Original Article

Anredera cordifolia (Ten.) Steenis and *Sonchus arvensis* L. Inhibit Gentamicin-induced Nephrotoxicity: The Role of Urinary N-acetyl Beta-D-glucosaminidase

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Abstract

Introduction: *Anredera cordifolia* (Ten.) v Steenis and *Sonchus arvensis* L. have been used traditionally to treat many diseases such as inflammation, hypercholesterolemia, and kidney stones. This study investigated the renoprotective effect of the best combination of *A. cordifolia* (Ten.) Steenis and *S. arvensis* L. against gentamicin-induced nephrotoxicity in rats reduced the urinary N-acetyl beta-D-glucosaminidase (NAGase) specific marker. **Materials and Methods:** This study used male Wistar rats, weighing 200–300 g. The experiment consisted of a negative control group, a positive control group, *A. cordifolia* 100 mg/kg (body weight) b.w. group (AE), *S. arvensis* 100 mg/kg b.w. group (SE), *A. cordifolia* 50 mg/kg b.w. + *S. arvensis* 50 mg/kg b.w. (AE50 + SE50), *A. cordifolia* 100 mg/kg b.w. + *S. arvensis* 100 mg/kg b.w. (AE100 + SE100), *A. cordifolia* 75 mg/kg b.w. + *S. arvensis* 25 mg/kg b.w. (AE75 + SE25), and *A. cordifolia* 25 mg/kg b.w. + *S. arvensis* 75 mg/kg b.w. (AE25 + SE75). All groups were given the extract according to the group for 14 days orally. On day 15, all groups except the negative control group induced renal failure by administering gentamicin 100 mg/kg b.w. for 8 days along with the extract. On day 22, the evaluation was carried out by measuring urea, creatinine, and NAGase. **Results:** All treatment groups showed significantly decreased levels of creatinine and urea in serum and urinary NAGase when compared with the positive control group ($P < 0.05$). The AE75 + SE25 group showed the smallest elevated levels of creatinine (14, 36%) and urea (17.4%) in serum and urinary NAGase (29.4%) when compared with the positive control group ($P < 0.05$). **Conclusion:** The combination of *A. cordifolia* 75 mg/kg b.w. and *S. arvensis* 25 mg/kg b.w. extract showed a better nephroprotective effect in decreasing the NAGase as the early biomarker in kidney failure.

Keywords: *Anredera cordifolia, gentamicin, N-acetyl beta-D-glucosaminidase, Sonchus arvensis*

Introduction

Anredera cordifolia (Ten.) v Steenis and *Sonchus arvensis* L. have been used traditionally to treat many diseases worldwide. *A. cordifolia* (Ten.) v Steenis is known as Madeira vine (Western), enredadera papa (Brazil), binahong (Indonesia), de san chi (China), madeiraranker (South Africa), and madeiraranka (Sweden).[1,2] In China, *A. cordifolia* was used as analgesics, symptomatic therapy of diabetes, hypoglycemic agents, and anti-inflammatory medication.[3] In Indonesia, it was used to treat acute gastritis, headache, heartburn, mouth sores, wound, postoperative inflammation, hemorrhoids, gout, rheumatism, hypercholesterolemia, vertigo, typhus, sore throat, and migraines.[1] Simultaneously, *S. arvensis* was used to treat asthma, depression, coughs, kidney stones, and diarrhea.[4,5] In China, it was used to treat fever, inflammation, rheumatism, diarrhea, and antidote to snake bite.[6]

Gentamicin is an aminoglycoside antibiotic to treat infections caused by Gram-negative bacteria. Nephrotoxicity is one of the limitations of aminoglycoside therapy. Gentamicin accumulates in the renal cortex, which induces changes in renal morphology.[7] There are reports which suggest the role of reactive oxygen species (ROS)/nitrogen species, in association with increased lipid peroxide formation and decreased activity of antioxidant enzymes in gentamicin-induced nephrotoxicity.[8] Gentamicin accumulation in the proximal tubule epithelial cells induces changes in the function of many cell organelles.[7] The damaged tubules will release N-acetyl beta-D-glucosaminidase (NAGase) as a particular enzyme in detecting acute kidney failure.[9]

