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ORIGINAL RESEARCH

Nephroprotective Activity of *Angelica keiskei* (Miq). Koidz. on Cisplatin-Induced Rats: Reducing Serum Creatinine, Urea Nitrogen, KIM-1, and Suppressing NF-kappaB p65 and COX-2

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Background: The sap of *Angelica keiskei* (Miq). Koidz. has been reported for its abundance of chalcone contents. Chalcones have been known for their effective nephroprotective activity toward cisplatin-induced renal cells and mice.

Purpose: To investigate the effect of *A. keiskei* sap extract (ASEE) on kidney function parameters (serum creatinine, urea nitrogen, and kidney injury molecule-1) and the expression of NF-kappaB p65 and COX-2 in cisplatin-induced Wistar rats.

Methods: In vivo nephroprotective activity of ASEE at 1000 and 1500 mg/kg BW/day doses for 10 days on cisplatin (5 mg/kg BW) induced nephrotoxicity was evaluated on Wistar rats. Quercetin 20 mg/kg BW/day was used as the control drug. Cisplatin inducement was given on day 7. The BW was measured every day. On day 11, the rats were euthanized, and their blood was taken intracardially for creatinine and urea nitrogen analysis. Histopathological analysis was carried out on the right kidney, and KIM-1 levels in the left kidney were measured. The Western blot technique evaluated the NF-kappaB p65 and COX-2 expression in the kidney. All data obtained were compared to the cisplatin group (negative control). The total flavonoids and chalcones in ASEE were also determined.

Results: Pretreatment with ASEE reduces the BW of Wistar rats, and significantly reduces creatinine and KIM-1 levels, but does not significantly reduce the levels of urea nitrogen, the expression of NF-kappaB p65, and COX-2 in the kidney of cisplatin-induced Wistar rats. The total flavonoid content in ASEE is 8.755 g QE/100 g extract and the total chalcone content is 5.532 g IBCE/100 g extract.

Conclusion: The sap of *Angelica keiskei* (Miq). Koidz. reveal the potential to protect the kidneys against cisplatin-induced toxicity. The nephroprotective activity may be attributed to the antioxidant and anti-inflammatory properties of the flavonoids and the chalcones contained in the sap of *Angelica keiskei* (Miq). Koidz.

Keywords: antioxidants, apoptosis, chalcones, chemotherapy, Japanese celery, kidney injury

Introduction

Cisplatin activates the crosslinking of deoxyribonucleic acid (DNA) and the formation of DNA adducts with a non-specific mechanism of action, thus triggering apoptosis in both cancer cells and normal tissues. The adverse effects of cisplatin are dose-dependent and include nephrotoxicity, among other organ toxicities.[1,2] Studies in animal models have described that cisplatin induces DNA damage, mitochondrial pathology, oxidative stress, and endoplasmic reticulum stress in the kidneys, thus confirming the pathogenesis of cisplatin nephrotoxicity.[3] It is observed that approximately 30% of patients treated with a single dose of cisplatin experience a notable decrease in their kidney function, due to damage in

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the tubular, marked by elevated plasma creatinine and urea levels.[4] Importantly, many studies have reported the role of plant flavonoids in protecting the kidneys by improving antioxidant status, mitigating excessive reactive oxygen species (ROS) levels, and decreasing oxidative stress.[5–8] Moreover, a chalcone derivative was reported for its strong anti-necroptosis and protective activity by directly binding to RIPK1 (the main regulator of epithelial cell survival, homeostasis, and inflammation) and inhibiting RIPK1-RIPK3-mixed-lineage kinase domain-like protein (MLKL) signaling pathway.[9]

The stem of *Angelica keiskei* (Miq). Koidz. contains flavonoids such as quercetin and luteolin, polyphenols, and chalcone compounds including xanthoangelol, 4-hydroxyderricin, and isobavachalcone.[10,11] *A. keiskei* has been reported for its numerous pharmacology activities such as nephroprotective,[12] antiobesity,[13] antidiabetic,[11,14] anti-inflammatory,[15,16] antitumor,[17] and anti-hyperlipidemia.[18,19] Interestingly, xanthoangelol D, a chalcone isolated from *A. keiskei* root, exhibited a suppression of the NF-kappaB activation as reported by Sugii and colleagues.[20] Another study reported the potential of chalcone in alleviating polycystic kidney disease by inhibiting cystic fibrosis transmembrane conductance regulator (CFTR) expression and reducing extracellular signal-regulated kinase 1/2 (ERK1/2) and mammalian target of rapamycin (mTOR)/S6K signaling pathways as well as activating AMP-activated protein kinase (AMPK) expression.[21]

Considering the potential of *Angelica keiskei* as a nephroprotective agent, due to its flavonoids and chalcone content, this study aims to explore the effect of *the sap of A. keiskei* on the serum creatinine, urea nitrogen, and KIM-1 levels, and the expression of NF-kappaB p65 and COX-2 in animal models.

Material and Methods

Plant Materials

The stem specimen (Figure 1) and sap were collected from Mount Rinjani, Indonesia. The plant specimen was identified by Arifin Surya Dwipa Irsyam (https://www.scopus.com/authid/detail.uri?authorId=57211286941; https://herbarium.sith.

Figure 1 The stem of *Angelica keiskei* (Miq.) Koidz., collected at Sembalun Bumbung Village, East Lombok Regency, West Nusa Tenggara, Indonesia (Google maps −8.39887349095366, 116.54712323344886). The yellow sap is shown by yellow arrows.

itb.ac.id/profil-kurator/), the certified botanist at the School of Life Sciences and Technology, Bandung Institute of Technology (Bandung, West Java, Indonesia) (https://herbarium.sith.itb.ac.id/koleksi/), and was confirmed as *Angelica keiskei* (Miq). Koidz. of plant family Apiaceae (document 2847/ITI.C11.2/2023).

