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Original Article

The therapeutic effect of *Centella asiatica* hydroalcoholic extract on gentamicin-induced nephrotoxicity

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Abstract

Introduction: Antioxidant and anti-inflammatory features of *Centella asiatica* (*Centella*) hydroalcoholic extract is documented in various diseases. This study aimed to investigate the effect of *Centella* hydroalcoholic extract on gentamicin (GM) induced nephrotoxicity.

Methods: In this study, 28 male Wistar rats were studied in 4 groups namely: control, sham, GM+normal saline (NS) and GM+*Centella* extract. In order to nephrotoxicity induction, gentamicin (100mg/kg) was injected as ip for seven days and then *Centella* (100 mg/kg/ip) administered for 7 consecutive days. Finally, the blood samples were collected from heart in order to measure the plasma creatinine (Cr) and urea nitrogen levels. Oxidative stress indices were also measured through assessing the malondialdehyde (MDA) and ferric reduction antioxidant power (FRAP) levels in right kidney. Moreover, histological damages were assessed through studying hematoxylin-eosin stained left kidney sections.

Results: There was a significant increase in Cr, urea nitrogen and MDA levels, as well as renal tissue damages, while FRAP level reduced in the group received GM+NS compared to the sham one. Treatment with *Centella* resulted in a significant decrease in plasma Cr, urea nitrogen, MDA and tissue damages in the GM+*Centella* extract group compared to the GM+NS group. Moreover, FRAP level increased significantly in the GM+*Centella* group compared to the GM+NS group.

Conclusion: Treatment with *Centella* extract is effective for ameliorating the kidney damages in gentamicin-induced nephrotoxicity in rats.

Keywords:
Centella asiatica;
Gentamicin;
Nephrotoxicity;
Oxidative stress

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Introduction

Gentamicin, an aminoglycoside antibiotic, plays a critical and effective role in the treatment of Gram-negative bacteria; however, it has also been documented to be one of the most leading causes of 20% of renal failures in health care units (Abdelrahman, 2018). The history of kidney disease dates back to thousands of years ago (Changizi-

Ashtiyani and Cyrus, 2010; Shamsi et al., 2014). The nephrotoxicity mechanism posed by gentamicin is not still fully understood (Ehsani et al., 2017), even though, the reactive oxygen species (ROS) play a vital role in the gentamicin-induced nephrotoxicity mechanism. The production and accumulation of ROS induces apoptosis, tubular necrosis and increases the infiltration of leukocytes (Jaikumkao et al., 2016).

There is a rich literature on the use of medicinal plants and their extracts in the treatment of various diseases, including kidney diseases. The presence of some vital active ingredients such as polyphenols, especially flavonoids and phenolic acids, in medicinal herbs has made them more effective and diverse (Rezaei et al., 2016; Zarei et al., 2014). Flavonoids are one of the main types of polyphenols with numerous medicinal functions; however, their role in kidney diseases has been less concerned. The protective effects of several flavonoids against many nephrotoxic agents, such as gentamicin, alcohol and nicotine has been proved in previous studies (Vargas et al., 2018).

Centella asiatica (Centella) from the *Umbelliferae* (*Apiaceae*) family has been used as a medicinal herb in India, China and Sri Lanka throughout the past thousands of years (Chandrika and Kumara, 2015). The primary active constituents in *Centella* are asiatic acid (AA, a pentacyclic triterpenoid and the main ingredient), madecassoside, madasiatic, fatty acids, amino acids, brahmoside, glycosides isothankuniside and flavonoids. The presence of these compounds causes the plant to have antioxidant and free radicals scavenging properties (Kumari et al., 2016; Ratz-Łyko et al., 2016). The therapeutic effect of *Centella* has been reported for a variety of diseases (Puttarak et al., 2017; Visweswari et al., 2010). It has been revealed that AA protects individuals against neuronal apoptosis through reducing ROS levels and that it is effective in the treatment of diseases such as Parkinson's and Alzheimer's (Lokanathan et al., 2016). In addition, the protective effect of *Centella* extract in renal diseases is confirmed in several studies (Maneesai et al., 2017; Yuyun et al., 2018). Yang's et al. (2018) demonstrated the protective effects of AA in *Centella* on the cisplatin-induced acute renal failure model. Moreover, Kamble and Patin (2018) concluded that the AA has protective effect in doxorubicin toxicity in rats in a dose-dependent manner. In a study conducted by Choi et al. after induction of liver damage by dimethylnitrosamine, *Centella* extract gavage for five days significantly decreased malondialdehyde (*MDA*) and inflammatory mediators, and increased antioxidant enzymes (Choi et al., 2016). There is, however, a gap in the literature on the effectiveness of the *Centella* hydroalcoholic extract in gentamicin induced nephrotoxicity model. Hence the present study aimed to evaluate the effect of *Centella* administration in gentamicin induced nephrotoxicity model.