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A. cordifolia (Ten.) Steenis and *S. arvensis* L. are known to possess potent antioxidant properties. *A. cordifolia* exhibits antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, with an IC_{50} value of 68.07 µg/mL.[10,11] Meanwhile, *S. arvensis* has antioxidant activity against DPPH and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), with an IC_{50} value of 3.4–65.7 µg/mL.[6] An alteration in the antioxidant enzyme activities with nitric oxide (NO) and NAG levels in rat studies indicate increased ROS activity. The oxidative mechanism function is demonstrated by the increase in renal tissue and urinary NAG levels with a decrease in renal superoxide dismutase (SOD) activities.[12]

In a previous study, *A. cordifolia* and *S. arvensis* at each dose 100 mg/kg body weight (b.w.) showed improvement in rat renal failure by decreased serum creatinine levels induced by gentamicin.[13,14] In an acute toxicity analysis, both *A. cordifolia* and *S. arvensis* extracts were well tolerated after a single administration. At the maximum dose of 15 g/kg b.w., no mortality was observed.[15,16] Therefore, this study was carried out to evaluate the renoprotective effect of the best combination of *A. cordifolia* (Ten.) Steenis and *S. arvensis* L. against gentamicin-induced nephrotoxicity in rats reduced the urinary NAGase-specific marker in acute kidney failure.

Materials and Methods

Plant material

The fresh *A. cordifolia* (Ten.) Steenis and *S. arvensis* (Linn.) leaves were collected from the Herbal Jaya Garden, Tawangmangu, Karanganyar, Central Java. They were identified and authenticated by Herbarium Bandungense, School of Life Sciences and Technology, Institut Teknologi Bandung (No. 652/I1.CO2.2/PL/2018).

Chemicals

PT Indofarma supplied gentamicin injections and physiological sodium chloride. Rajawali Nusindo, Indonesia provided urea and creatinine kits. Rat NAGase ELISA kits were obtained Elabscience (USA). Aquadest, sodium carboxymethyl cellulose, and all other chemicals were of analytical grade.

Standardization of extract of *A. cordifolia* and *S. arvensis*

According to the Indonesian Herbal Pharmacopoeia, standardization of *A. cordifolia* and *S. arvensis* extracts includes water content and water-soluble and ethanol-soluble extract. The phytochemical screening provides for examining secondary metabolites of compounds such as alkaloids, flavonoids, polyphenol, steroids/triterpenoids, quinones, tannins, and saponins.[17]

Experimental design

This study used male Wistar rats weighing 200–300 g and were obtained from PT Biofarma. The animals were placed in 12 h light/dark cycle, temperature of (22 ± 3)°C, and humidity of 60–70%. The experiment consisted of a negative control group (normal rat), a positive control group (kidney failure induction without treatment), *A. cordifolia* 100 mg/kg b.w. group (AE), *S. arvensis* 100 mg/kg b.w. group (SE), combination of *A. cordifolia* 50 mg/kg b.w. and *S. arvensis* 50 mg/kg b.w. (AE50 + SE50), combination of *A. cordifolia* 100 mg/kg b.w. and *S. arvensis* 100 mg/kg b.w. (AE100 + SE100), combination of *A. cordifolia* 75 mg/kg b.w. and *S. arvensis* 25 mg/kg b.w. (AE75 + SE25), and combination of *A. cordifolia* 25 mg/kg b.w. and *S. arvensis* 75 mg/kg b.w. (AE25 + SE75).

All groups were given the extract according to the group for 14 days orally. On day 15, all groups except the negative control group induced renal failure by administering gentamicin 100 mg/kg b.w. for 8 days along with the extract. On day 22, the animals were sacrificed, and their serum was taken. Evaluation of kidney function was carried out by measuring the levels of urea, creatinine, and NAGase at the end of therapy. All experimental animal protocols have been approved by the Institutional Animal Ethics Committee (IAEC), following the Committee's guidelines for the Objective of Supervision and Control of Animal Experiments (No. 09/KEPHP-ITB/12–2019).