Chemicals and Drugs

The chemicals used were technical grade ethanol 96% solvent for plant extraction (BrataChem Indonesia), analytical-grade ethanol (Merck), quercetin (Tokyo Chemical Industry; CAS No. 849061–97-8), isobavachalcone (Sigma-Aldrich; CAS No. 20784–50-3), pharmaceutical grade carboxymethylcellulose sodium, cisplatin for injection 10 mg/10 mL (Kalbe), ketamine for injection (Fatro Iberica), Creatinine Kinetic Colorimetric Method (Linear Chemicals), Urea/Blood Urea Nitrogen BR kit (Linear Chemicals), KIM-1 ELISA kit (Elabscience; Catalog No. E-EL-R3019), phosphate buffer pH 7.4 (Merck), formalin 10% (Mid Chem), hematoxylin (Himedia) and eosin (Himedia), rabbit anti-COX-2 (Cell Signaling Technology; 12282S), human/mouse/rat beta-actin antibody (Biotechne; Catalog No. MAB8929), NF-kappaB p65 SC-8008 (Santa Cruz Biotechnology, Inc.) for detection of NF-kappaB p65 of rats by Western Blotting, IRDye® 800CW goat anti-mouse IgG secondary antibody (LICORbio 925–32,210), bovine serum albumin, sodium dodecyl sulfate 15%, nitrocellulose membrane (Merck), phosphate buffer saline-Tween 20 (PBST) 0.1%, and prestained protein marker (8–195 kDa) Cat. No. 86941 (https://www.diagnocine.com/Product/Prestained-Protein-Marker-8195-kDa/86941).

Instruments and Glassware

The instruments used were a rotary evaporator (IKA RV 10), freeze dryer (Ihanil, Vac 8), hot plate (Thermo Scientific), UV-visible spectrophotometer (Analytik Jena Specord 200), chemical glassware (Pyrex), digital analytical balance (Mettler Toledo Dragon 204), microcentrifuge (Eppendorf®), oral gavage, microscope (Primostar 3 binoculars; Carl Zeiss AG), incubator (Heratherm), multimode reader (Tecan, Infinite 200PRO NanoQuant), micropipette (Eppendorf), Western Blot instrument (Invitrogen Thermo Scientific dan Biorad), heat block (Cleaver), and rocker shaker (Cleaver).

Plant Extraction

The sap was extracted by following a previous procedure of Aulifa et al (2022)[10] as follows: approximately 2 L of the sap was dried at −80°C, and eventually 60 g of the dry sap powder was macerated in ethanol 96% solvent (1:10) for 5×24 h. The filtrate was collected and evaporated at 60°C, 85 rpm for 40 minutes until a thick ethanol extract of *A. keiskei* sap (abbreviated as ASEE) was obtained with a weight of 49.25 g or 82.08% w/w. The viscous ASEE was determined for its total flavonoids and chalcone content.

Identification of Flavonoids in ASEE and Determination of Total Flavonoid Content (TFC)

Identification of flavonoids in the extract was carried out by following previous procedures using the $AlCl_3$ reagent.[22,23] The total flavonoid content (TFC) was determined by adopting previous procedures as follows:[24,25] Accurately weighed quercetin 10 mg was dissolved in 10 mL of analytical-grade ethanol to obtain 1000 µg/mL. The solution was diluted to 80 µg/mL and used as the standard. Parallelly, 10 mg of ASEE was weighed and dissolved in 10 mL of analytical-grade ethanol. The solution was diluted to 10 µg/mL. In each of the six volumetric flasks of 10 mL, 2 mL of ASEE solution was put into, and added with quercetin solution of 0; 0.1; 0.2; 0.4; 0.8; and 1.6 µL for the standard addition method (spiking various volume of standard quercetin into ASEE solution). The absorbance of the solution was measured at the maximum wavelength of quercetin (λ_{max} 374 nm).

Determination of Total Chalcones

The total chalcones were determined by following previous procedures:[26,27] Accurately weighed isobavachalcone (IBC) 10 mg was dissolved in 10 mL of analytical-grade ethanol to obtain 1000 µg/mL. The solution was diluted to 80 µg/mL and used as the standard. Parallelly, 10 mg of ASEE was weighed and dissolved in 10 mL of analytical-grade ethanol.

The solution was diluted to 10 µg/mL. In each of the six volumetric flasks of 10 mL, 2 mL of ASEE solution was put into, and added with IBC solution of 0; 0.25; 0.5; 0.75; 1.0; 1.25; and 1.5 µL for the standard addition method (spiking various volume of standard IBC into ASEE solution). The absorbance of the solution was measured at the maximum wavelength of IBC (λ_{max} 367 nm).

Animals

Twenty-five male Wistar rats, 6–8 weeks, 200–220 g, were used in this study. The rats were confirmed as healthy by a veterinarian examination (Document No. 121/TRM/SK/X/2023). The rats were randomly placed in cages (n = 5 per cage) with net-shaped wire lids (2430 cm^2 of base area and 37 cm of height), and rice husk mats, cleaned and replaced every 2 days. Animals were set at 12 hours of a light–dark cycle, given free standard pellet feed (containing 5% low fiber, 20% protein, and 5–10% fat), and drank water. The body weight (BW) of the rats was measured every day. The study was approved by the Research Ethics Committee of Padjadjaran University (Document No. 1241/UN6.KEP/EC/2023), which follows The Guide for the Care and Use of Laboratory Animals (NRC 2011; eighth edition) (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf).[28]

The rats were divided into 5 groups, (1) normal (treated with CMC Na 0.3% suspension for 10 days), (2) negative (nephrotoxicity induced with cisplatin 5 mg/kg BW on day 7), (3) positive (treated with quercetin 20 mg/kg BW for 10 days and nephrotoxicity induced with cisplatin 5 mg/kg BW on day 7), and two test groups which were treated with (4) ASEE 1000 mg/kg BW, and (5) ASEE 1500 mg/kg BW for 10 days and nephrotoxicity induced with cisplatin 5 mg/kg BW on day 7. The doses of ASEE were calculated from the results of TPC and TFC.