Materials and methods

Animals and experimental design

The present experimental study was performed on 28 male Wistar rats weighing 180-200g. Throughout the study, the provisions of the Helsinki declaration and all ethics codes on working with laboratory animals approved by Iran's Ministry of Health and Medical Education were fully observed. The protocol was approved by the Ethics Committee under Research No. IR.ARAKMU.REC.1397.97. The rats were purchased from the Animal Breeding Center at Arak University of Medical Sciences, Markazi, Iran. All animals were kept at a 24-22°C and the ambient conditions were 12 hours of brightness/ 12 hours of darkness and free access to standard water and food.

Experimental groups

The animals in this study were assigned into four groups (n =7) as follows: (1) control, no intervention such as injection, surgery or gavage was performed; (2) sham group, the rats received 2ml/day of normal saline (NS) as ip injection during the first and second weeks; (3) gentamycin and normal saline (GM+NS), the rats received 100mg/kg/day of gentamicin (Alborz, Iran) in the first week (Stojiljkovic et al., 2012) and 2ml/day of NS in the second week as ip injection and (4) gentamicin and *Centella* (GM+*Centella*), the rats received gentamicin 100mg/kg/day in the first week and 100mg/kg the hydroalcoholic extract of *Centella asiatica* in the second week as ip injection.

Preparation of *Centella asiatica* extract

Herbal species were collected from Anzali (Gilan,

Iran) in June and identified by a medicinal plant specialist (voucher No.: Hfbu-2019300). Extraction process was performed using percolation method, as described below. To this end, 500ml of 70% ethanol was added to 100g dry weight of *Centella* leaves and the mixture was stirred at room temperature for 72 hours. The supernatant was then filtered using Whitman Filter No. 1. After the filtration process, the dark green solution in the incubator was evaporated under reduced pressure at 50°C and it was then lyophilized (1mg of dried extract of *Centella* leaves was equivalent to 5.26mg dry leaves) (Binti et al., 2018).

Sample collection method

At the end of the treatment period and after anesthesia, blood samples were taken from the heart for plasma preparation. The plasma creatinine (Cr) and urea nitrogen were measured using an auto-analyzer (Biotjencal, Italy). Furthermore, the rats' kidneys were also removed. The left kidneys were placed in 10% formalin solution for hematoxylin-eosin staining. The right kidneys were transferred to the nitrogen tanks in order to oxidative stress parameters study through measuring malondialdehyde (MDA) and total antioxidant activities of all defense mechanisms, ferric reduction antioxidant power (FRAP) (Moosavi et al., 2011).

Measuring oxidative stress

Renal tissue was homogenized in PBS and 200µl of the homogenized solution was added to a tube containing 20% acetic acid, 0.8% thiobarbituric acid and 8.1% sodium dodecyl sulfate and then the solution was heated in water bath (DUBNOFF, USA) at 95°C for 60min. After cooling down and adding 4ml n-butanol, the samples were centrifuged (4000rpm). The light absorption of the supernatant layer was measured at 532nm wavelength using a spectrophotometer (Spectrolab 7500 UV, UK). Tetraethoxypropane was used as an external standard (Azarkish et al., 2017).