Collection of samples and biochemical assays

Blood and urine samples were collected at the end of therapy. Serum, obtained from the blood samples through centrifugation, was used to measure creatinine and urea levels. Meanwhile, urine was used to measure NAGase. Serum urea was determined with the urease enzyme kit. Urease will hydrolyze urea to form carbon dioxide and ammonia.[18] Serum creatinine was determined with Jaffe's kinetic method, and absorbance was measured by a spectrophotometer at 546 nm.[19]

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). The data obtained were analyzed by one-way analysis of variance in Prism software version 8, followed by Tukey's *post hoc* test. Differences were considered to be statistically significant when $P \leq 0.05$.

Results

Standardization and phytochemical characterization

The crude drug of *A. cordifolia* and *S. arvensis* leaves was extracted using ethanol 96%. The sample was dry extract that was obtained by evaporating the solvent from liquid extract. The standardization of extract was determined with the distilled water content, water-soluble extract content, and ethanol-soluble extract content and phytochemical screening extract of *A. cordifolia* (Ten.) and *S. arvensis* L. leaves. The results of general standard determination and phytochemical screening of extracts can be seen in Table 1.

The differences in place and growth conditions will affect the plant's phytochemical content, especially the growth of medicinal plants.[20] The results of the characterization of extracts showed that extracts' water content met the Indonesian

Herbal Pharmacopoeia requirements, i.e., not greater than 10%.[17] The extract contains water that can cause contamination of microorganisms. Ethanol-soluble extract content in crude drugs was higher than water-soluble extract content, showing that the main compound of the extract is polar compound.[21] Determination of water-soluble extract and ethanol-soluble extract content provides an overview of the yield from the extraction process. The results show that ethanol can dissolve more compounds than water, so ethanol was chosen as the solvent.

Nephroprotective activity

The effects of *A. cordifolia* and *S. arvensis* treatment on gentamicin-induced nephrotoxicity on serum creatinine levels are shown in Figure 1. Administration of gentamicin elevated the levels of creatinine in serum. The positive control group showed the highest levels of creatinine (4.69 ± 0.5 mg/dL). All of the treatment groups showed a decrease in serum creatinine levels significantly when compared with the positive control group ($P < 0.05$). The combination of the *A. cordifolia* 75 mg/kg b.w. and *S. arvensis* 25 mg/kg b.w. group showed the lowest creatinine levels (0.67 ± 0.1 mg/dL). Also, it showed the smallest elevated levels of creatinine in serum (14.36%) when compared with the positive control group ($P < 0.05$).

Table 1: Characteristics of ethanolic extract of *A. cordifolia* and *S. arvensis*

Parameters	*A. cordifolia*	*S. arvensis*
Water content (%)	5 ± 0.1	2.08 ± 0.25
Water-soluble extract content (%)	8.46 ± 0.28	4.64 ± 0.05
Ethanol-soluble extract (%)	11.13 ± 1.27	7.15 ± 0.63
Alkaloid	+	+
Flavonoid	+	+
Polyphenol	+	+
Steroid/triterpenoid	+	+
Quinon	−	+
Hydrolysate tannin	−	+
Condensed tannin	−	−
Saponin	−	−

A similar pattern was observed for the urea levels in the animal subjects [Figure 2]. Gentamicin elevated the levels of urea in serum. The positive control group showed the highest levels of urea (445.67 ± 53.68 mg/dL). All of the treatment groups showed decreased urea levels in serum significantly when compared with the positive control group ($P < 0.05$). The combination of the *A. cordifolia* 75 mg/kg b.w. and *S. arvensis* 25 mg/kg b.w. group showed the lowest urea levels (76.40 ± 16.4 mg/dL). Also, it showed the smallest elevated levels of urea in serum (17.4%) when compared with the positive control group ($P < 0.05$).

At last, gentamicin-induced nephrotoxicity was showed by urinary NAGase as injury markers of damage in renal proximal tubular cells [Figure 3]. The positive control group showed the highest urinary NAGase (46.56 ± 7.79 ng/mL). All of the treatment groups showed decreased urinary NAGase levels when compared with the positive control group ($P < 0.05$). The combination of the *A. cordifolia* 75 mg/kg b.w. and *S. arvensis* 25 mg/kg b.w. group showed the lowest NAGase levels (13.71 ± 7.92 ng/dL). Also, it showed the smallest elevated levels of NAGase in serum (29.4%) when compared with the positive control group ($P < 0.05$).

