dunn's test (p<0.05) | ANOVA and post hoc Tukey's test (p<0.05) | Kruskal–Wallis and Dunn's test (p<0.05) | ANOVA and post hoc Tukey's test (p<0.05) | Kruskal–Wallis and Dunn's test (p<0.05) |

**Notes**: Statistical analysis was done using IBM SPSS Statistics version 25.0 for Windows. *Indicated a significant difference (p<0.05) compared to the negative control.

creatinine in cisplatin-induced mice.[41] Panduratin A, a phytoconstituent extracted from *B. rotunda*, confirmed its inhibitory activity towards the apoptotic signaling pathways in cisplatin-induced HCT116 and A549 cells without affecting the anticancer activity of cisplatin.[42]

The loss of weight of cisplatin-induced rats indicates a reduced appetite in the rats probably due to the pain generated from the toxicity they suffer. However, the pain due to toxicity may influence the lack of sleep in the rats, which subsequently decreases Cu/Zn-SOD activity in the hippocampus, alters the metabolism of ROS, and causes weight loss.[43] Similar to our result, a previous study confirmed that all six-week-old male Wistar rats treated with cisplatin significantly decreased appetite after 3 weeks and endured 31% weight loss.[42] Treatment with cisplatin in patients with lung cancer led to gastrointestinal dysfunctions, in which one of the observed symptoms was weight loss.[44]

Our findings revealed a sharp rise of BUN and SCr in the cisplatin-induced control group compared to those of the normal group. The mechanisms connected to cisplatin treatment are predicted to be caused by the production of proinflammatory cytokines and ROS, which stimulate inflammation, oxidative stress, and the activation of apoptotic caspases. The apoptotic pathway then triggers kidney injury, steering to the reduction of GFR and elevation of BUN and sCR.[45] The mean GFR in the healthy SD rats is 1080 ±130 µL/minute/100 g BW.[46] There was a non-linear relationship between SCr and GFR where a small increase in creatinine represents a significant decrease in GFR. These changes in sCr have been used to diagnose AKI.[47] An increase in SCr, urea, TNF-α, and lipid peroxidation confirms the manifestation of nephrotoxicity.[48] The ROS produced by cisplatin toxicity eventually enhances the lipid peroxidation process and leads to the elevation of MDA levels.[49,50]

In our work, mRNA was isolated from the kidney cortex of the rats. The kidney cortex is the outer part of the kidney, in which the glomerulus and convoluted tubules are located. The protein-coding transcripts mRNA is present in the kidney cortex.[51,52] Our results confirm that cisplatin stimulates the expression of *KIM −1, NGAL, Casp3*, and *Casp7* as shown in the negative control group, whereas quercetin (positive control) and all doses of EEBR significantly suppress the expression of *Kim-1* and *NGAL* genes. Moreover, the expression of the *Casp3* gene is significantly attenuated by higher doses of EEBR (250 mg/kg BW and 500 mg/kg BW). Interestingly, only EEBR not quercetin, significantly attenuates the expression of the *Casp7* gene.

*KIM −1* and *NGAL* levels in the serum have been proven to be more sensitive and rapid in detecting AKI during nephrotoxicity,[4] particularly when there is no reference to calculate the relative increase of SCr to diagnose AKI.[53] A recent study in animal disease models by Wang and co-workers (2020) described that serum NGAL was reported to successfully predict early diagnosis of cisplatin-induced AKI but is less accurate in later stages compared to BUN and SCr.[54] *KIM −1* is highly upregulated in proximal tubular cells due to the polarity loss of these cells.[55] Many clinical cases regarding the elevation of *KIM −1* in patients with cisplatin anticancer therapy have been published, among them is an observation on *KIM-1* level elevation in the urine of adult patients receiving cisplatin chemotherapy.[56]