To measure the FRAP, the solution was first prepared using a mixture of 300mmol/l acetate buffer (pH=3.6) and 10mM TPTZ (Merck, Germany) in chloridric acid (40mmol/l) and ferric chloride solution (2mmol/l). Then 1.5ml of the solution was poured into the test tube at 37°C and 50µl from the homogenized sample was added to the above solution in order to initiate the reaction. Light absorption variations were measured at a wavelength of 593nm. A standard curve was plotted using $FeSO_4$ $7H_2O$. FRAP values are reported in µmol/l (Qasem et al., 2018).

Histological study method

To investigate tissue damages in kidneys, the cortex, external and internal medulla were studied using optical microscopy. Bowman's space enlargement, cell necrosis, vascular congestion and intratubular proteinaceous cast formation were evaluated and graded. In this study, the increased size of Bowman space in gentamicin group rats compared to the sham group, was considered as one hundred percent damage and the percentage of dilation was calculated in other rats to be compared. The cell necrosis, vascular congestion and *intratubular cast* formation in ten microscopic fields were calculated according to the previous studies (Najafi et al., 2015). These percentages were calculated and the following grades were employed: grade 0 for no damage, grade 1 for 1-20% damage, grade 2 for 21-40% damage, grade 3 for 41-60% damage, grade 4 for 61-80% damage and grade 5 for 81-100% damage. Then the degree of total histopathologic damages, which was equal to the sum of all degrees of damages, was calculated and statistical analysis was performed to examine the total degree of damage.

Statistical analysis

Data were analyzed using SPSS software version 18. One-way ANOVA and Duncan's post hoc tests were used to show the significance of the data. The exact *P*-value was determined using the LSD test. Nonparametric data analysis was also performed using *Kruskal–Wallis* and Mann-Whitney tests. Data were reported as mean±SEM and $P<0.05$ was set as the level of significance.

Results

***Centella asiatica* treatment improves kidney function**

Table 1 shows a significant increase ($P<0.001$) in the plasma Cr and urea nitrogen parameters (21% and 38%, respectively) in the gentamicin group in comparison to the sham group. Treatment with *Centella* extract resulted in a significant decrease in

Table 1: Serum creatinine and urea-nitrogen concentration in rats with gentamicin (GM) induced nephrotoxicity and received *Centella asiatica* (100mg/kg) for 7 days (GM+*Centella*), GM+normal saline (NS), sham or control groups. $^{***}P$<0.001 in comparison to sham group; $^{†††}P$<0.001 in comparison to GM group.

Parameter	Control	Sham	GM+NS	GM+*Centella*
Plasma creatinine, (mg/dl)	0.52 ± 0.014	0.56 ±0.02	0.71 ± 0.02 ***	0.54 ± 0.03 †††
Plasma urea nitrogen (mg/dl)	23.6 ± 1.65	25.4 ± 0.071	41.3 ± 2.86 ***	30.5 ± 2.15 †††