The Effect of ASEE on the Body Weight, Serum Creatinine, and Blood Urea Nitrogen of Cisplatin-Induced Wistar Rats

The BW of the rats was measured every day. On day 11, the rats were euthanized using ketamine injection at a dose of 100 mg/kg BW, and their blood was taken intracardially for creatinine and urea nitrogen analysis. The whole blood was centrifugated at 12,000 rpm 40 °C for 10 minutes, the plasma was separated, and its creatinine (sCr) and urea nitrogen (BUN) levels were measured by following the instructions of the Creatinine Kinetic colorimetric method (Linear Chemicals) and Urea/BUN BR kit (Linear Chemicals). All data obtained were compared to the cisplatin group (negative control).

The Effect of ASEE on the Kidney Injury Molecule-1 (KIM-1) Levels of Cisplatin-Induced Wistar Rats

The left kidney of the rats was separated, cleaned, and washed with saline solution. About 100 mg of the kidney tissue was homogenized in a phosphate buffer solution of pH 7.4, and centrifugated at 4 °C 10,000 rpm. KIM-1 levels were measured by strictly following the instructions in the KIM-1 ELISA kit (Elabscience; Catalog No. E-EL-R3019). All data obtained were compared to the cisplatin group (negative control).

Kidney Histopathological Analysis

The right kidney of the rats was separated, cleaned, washed with saline solution, and soaked in 10% formalin solution. The histopathology procedures were carried out by following previous methods,[29,30] visualized using a Primostar 3 binoculars microscope (Carl Zeiss AG), and photographed using a digital camera (Infinity1®). All data obtained were compared to the cisplatin group (negative control).

The Effect of ASEE on the Levels of NF-kappaB p65 and COX-2 of Cisplatin-Induced Wistar Rats

The Western blot technique was used to measure the protein levels of NF-kappaB p65 and COX-2 in the kidney according to the method described elsewhere.[31–36] All data obtained were compared to that of the cisplatin group (negative control).

Statistical Analysis

Analysis of the data was performed using GraphPad Prism 8.1.2. The difference in the nephroprotective activity of test animals was analyzed using the one-way or two-way analysis of variance (ANOVA) test at a confidence level of 95%. The Bonferroni test determined the mean significant difference between each group with $p < 0.05$.

Results

Identification of Flavonoids in ASEE, Total Flavonoid Content, and Total Chalcones

The absorption spectra of ASEE with or without the addition of $AlCl_3$ and sodium acetate are presented in Figure 2a. All spectra confirm the presence of Al(III)-flavonoid complexes as indicated by the bathochromic shift. The method is based on the forming of Al(III)-flavonoid chelates.[37] The total flavonoid content of ASEE calculated using a quercetin standard curve (linear regression equation $y = 0.0092x + 0.0851$; $R^2 = 0.9991$) is 87.55 mg QE/g extract. The total chalcones in ASEE calculated using an isobavachalcone (IBC) standard curve (linear regression equation $y = 0.0696x + 0.0385$; $R^2 = 0.9970$) is 55.32 mg IBCE/g extract. The spectrum of ASEE spiked with increased concentrations of quercetin is depicted in Figure 2b and ASEE spiked with increased concentrations of IBC is depicted in Figure 2c.

ASEE Reduces BW, sCr, and BUN of Cisplatin-Induced Wistar Rats

Pretreatment with ASEE 1000 mg/kg BW (green) and 1500 mg/kg BW (blue) from day 1 to day 7 before cisplatin inducement reduces the BW of Wistar rats. Rats in the normal group (treated daily with CMC Na suspension 0.3%) showed an increase in BW during 11 days of observation (Figure 3a) and significant differences in the BW of day 7 compared to day 11 (Figure 3b). The difference in BW on day 7 and day 11 of rats treated with ASEE 1000 mg/kg BW is a 4.865% decrease; meanwhile, rats treated with ASEE 1500 mg/kg BW show a 6.452% of BW reduction.

Cisplatin inducement at a dose of 5 mg/kg BW increases serum creatinine levels (sCr = 1.15 mg/dL) compared to the normal control group treated with sodium carboxymethylcellulose without cisplatin inducement (sCr = 1.082 mg/dL), although the increase is not statistically significant. Interestingly, pretreatment with ASEE 1000 mg/kg BW (sCr = 0.993 mg/dL) and ASEE 1500 mg/kg BW (sCr = 0.999 mg/dL) significantly reduced the sCr levels with p values of respectively, 0.0035 and 0.0050, compared to the negative control (cisplatin-induced rats without treatment) group (Figure 4a).

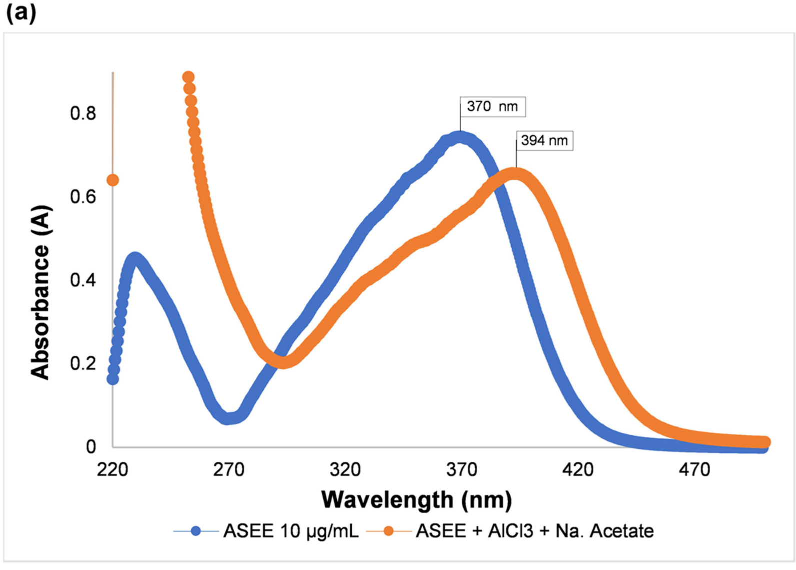
Cisplatin inducement at a dose of 5 mg/kg BW significantly increases blood urea nitrogen levels (BUN = 20.833 mg/dL) compared to the normal control group ($p = 0.0341$). Interestingly, pretreatment with ASEE 1000 mg/kg BW (BUN = 12.5 mg/dL) and ASEE 1500 mg/kg BW (BUN = 16.66 mg/dL) reduces the BUN levels although not significant, with p values of respectively, 0.1551 and 0.6439, compared to the negative control (Figure 4b).