## Conclusion

Our findings revealed that oxidative stress, inflammation, and cell death, might critically contribute role in the pathogenesis of cisplatin-induced nephrotoxicity. Pretreatment with the ethanol extract of *Boesenbergia rotunda* (EEBR) protects the kidney by decreasing creatinine, urea nitrogen, glutamic pyruvate transaminase, and malondialdehyde levels in the blood and reducing the expression of *Havcr1 (KIM-1), Lcn2 (NGAL), Casp3*, and *Casp7* genes in the kidney cortex of cisplatin-induced rats. EEBR did not significantly alter the BW and the % kidney relative weight which further confirms its safety. Taken together, the ethanol extract of *Boesenbergia rotunda* of the Zingiberaceae family is a potential candidate for add-on therapy in cisplatin-treated patients, but further studies are needed to verify its efficacy and safety.

## Data Sharing Statement

The data generated in the present study may be requested from the corresponding author upon reasonable request.

## Acknowledgments

The authors thank the Rector of Universitas Padjadjaran via the Directorate of Research and Community Engagement for funding both the research project and the APC.

## Funding

This research was funded by the Rector of Universitas Padjadjaran via the Directorate of Research and Community Engagement in the scheme of Academic-Leadership Research Grant No. 1959/UN6.3.1/PT.00/2021 and 1959/ UN6.3.1/ PT.00/2022 and the APC was funded by the Rector of Padjadjaran University via the Directorate of Research and Community Engagement.

## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Kelland L. The resurgence of platinum-based cancer chemotherapy. *Nat Rev Cancer*. 2007;7(8):573–584. doi:10.1038/nrc2167
2. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. *Eur J Pharmacol*. 2014;740:364–378. doi:10.1016/j.ejphar.2014.07.025
3. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of cisplatin nephrotoxicity. *Toxins (Basel)*. 2010;2:2490–2518.
4. Al-Naimi MS, Rasheed HA, Hussien NR, Al-Kuraishy HM, Al-Gareeb AI. Nephrotoxicity: role and significance of renal biomarkers in the early detection of acute renal injury. *J Adv Pharm Technol Res*. 2019;10(3):95–99. doi:10.4103/japtr.japtr_336_18