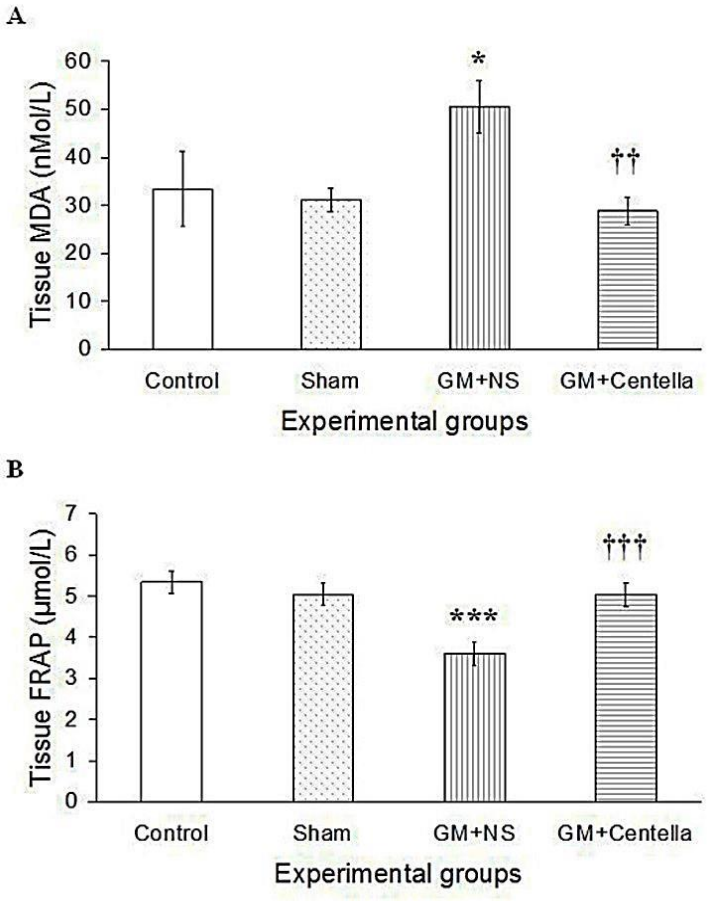

Fig.1. Effects of *Centella asiatica* on (A) renal tissue malondialdehyde (MDA) and (B) ferric reducing/antioxidant power (FRAP) following gentamicin-induced nephrotoxicity in rats with gentamicin (GM) induced nephrotoxicity and received *Centella asiatica* (100mg/kg) for 7 days (GM+*Centella*), GM+normal saline (NS), sham or control groups. $^{*}P$<0.05 and $^{***}P$<0.001 in comparison to sham group; $^{††}P$<0.01 and $^{†††}P$<0.001 in comparison to GM group.

Cr and urea nitrogen concentrations, compared to the gentamicin group (P<0.001).

Centella asiatica treatment improves oxidative stress

The MDA level in the gentamicin group was 33% higher than that in the sham group (P<0.05) and the FRAP was 28% lower in comparison to the sham group (P<0.001). Moreover, the results revealed a significant decrease (P<0.01) in MDA (52%) and a significant increase (P<0.001) in FRAP (28%) for the *Centella* group, compared to the gentamicin group (Fig. 1).

Centella asiatica treatment reduces tissue damage

As shown in Table 2, gentamicin injection throughout seven consecutive days in the rats caused obvious tissue damage in the renal cortex and medulla, compared to the sham group. So that the size of

Table 2: Effects of *Centella asiatica* extract on renal histopathologic scores induced by gentamicin.

Histopathologic damages		Experimental groups			
		Control	Sham	GM+NS	GM + *Centella*
Cortex	Bowman space enlargement	0	0	2.81	3.21
	Proximal tubal damage	0	0	1.96	1.28
	The thick ascending limb (TAL) of Henle's loop	0	0	1.34	1.08
Outer medulla	Pars Recta damage	0	0	1.72	0.82
	The thick ascending limb (TAL) of Henle's loop	0	0	1.31	1.11
	Vascular congestion	0	0	1.16	0.87
	Intra tubular proteinaceous casts	0	0	2.57	2.20
Inner medulla	Vascular congestion	0	0	0. 82	0.54
	Intra tubular proteinaceous casts	0	0	2.52	2.26
Total histopathologic scores		0	0	16.21 ***	13.67 ***††

Scores of histopathologic damages in the rats with no intervention (control), normal saline injection (sham), gentamicin and normal saline injection (GM+NS), or gentamycin and *Centella* extract (GM+*Centella*). $^{***}P<0.001$ in comparison to sham group; $^{††}P<0.01$ in comparison to GM group.