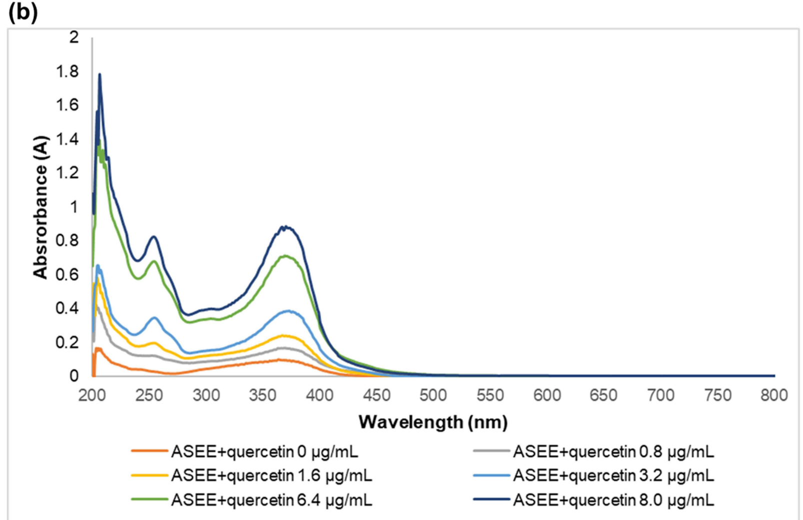
ASEE Reduces the KIM-1 Levels of Cisplatin-Induced Wistar Rats

KIM-1 levels were calculated using a linear regression KIM-1 standard curve with an equation of $y = 0.1234\,x + 0.0145$ and $R^2 = 0.9971$ (Figure 5a). Cisplatin inducement at a dose of 5 mg/kg BW significantly increases KIM-1 levels (KIM-1 = 8.178 ng/dL) compared to the normal control group (KIM-1 = 0.195 ng/dL; $p = 0.0032$). Interestingly, pretreatment with ASEE 1000 mg/kg BW (KIM-1 = 6.074 ng/dL) and ASEE 1500 mg/kg BW (KIM-1 = 6.367 ng/dL) reduces the KIM-1 levels although not significant compared to the negative control (Figure 5b).

The Effect of ASEE on the Kidney Histopathology of Cisplatin-Induced Wistar Rats

The effects of ASEE on the microscopic histopathology examination of cisplatin-induced Wistar rat kidney tissue are presented in Figure 6. Cisplatin inducement at a dose of 5 mg/kg BW causes amyloid sedimentation, glomerular atrophy,





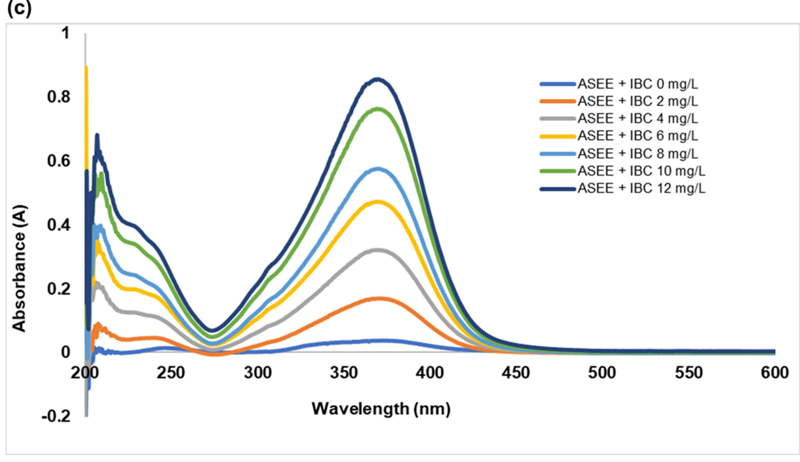
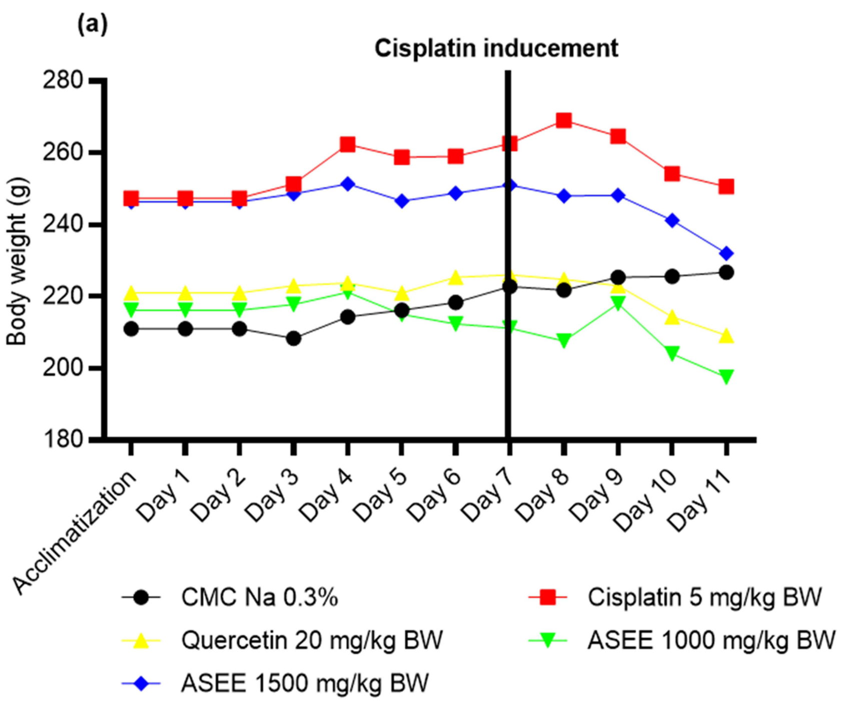