Discussion

Gentamicin is a broad-spectrum aminoglycoside antibiotic used to treat infections such as those caused by Gram-negative bacteria. Still, gentamicin can cause nephrotoxicity due to the selective accumulation of gentamicin in renal

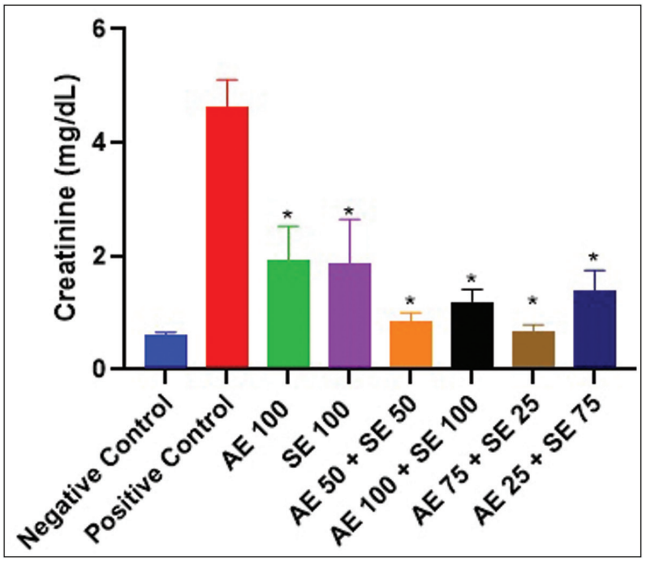

Figure 1: Effect of extract of *A. cordifolia* (Ten.) Steenis, *S. arvensis* L, and their combinations on gentamicin-induced nephrotoxicity reduced the serum creatinine level. All values are expressed as mean ± SEM ($n = 3$); *means $P < 0.05$ compared with the positive control

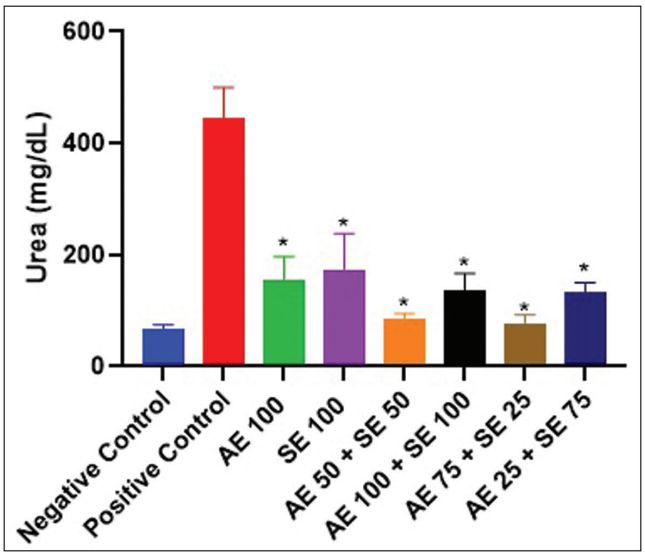

Figure 2: Effect of extract of *A. cordifolia* (Ten.) Steenis, *S. arvensis* L, and their combinations on gentamicin-induced nephrotoxicity reduced the serum urea level. All values are expressed as mean ± SEM ($n = 3$); *means $P < 0.05$ compared with the positive control

cortex.[7,22] Gentamicin causes acute renal failure in 10–15% of the patients,[22] and about 30% of the patients experienced nephrotoxicity after receiving gentamicin for more than 7 days.[23] Nephrotoxicity of gentamicin can increase biochemistry kidney function markers significantly, such as creatinine and urea.[24-27] Creatinine and urea are nitrogenous end products of metabolism that may influence the glomerular filtration rate and cause elevated serum levels indicated with renal damage.[28]

NAGase is a lysosomal enzyme that plays a role in the degradation of glycoproteins in the renal tubule epithelium.[29] The damage of the proximal tubules will release NAGase. NAGase cannot get past glomerular filtration and elevated the urinary concentrations. This condition is used as early detection of kidney damage. In *in vivo* study, NAGase is more specific than creatinine and urea in serum.[30] NAGase is a particular kidney marker for the damage proximal convoluted tubule. Gentamicin can trigger the response after 8 h of administration.[31] Clinical study also reported NAGase as an early marker of mild tubular injury.[32]