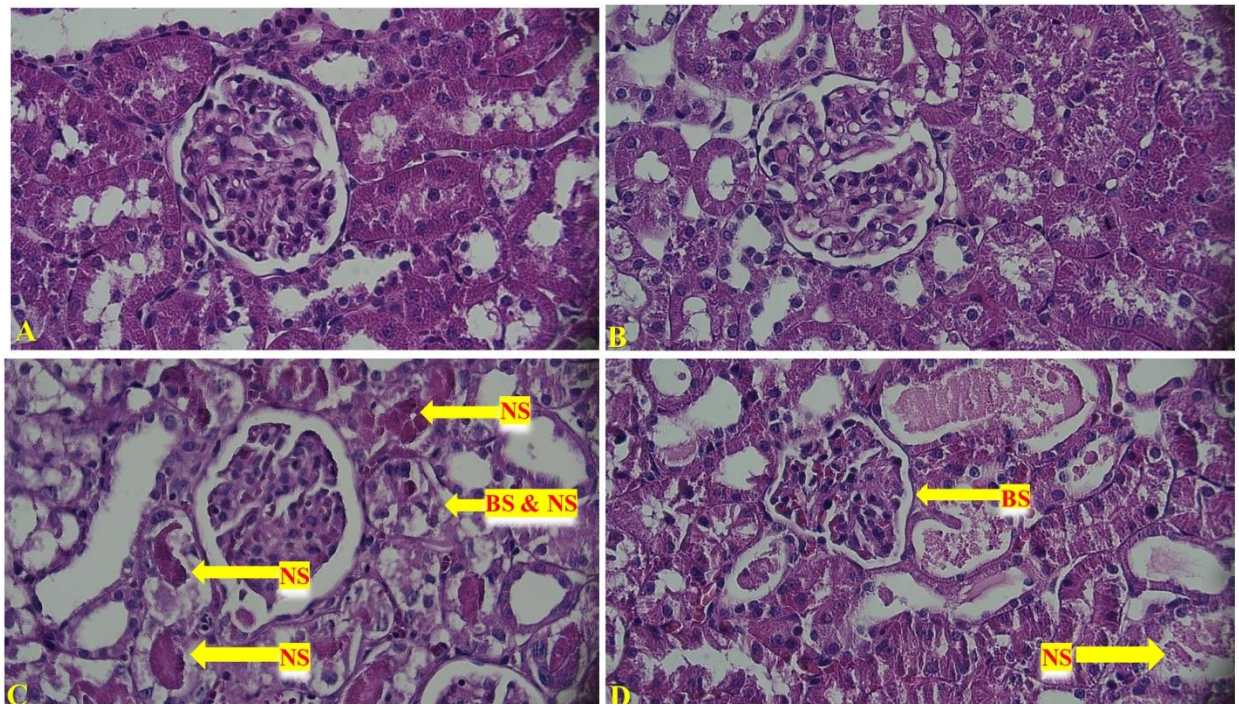

Fig.2. Histopathologic alterations in rat kidneys following gentamicin administration and *Centella asiatica* treatment. Cross-sectional view of the kidneys to represents the size of the Bowman's space (BS) and the necrosis of the cell (NS) in the studied groups: A) with no intervention (control); B) normal saline (sham); C) gentamicin and normal saline and D) gentamicin and *Centella* extract (400x magnification and hematoxylin-eosin staining).

Bowman's space was 2.81, proximal tubular injury with 1.96 degrees and the thick ascending limb damage with grade 1.34 in the renal cortex of the gentamicin group were observed compared to the sham group. In the external medulla of the kidney, gentamicin injections also resulted in damage to the pars recta and thick ascending limb of Henle's loop at 1.72 and 1.32 degrees, respectively. Furthermore, the vascular congestion and the formation of intratubular proteinaceous casts were evident in the gentamicin group (Table 2 and Fig. 2).

Treatment with *Centella* extract for seven days decreased renal tissue damages in comparison to gentamicin group. So that the injuries in proximal tubule and the thick ascending limb decreased by 1.68 and 1.78, respectively; however, there was no decrease in the Bowman's space. Other tissue injuries, including vascular congestion and intra-tubular protein cast, were decreased in the group treated with *Centella*. In general, *Centella* extract significantly decreased the histopathologic damages (P<0.01) compared to the gentamicin group. Nevertheless, the histopathologic damage was still significantly higher in the treated group than in the sham group (Table 2 and Fig. 2).