Figure 2 The absorption spectra of the ethanol extract of (**a**) *A. keiskei* sap (ASEE) with (red) and without (blue) the addition of aluminum chloride and sodium acetate. The addition of aluminum chloride and sodium acetate to ASEE resulted in a bathochromic shift from 370 nm to 394 nm due to the formation of a flavonoid-aluminum complex; (**b**) ASEE spiked with increased concentration of quercetin; (**c**) ASEE spiked with increased concentration of isobavachalcone.



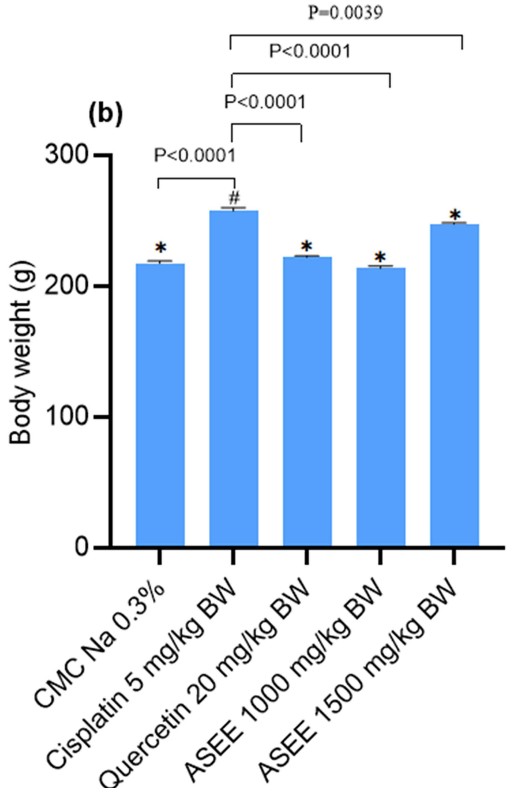
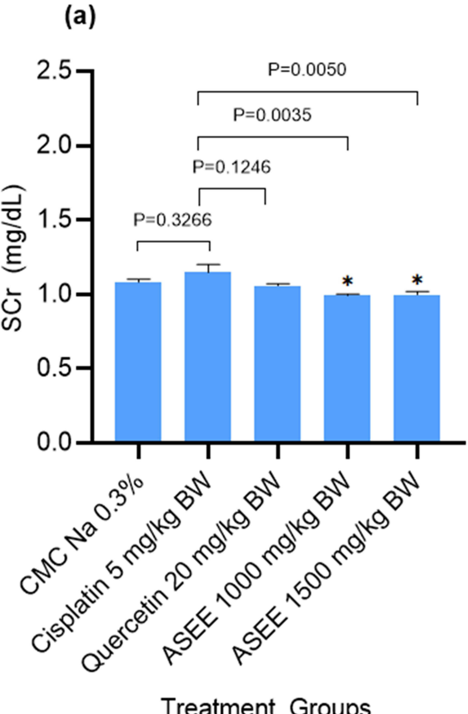


Figure 3 (**a**) Pretreatment with ASEE 1000 mg/kg BW (green) and 1500 mg/kg BW (blue) from day 1 to day 7 before cisplatin inducement reduces the BW of Wistar rats. Rats in the normal group (treated daily with CMC Na suspension 0.3%) showed an increase in BW during 11 days of observation; (**b**) Statistical analysis was performed using the two-way ANOVA and Dunnett's test. Significant difference is defined if $p < 0.05$ and depicted as # (when compared to the Na CMC group or the normal control group), and depicted as * (when compared to the cisplatin group).



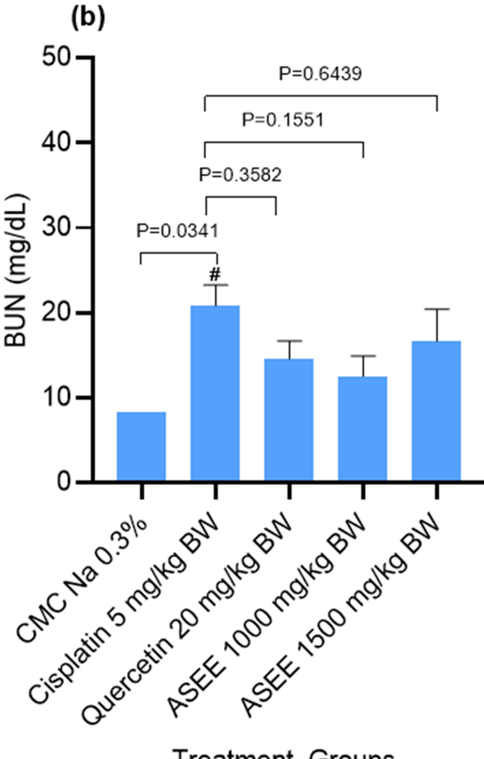
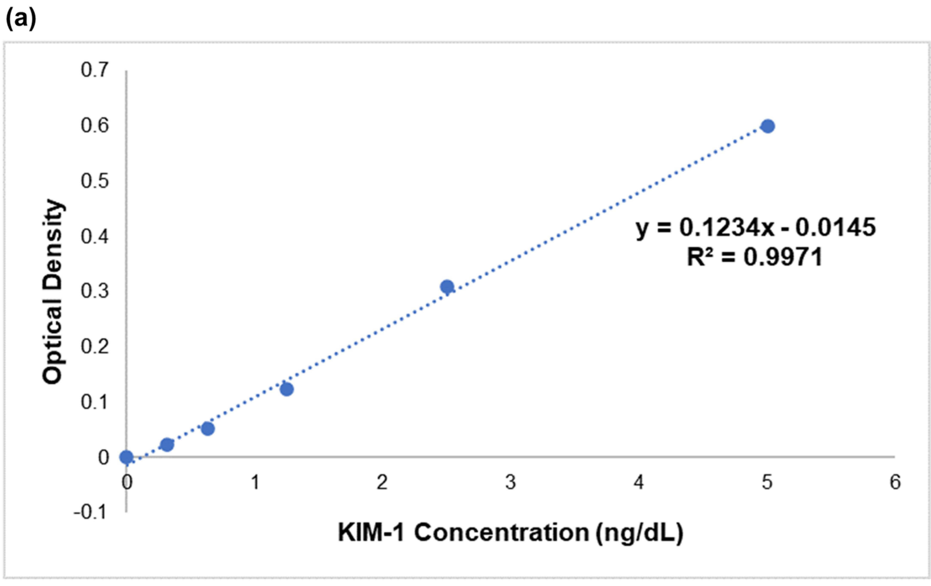