5. Tanase DM, Gosav EM, Radu S, et al. The predictive role of the biomarker kidney molecule-1 (KIM-1) in acute kidney injury (AKI) cisplatin-induced nephrotoxicity. *Int J Mol Sci*. 2019;20(20):5238. doi:10.3390/ijms20205238
6. Mishra J, Mori K, Ma Q, Kelly C, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. *Am J Nephrol*. 2004;24(3):307–315. doi:10.1159/000078452
7. Shahbazi F, Sadighi S, Dashti-Khavidaki S, Shahi F, Mirzania M. Urine ratio of neutrophil gelatinase-associated lipocalin to creatinine as a marker for early detection of cisplatin-associated nephrotoxicity. *Iran J Kidney Dis*. 2015;9(4):306–310.
8. Kaushal GP, Kaushal V, Hong X, Shah SV. Role and regulation of activation of caspases in cisplatin-induced injury to renal tubular epithelial cells. *Kidney Int*. 2001;60(5):1726–1736. doi:10.1046/j.1523-1755.2001.00026.x
9. Jiang M, Wang CY, Huang S, Yang T, Dong Z. Cisplatin-induced apoptosis in p53-deficient renal cells via the intrinsic mitochondrial pathway. *Am J Physiol Renal Physiol*. 2009;296(5):F983–F993.
10. Yang C, Kaushal V, Haun RS, Seth R, Shah SV, Kaushal GP. Transcriptional activation of caspase-6 and −7 genes by cisplatin-induced p53 and its functional significance in cisplatin nephrotoxicity. *Cell Death Differ*. 2008;15(3):530–544. doi:10.1038/sj.cdd.4402287
11. Bien Z, Fowler AJ, Robbins AJ, Pearse RM, Prowle JR, Wan YI. Trends in hospital admissions associated with an acute kidney injury in England 1998–2020: a repeated cross-sectional study. *SN Compr Clin Med*. 2022;4:53. doi:10.1007/s42399-022-01127-y
12. Hidayat H, Pradian E, Kestriani ND. Incidence, length of stay, and mortality of acute kidney injury patients at the ICU of Dr. Hasan Sadikin Bandung Hospital. *J Anestesi Perioperatif*. 2020;8:108–118. doi:10.15851/jap.v8n2.2054
13. Farooqui MA, Almegren A, Binrushud SR, et al. Incidence and Outcome of Acute Kidney Injury in Patients Hospitalized With Coronavirus Disease-19 at a Tertiary Care Medical Center in Saudi Arabia. *Cureus*. 2021;13(10):e18927. doi:10.7759/cureus.18927
14. Hansrivijit P, Gadhiya KP, Gangireddy M, Goldman JD. Risk Factors, Clinical Characteristics, and Prognosis of Acute Kidney Injury in Hospitalized COVID-19 Patients: a Retrospective Cohort Study. *Medicines*. 2021;8(1):4. doi:10.3390/medicines8010004
15. Sujana D, Saptarini NM, Sumiwi SA, Levita J. Nephroprotective activity of medicinal plants: a review on in silico-, in vitro-, and in vivo-based studies. *J Appl Pharm Sci*. 2021;11:113–127. doi:10.7324/JAPS.2021.1101016
16. Trevisani F, Di Marco F, Quattrini G, et al. Acute kidney injury and acute kidney disease in high-dose cisplatin-treated head and neck cancer. *Front Oncol*. 2023;13:1173578.
17. Liu JQ, Cai GY, Wang SY, et al. The characteristics and risk factors for cisplatin-induced acute kidney injury in the elderly. *Ther Clin Risk Manag*. 2018;14:1279–1285.
18. Oda H, Mizuno T, Ikejiri M, et al. Risk factors for cisplatin-induced acute kidney injury: a pilot study on the usefulness of genetic variants for predicting nephrotoxicity in clinical practice. *Mol Clin Oncol*. 2020;13(5):58.
19. Money ME, Hamroun A, Shu Y, et al. Case report and supporting documentation: acute kidney injury manifested as oliguria is reduced by intravenous magnesium before cisplatin. *Front Oncol*. 2021;11:607574. doi:10.3389/fonc.2021.607574
20. Jitvaropas R, Saenthaweesuk S, Somparn N, Thuppia A, Sireeratawong S, Phoolcharoen W. Antioxidant, antimicrobial and wound healing activities of Boesenbergia rotunda. *Nat Prod Commun*. 2012;7(7):909–912.