Treatment with *A. cordifolia, S. arvensis*, and their combination with gentamicin can decrease creatinine and urea in serum. Those suggest the nephroprotective effect of *A. cordifolia* and *S. arvensis* extract. The nephroprotective effect of different extracts in gentamicin-induced nephrotoxicity has been studied previously.[8,33] *A. cordifolia* and *S. arvensis* have been studied to reduce the level of creatinine and urea in rat kidney failure.[13,34] In this study, the combination of *A. cordifolia* 75 mg/kg b.w. and *S. arvensis* 25 mg/kg b.w. shows the best effect in reducing the level of creatinine and urea in serum. Furthermore, that combination also offers the lowest NAGase as specific kidney tubular injury.

Several studies are suggesting that gentamicin also increases ROS production in mitochondria.[35] Superoxide anions and hydroxyl radicals are the most critical ROS. They can cause cell damage and cell death by inhibiting electron transport pathways, suppressing cellular respiration and ATP production, and destabilizing cell membranes resulting in necrosis. ROS causes oxidative stress that leads to apoptosis cell.[7] Oxidation reaction also occurs in the mitochondria of the kidney. As a result, oxidative stress can progress kidney disease.[36]

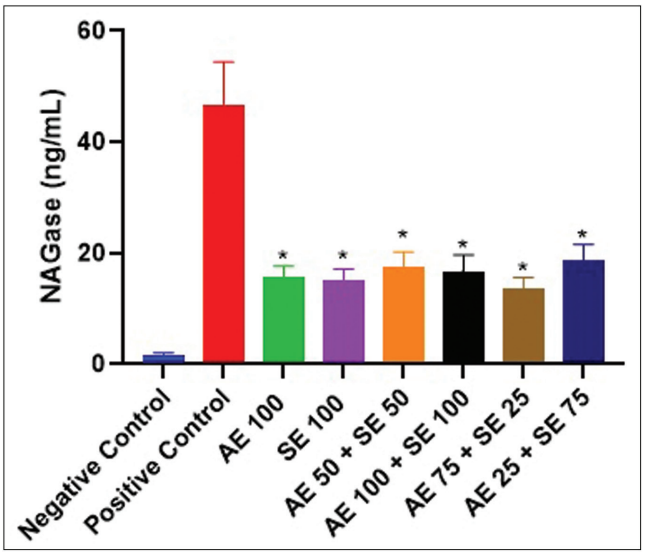

Figure 3: Effect of extract of *A. cordifolia* (Ten.) Steenis, *S. arvensis* L, and their combinations on gentamicin-induced nephrotoxicity reduced the urinary NAGase level. All values are expressed as mean ± SEM (*n* = 3); *means *P* < 0.05 compared with the positive control

The phytochemical screening showed that *A. cordifolia* and *S. arvensis* contain flavonoids. Several studies reported that *A. cordifolia* and *S. arvensis* have an antioxidant effect.[6,37] Vitexin is one of the compounds isolated from *A. cordifolia* leaves extracts. In albino rats, vitexin offered substantial protection against gentamicin- and cisplatin-induced nephrotoxicity.[38] Meanwhile, flavonoids from the flavone class, such as luteolin and luteolin 7-O glucoside, are found in *S. arvensis*.[39] In mice, luteolin can reduce cisplatin-induced nephrotoxicity. In this study, the single extract of *A. cordifolia* or *S. arvensis* and the combination of extract show the nephroprotective effect. The combination of the two extracts can increase the potential antioxidant effect. *A. cordifolia* is more dominant than *S. arvensis* in the nephroprotective effect because the dose of *A. cordifolia* is bigger than that of *S. arvensis*. This study is only carried out in animals, and the lack of histology parameters should be the study's limitation. More studies should be performed using specific staining in histology kidney to reassure the nephroprotective mechanism of *A. cordifolia* and *S. arvensis*.

Conclusion

The combination of *A. cordifolia* 75 mg/kg b.w. and *S. arvensis* 25 mg/kg b.w. extract showed a better nephroprotective effect in decreasing NAGase as the early biomarker in kidney failure.

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Conflicts of interest

The authors declared no conflict of interest.

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