Figure 4 The effects of ASEE on the (**a**) serum creatinine and (**b**) blood urea nitrogen of cisplatin-induced Wistar rats. ASEE doses of 1000 mg/kg BW and 1500 mg/kg BW significantly reduce creatinine ($p = 0.0035$ and $p = 0.0050$, respectively) but do not significantly reduce the levels of urea nitrogen ($p = 0.1551$ and $p = 0.6439$, respectively). Statistical analysis was performed using the two-way ANOVA and Dunnett's test. Significant difference is defined if $p < 0.05$ and depicted as # (when compared to the Na CMC group or the normal control group), and depicted as * (when compared to the cisplatin group).



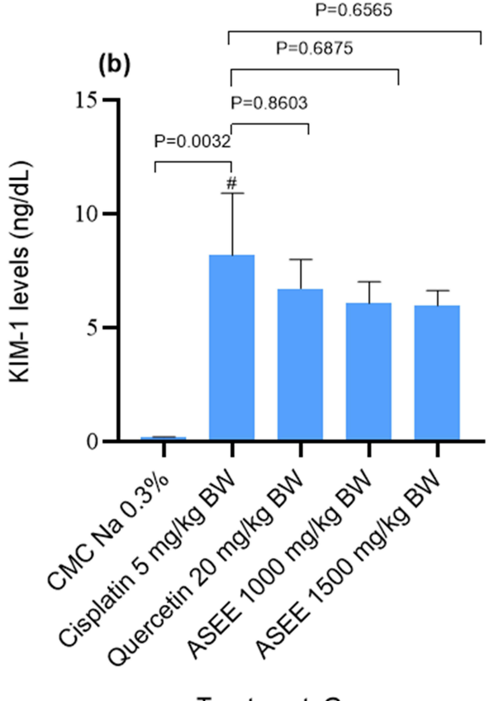


Figure 5 The KIM-1 levels were calculated using a linear regression KIM-1 standard curve with an equation of y = 0.1234x + 0.0145 and R2 = 0.9971 (**a**) and (**b**) the effects of ASEE on the KIM-1 levels of cisplatin-induced Wistar rats. ASEE doses of 1000 mg/kg BW and 1500 mg/kg BW reduce KIM-1 levels (6.074 ng/dL; p = 0.6875 and 6.367 ng/dL; p = 0.6565, respectively) although not significant compared to the negative control (cisplatin-induced) group. Statistical analysis was performed using the one-way ANOVA and Post Hoc Bonferroni test. Significant difference is defined if $p < 0.05$ and depicted as # (when compared to the Na CMC group or the normal control group).

and necrosis of the epithelial tubule. Pretreatment with ASEE or quercetin reduces amyloid sedimentation, but glomerular atrophy and necrosis of the epithelial tubule are still observed.

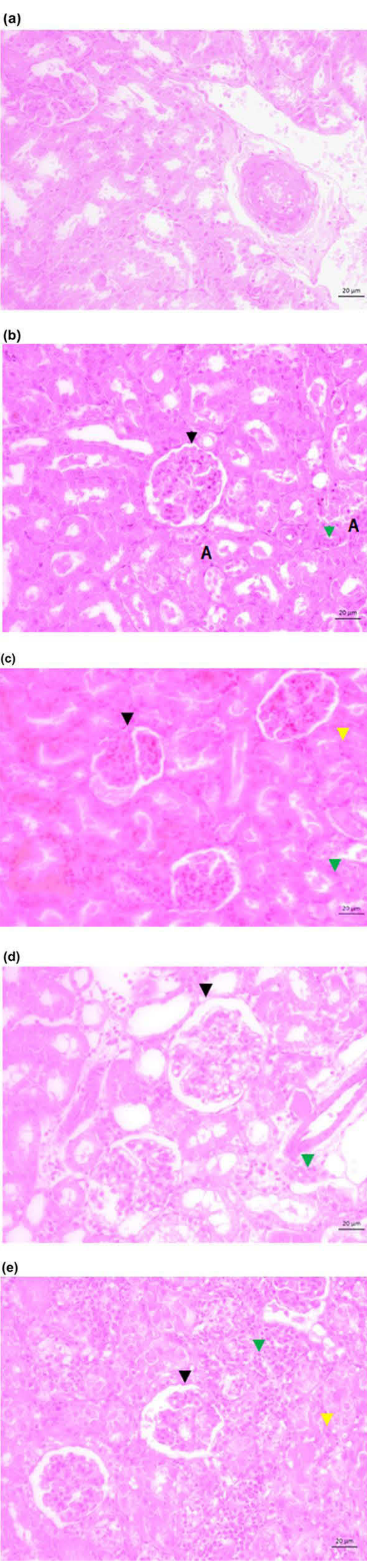


Figure 6 The effects of ASEE on the microscopic histopathology examination of kidney tissue of (**a**) Na CMC group (the normal control group); (**b**) cisplatin 5 mg/kg-induced group; (**c**) quercetin 20 mg/kg group; (**d**) ASEE dose of 1000 mg/kg group; (**e**) ASEE dose of 1500 mg/kg group. A indicates amyloid sedimentation; black arrowhead indicates glomerular atrophy; green arrowhead indicates necrosis of epithelial tubule; yellow arrowhead indicates infiltration of inflammatory cells.