21. Atun S, Handayani S, Rakhmawati A. Potential bioactive compounds isolated from *Boesenbergia rotunda* as antioxidant and antimicrobial agents. *Pharmacogn J*. 2018;10:513–518. doi:10.5530/pj.2018.3.84
22. Rithichai P, Jirakiattikul Y, Poljan P, Youngvises N, Itharat A. Growth, bioactive compound accumulation and antioxidant activity in rhizomes and storage roots of *Boesenbergia rotunda* (L.) Mansf. *Agric Nat Resour*. 2022;56:299–306.
23. Chahyadi A, Hartati R, Wirasutisna KR. *Boesenbergia pandurata* Roxb. an Indonesian medicinal plant: phytochemistry, biological activity, plant biotechnology. *Procedia Chem*. 2014;13:13–37. doi:10.1016/j.proche.2014.12.003
24. Isa NM, Abdelwahab SI, Mohan S, et al. In vitro anti-inflammatory, cytotoxic and antioxidant activities of boesenbergin A, a chalcone isolated from Boesenbergia rotunda (L.) (fingerroot). *Braz J Med Biol Res*. 2012;45(6):524–530.
25. Saah S, Siriwan D, Trisonthi P. Biological activities of *Boesenbergia rotunda* parts and extracting solvents in promoting osteogenic differentiation of pre-osteoblasts. *Food Biosci*. 2021;41:101011.
26. Wang T, Liu C, Shu S, Zhang Q, Olatunji OJ. Therapeutic efficacy of polyphenol-rich fraction of Boesenbergia rotunda in diabetic rats: a focus on hypoglycemic, antihyperlipidemic, carbohydrate metabolism, antioxidant, anti-inflammatory and pancreato-protective activities. *Front Biosci*. 2022;27:206.
27. Sujana D, Saptarini NM, Sumiwi SA, Levita J. The protective effect of *Boesenbergia rotunda* extract on cisplatin-exposed human embryonic kidney-293 cells by inhibiting the expression of kidney injury molecule-1, neutrophil gelatinase associated-lipocalin, NF-κB, and caspases. *J Herbmed Pharmacol*. 2023;12:147–152.
28. Keoni CL, Brown TL. Inhibition of apoptosis and efficacy of pan-caspase inhibitor, Q-VD-OPh, in models of human disease. *J Cell Death*. 2015;8:1–7.
29. Herawati IE, Lesmana R, Levita J, Subarnas A. Cytotoxicity, Apoptosis, Migration Inhibition, and Autophagy-Induced by Crude Ricin from Ricinus communis Seeds in A549 Lung Cancer Cell Lines. *Med Sci Monit Basic Res*. 2022;28:e936683.
30. Yang L, Xing G, Wang L, et al. Acute kidney injury in China: a cross-sectional survey. *Lancet*. 2015;386:1465–1471. doi:10.1016/s0140-6736(15)00344-x
31. Khasanah D, Permana YS, Rahman A, Saraswati PT. Nephroprotective activity of fingerroot (*Boesenbergia pandurata*) extract against cisplatin-induced nephrotoxicity in mice: molecular, biochemical, and histopathological approach. *J Asian Med Student Assoc*. 2020;8:13.
32. Hassan SM, Khalaf MM, Sadek SA, Abo-Youssef AM. Protective effects of apigenin and myricetin against cisplatin-induced nephrotoxicity in mice. *Pharm Biol*. 2017;55(1):766–774. doi:10.1080/13880209.2016.1275704
33. Vargas F, Romecín P, García-Guillén AI, et al. Flavonoids in kidney health and disease. *Front Physiol*. 2018;9:394. doi:10.3389/fphys.2018.00394
34. Thongnuanjan P, Soodvilai S, Fongsupa S, et al. Protective effect of panduratin a on cisplatin-induced apoptosis of human renal proximal tubular cells and acute kidney injury in mice. *Biol Pharm Bull*. 2021;44(6):830–837. doi:10.1248/bpb.b21-00036
35. Lin MT, Ko JL, Liu TC, Chao PT, Ou CC. Protective effect of D-Methionine on body weight loss, anorexia, and nephrotoxicity in cisplatin-induced chronic toxicity in rats. *Integr Cancer Ther*. 2018;17:813–824.
36. Ramanathan L, Gulyani S, Nienhuis R, Siegel JM. Sleep deprivation decreases superoxide dismutase activity in rat hippocampus and brainstem. *Neuroreport*. 2002;13:1387–1390.