The Effect of ASEE on the Expression of NF-kappaB p65 of Cisplatin-Induced Wistar Rats

Intraperitoneal exposure of cisplatin single dose at 5 mg/kg BW resulted in an increase of NF-kappaB p65 and COX-2 levels in the kidney tissue of Wistar rats. The relative expression of NF-kappaB p65 and COX-2 influenced by ASEE pretreatment was measured using the Western blot technique (Figure 7a and b). The protein bands of COX-2 (72 kDa), NF-kappaB p65 (65 kDa), and beta-actin (42 kDa) expression in the kidney tissue of cisplatin-induced Wistar rats are depicted in Figure 7c. It is confirmed that pretreatment with ASEE can reduce the expression of NF-kappaB p65 and COX-2 expression although not significant compared to the cisplatin group.

Discussion

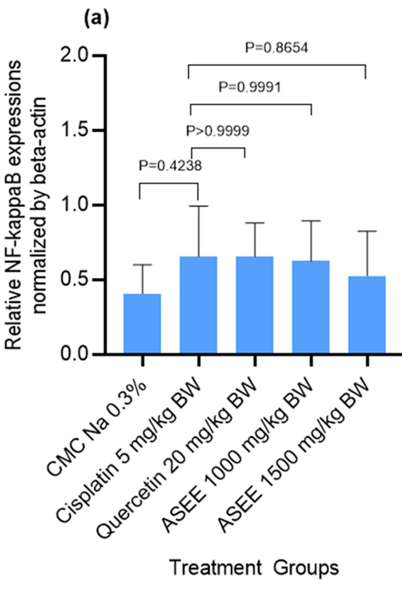
The sap of *A. keiskei* collected at Sembalun Bumbung Village, East Lombok Regency, West Nusa Tenggara, Indonesia, shows a yellow color and specific odor. In our study, the TFC in the ethanol extract of *A. keiskei* sap (ASEE) is 87.55 mg quercetin equivalent (QE)/g extract and the total chalcones is 55.32 mg isobavachalcone equivalent (IBCE)/g extract. Our results are comparable to a previous study by Zhang and colleagues. It was reported that the total flavonoids in *A. keiskei* powder supplied by Shandong Ashitaba Biotech Co., Ltd (Shandong, China), were better extracted with the ethanol solvent concentrations of 40–80%, with TFC values of 4.00 to 6.50 mg rutin equivalent (RE)/g extract.[10] Moreover, a recent study published in 2024 by Turck and co-workers described the total chalcone content in the sap of cultivated *A. keiskei* plants originating from certified organic farming was 1.778% (w/v).[38] It is delineated in a review article that 100 g of dried herb granules contained 198.7 mg of total chalcones, and the levels of chalcones in the leaves were 1.959 mg/100 g, in the stems was 2.63 mg/100 g, in the root bark was 10.51 mg/100 g, and in the root core was 1.44 mg/100 g.[39]

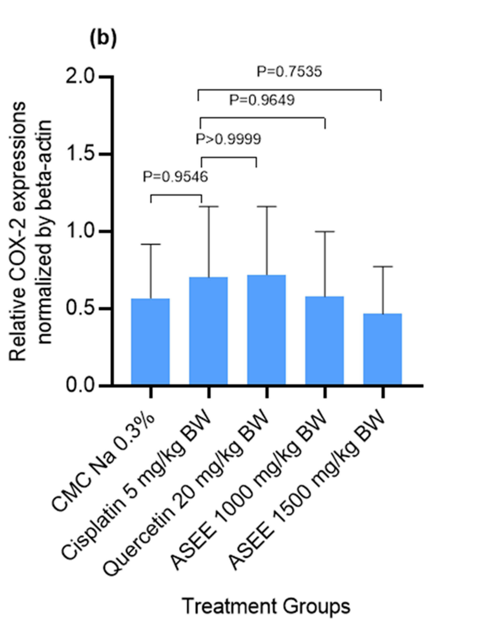
Oxidative stress contributes majorly to the pathophysiology of nephrotoxicity, which is characterized by an increase of creatinine in the blood, a reduction in urine output, and on the cellular level, it is marked by the expression of proinflammatory cytokines, due to the activation of NF-kappaB pathway.[40] In our study, rats intraperitoneally exposed to cisplatin at a single dose of 5 mg/kg BW have shown an increase in serum creatinine and BUN levels, and pretreatment with ASEE 1000 mg/kg BW and ASEE 1500 mg/kg BW reduced the sCr and BUN. Previous studies have also reported that cisplatin inducement resulted in long-term effects on the morphology and physiology of the rat kidney after single-dose intraperitoneal administration,[41,42] thus confirming the nephrotoxic effect this chemotherapy drug on the kidney proximal tubule[41] and increased lipid peroxidation, urine volume, and plasma creatinine levels of the rats.[42]

Growing evidence has shown the role of flavonoids and/or chalcones in mitigating excessive ROS levels, thus protecting the kidneys from damage due to oxidative stress.[5–9] Flavonoids exhibit protective effects on the kidneys by reducing the excessive ROS level or activating renal enzymatic and non-enzymatic antioxidants through various pathways.[5] Interestingly, a study on the effects of dietary *A. keiskei* on serum and liver lipid profiles of rats revealed no pathological impact on these organs, thus confirming its safety.[43]

Cisplatin inducement also significantly increases KIM-1 levels in the rats' blood, and pretreatment with ASEE 1000 mg/kg BW and ASEE 1500 mg/kg BW reduces the KIM-1 levels, thus confirming the nephroprotective activity of ASEE. KIM-1 is a proximal tubule transmembrane protein whose levels are significantly increased in the post-ischemic rat kidney and in patients with biopsy-proven acute tubular necrosis. KIM-1 was expressed in the patients' urines within 12 hours after the initial ischemic renal damage, while in animals, this protein develops parallelly with the manifestation of epithelial cell dedifferentiation and proliferation.[44] A multi-center study in France on 54 patients with kidney injury reported that blood KIM-1 levels were increased at diagnosis and decreased after therapy. KIM-1 was associated with the level of acute tubular necrosis and interstitial fibrosis/tubular atrophy on kidney biopsy, but not with interstitial infiltrate or with glomerular involvement.[45] KIM-1 levels correlate with inflammation and fibrosis in histological studies,[46] and modulate the activation of the NF-kappaB pathway and interaction with phosphatidylinositol3 kinase (PI3K).[47]

In our study, the intraperitoneal exposure of cisplatin single dose at 5 mg/kg BW resulted in an increase of NF-kappaB p65 and COX-2 levels in the kidney tissue of Wistar rats. The increase of NF-kappaB levels after cisplatin administration was also reported in several studies.[48–50]





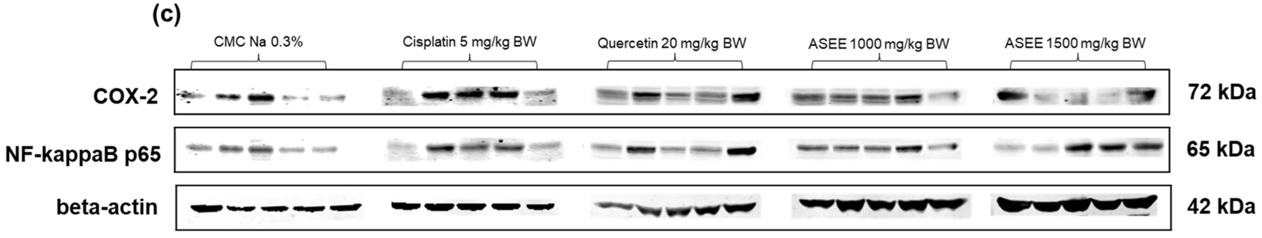


Figure 7 The effects of ASEE pretreatment on the relative expression of (**a**) NF-kappaB p65 and (**b**) COX-2 between treatment groups using Western blot analysis, normalized using beta-actin; (**c**) the protein bands of COX-2 (72 kDa), NF-kappaB p65 (65 kDa), and beta-actin (42 kDa) expression in the kidney tissue of cisplatin-induced Wistar rats.

The relative expression of NF-kappaB p65 and COX-2 influenced by ASEE pretreatment was measured using the Western blot technique and confirmed that pretreatment with ASEE can reduce the expression of NF-kappaB and COX-2

expression although not significant compared to the cisplatin group. Inflammation is the main mechanism of cisplatin-associated kidney injury. NF-kappaB signaling pathway activation steers to the elevated secretion of TNF-α, IL-1, and IL-6.[46] Cyclooxygenase−2 (COX−2) enzyme is produced in an inflammatory condition and works by catalyzing the conversion of arachidonic acid to prostaglandin. In the basal state, the COX2 gene is only expressed at a low level in the kidneys and the central nervous system. The expression of the COX2 gene is regulated by several transcription factors, among those is the NF-kappaB. NF−kappaB includes RelA (also known as p65), RelB, c−Rel, NF−kappaB1 (p50/p105), and NF−kappaB2 (p52/p100), with RelA (p65), the most frequently studied unit.[51] NF-kappaB remains inactive in the cytoplasm by binding to the inhibitory protein IkappaB-alpha and translocates into the nucleus upon activation.[52] The three Rel members of the family, RelA, RelB, and c-Rel, possess a C-terminal transcription activation domain that functions to regulate gene expression. All NF-kappaB proteins can undergo homodimerization or heterodimerization with the other NF-kappaB except p65, which only forms heterodimers.[53] As our ASEE contains flavonoids and chalcones, it is important to disclose that natural and synthetic derivatives of chalcones can modulate the NF-kappaB and STAT3 (signal transducer and activator of transcription 3) signaling pathways.[54]

Conclusion

The extract of *Angelica keiskei* (Miq). Koidz. (Apiaceae) sap has proven its nephroprotective activity in alleviating the levels of serum creatinine, urea nitrogen, and kidney injury molecule-1 (KIM-1), as well as suppressing the expression of proinflammatory cytokines COX-2 and NF-kappaB p65 in the kidney tissue of Wistar rats induced by intraperitoneal cisplatin single dose of 5 mg/kg BW. To our knowledge, this is the first report of the molecular mechanism by which this activity occurs. The underlying nephroprotective mechanism of *Angelica keiskei* (Miq). Koidz. sap is by disrupting the NF-kappaB signaling pathway, thus reducing the expression of NF-kappaB-dependent COX-2. This study also disclosed the presence of flavonoids (87.55 mg QE/g extract) and chalcones (55.32 mg IBCE/g extract). Flavonoids and chalcones contained in the sap of *Angelica keiskei* (Miq). Koidz. may contribute to the potential to protect the kidneys against cisplatin-induced toxicity, due to their antioxidant and anti-inflammatory properties. However, pretreatment with the extract of *Angelica* keiskei (Miq). Koidz. sap doses of 1000 mg/kg BW and 1500 mg/kg BW or quercetin although reducing amyloid sedimentation in the kidney tissue, cannot reduce glomerular atrophy, and necrosis of the epithelial tubule. Despite the limitations of the doses used, the ethanol extract of *Angelica keiskei* (Miq). Koidz. sap may be considered a potential candidate for a nephroprotective drug, particularly in patients with cisplatin chemotherapy, but further clinical studies are needed to verify its efficacy and safety in humans.

Data Sharing Statement

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

Additional Information

No additional information is available for this paper.

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Disclosure

The authors report no conflicts of interest in this work.

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