37. Oteki T, Ishikawa A, Sasaki Y, et al. Effect of rikkunshi-to treatment on chemotherapy-induced appetite loss in patients with lung cancer: a prospective study. *Exp Ther Med*. 2016;11:243–246.
38. McSweeney KR, Gadanec LK, Qaradakhi T, Ali BA, Zulli A, Apostolopoulos V. Mechanisms of cisplatin-induced acute kidney injury: pathological mechanisms, pharmacological interventions, and genetic mitigations. *Cancers (Basel)*. 2021;13:1572.
39. Mestry SN, Gawali NB, Pai SA, et al. *Punica granatum* improves renal function in gentamicin-induced nephropathy in rats via attenuation of oxidative stress. *J Ayurveda Integr Med*. 2020;11:16–23.
40. Sadick M, Attenberger U, Kraenzlin B, et al. Two non-invasive GFR-estimation methods in rat models of polycystic kidney disease: 3.0 Tesla dynamic contrast-enhanced MRI and optical imaging. *Nephrol Dial Transplant*. 2011;26:3101–3108.
41. Kangari P, Zarnoosheh Farahany T, Golchin A, et al. Enzymatic antioxidant and lipid peroxidation evaluation in the newly diagnosed breast cancer patients in Iran. *Asian Pac J Cancer Prev*. 2018;19:3511–3515.
42. Verlander JW, Glomerular Filtration. 6th. *Cunningham's Textbook of Veterinary Physiology*, 2020:480–488
43. Miranda KC, Bond DT, McKee M, et al. Nucleic acids within urinary exosomes/microvesicles are potential biomarkers for renal disease. *Kidney Int*. 2010;78:191–199.
44. Du Y, Hou L, Guo J, Sun T, Wang X, Wu Y. Renal neutrophil gelatinase-associated lipocalin and kidney injury molecule-1 expression in children with acute kidney injury and Henoch Schönlein purpura nephritis. *Exp Ther Med*. 2014;7:1130–1134.
45. Sabbisetti VS, Waikar SS, Antoine DJ, et al. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *J Am Soc Nephrol*. 2014;25:2177–2186.
46. George B, Wen X, Mercke N, et al. Time-dependent changes in kidney injury biomarkers in patients receiving multiple cycles of cisplatin chemotherapy. *Toxicol Rep*. 2020;7:571–576.
47. Rosdianto AM, Puspitasari IM, Lesmana R, Levita J. Inhibitory activity of *Boesenbergia rotunda* (L.) Mansf. rhizome towards the expression of Akt and NF-kappaB p65 in acetic acid-induced Wistar rats. *Evid-Based Complement Alternat Med*. 2020;2020:6940313.
48. Indonesian Herbal Pharmacopoeia. *The Republic of Indonesia Ministry of Health*. 2nd. Jakarta, Indonesia; 2017.
49. Sanchez-Gonzalez PD, Lopez-Hernandez FJ, Perez-Barriocanal F, Morales AI, Lopez-Novoa JM. Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumour activity. *Nephrol Dial Transplant*. 2011;26(11):3484–3495. doi:10.1093/ndt/gfr195
50. Soni H, Kaminski D, Gangaraju R, Adebiyi A. Cisplatin-induced oxidative stress stimulates renal Fas ligand shedding. *Ren Fail*. 2018;40 (1):314–322.
51. Ilić S, Stojiljković N, Veljković M, Veljković S, Stojanović G. Protective effect of quercetin on cisplatin-induced nephrotoxicity in rats. *Med Biol*. 2014;16:71–75.
52. Besseling PJ, Pieters TT, Nguyen ITN, et al. A plasma creatinine- and urea-based equation to estimate glomerular filtration rate in rats. *Am J Physiol Renal Physiol*. 2021;320:F518–F524.
53. Abdel-Hady H, El-Sayed M, Abdel-hady AA, et al. Nephroprotective activity of methanolic extract of *Lantana camara* and squash (*Cucurbita pepo*) on cisplatin-induced nephrotoxicity in rats and identification of certain chemical constituents of Lantana camara by HPLC-ESIMS. *Pharmacogn J*. 2018;10:136–147.
54. Wang W, Li Z, Chen Y, Wu H, Zhang S, Chen X. Prediction value of serum NGAL in the diagnosis and prognosis of experimental acute and chronic kidney injuries. *Biomolecules*. 2020;10(7):981.
55. Neelima S, Reddy PD, Bannoth SK. Nephroprotective activity of *Annona squamosa* leaves against paracetamol-induced nephrotoxicity in rats: in vitro and in vivo experiments. *Futur J Pharm Sci*. 2020;6:131.
56. Prozialeck WC, Edwards JR, Lamar PC, Liu J, Vaidya VS, Bonventre JV. Expression of kidney injury molecule-1 (Kim-1) in relation to necrosis and apoptosis during the early stages of Cd-induced proximal tubule injury. *Toxicol Appl Pharmacol*. 2009;238:306–314.