Discussion

In the present study, the treatment effect of *Centella* extract on gentamicin-induced nephrotoxicity model was examined. The results of this study showed that treatment with *Centella* leads to a decrease in plasma Cr and urea nitrogen, tissue MDA, as well as tissue damages, and increases the antioxidant capacity of the tissue (FRAP). The nephrotoxicity of gentamicin in proximal tubules is due to the destruction of lysosomes, which further results in the release of hydrolysis enzymes and consequently cell necrosis and proximal tubular obstruction (Miller et al., 2018). It was also revealed that the inhibition of membrane proteins, including Na^+/K^+-ATPase, also leads to cell death processes (Mingeot-Leclercq and Tulkens, 1999). Disruption of the membrane structures by gentamicin through the release of contractile hormones and platelet aggregation factors, which leads to blockage of the nephrons, changes glomerular permeability and decreases glomerular filtration (Miller et al., 2018; Valipour et al., 2016). In addition, gentamicin is actively reabsorbed in the proximal tubule and its concentration in tubular cells and its distribution in the circulation lead to a decrease in *creatinine clearance* (C_{Cr}) and an increase in plasma Cr and urea nitrogen (Jafarey et al., 2014). The fact is that serum creatinine still is an acceptable indicator to evaluate *glomerular filtration rate* (*GFR*) and has been the most sensitive serum marker in detecting minor C_{Cr} variations (Dalton, 2010).

In the present study, the gentamicin administration resulted in an increase in plasma Cr and urea nitrogen, compared to the sham group. This increase is probably due to a decrease in the *GFR*, thereby reducing absorption in the proximal tubule and activating the tubular-glomerular feedback. Aldahmash's et al. study on a 7-day gentamicin injection showed a significant damage to the kidney caused by increased blood urea nitrogen (BUN), eliminated glomerular polarity, reduced brush border and leukocyte infiltration (Aldahmash et al., 2016). In the present study, following the treatment with *Centella*, plasma Cr and urea nitrogen significantly decreased in the treatment group compared to the gentamicin group. Wang et al. (2013) documented the *Centella's* recovery effect on renal function. After inducing renal failure with intravenous adriamycin, they injected three different doses of *AA* and improved renal function by decreasing BUN, Cr and tissue inflammation through enhancing the expression of synaptopodin, nephrin, and podocin.

Other pathways involved in gentamicin nephrotoxicity are increased endothelin, monocyte-macrophage infiltration, production of ROS or oxidative stress and reduction of antioxidant defense systems (Jafarey et al., 2014; Oliveira et al., 2017). Oxidative stress occurs due to the imbalance in pro-oxidants and antioxidants performances and such an imbalance results in the formation of ROS such as anion superoxide, hydrogen peroxide and radical hydroxyl (Moosavi et al., 2011). ROS-induced damage plays an important role in nephrotoxicity pathophysiology and damages the tubular cells through the oxidation of proteins and lipids, thereby promoting DNA damage and induction of apoptosis (Oliveira et al., 2017). MDA is a major product of lipid peroxidation and the administration of gentamicin seems to cause a sharp increase in the MDA level as an oxidative stress marker in tubules and glomeruli (Aldahmash et al., 2016). In the present study, following the

administration of gentamicin, the MDA level increased in the gentamicin group, compared to the sham one; however, it significantly decreased after treatment with *Centella*. It has been shown that *Centella* has *free radical scavenging activities* (Arora et al., 2018).

The study of Masola et al. showed antioxidant effects of *Centella* extract on the diabetic nephropathy model as the MDA increased after streptozotocin-*induced diabetic nephropathy,* MDA decreased after seven days of treatment with *Centella* at doses of 500 and 1000mg/kg and the expression of glutathione S-transferase (GST) as an antioxidant also increased. Increased function of the GST leads to the conversion of highly-reactive metabolites towards less toxicity (Masola et al., 2018). In addition, high levels of polyphenols and flavonoids in the herbal extract play the most critical role in antioxidant activities (Lokanathan et al., 2016). In this study, the antioxidant properties of *Centella* were also evaluated using FRAP parameter. The FRAP decreased in the gentamicin group and treatment with *Centella* caused a significant increase in FRAP in comparison to the gentamicin group. This finding indirectly indicates an increase in antioxidant capacity of renal tissue by *Centella* administration.

The results of 30 days of ethanolic extract of *Centella* gavage on albino albumin-treated with isoniazid showed that the use of 100mg/kg of extract had an effective role in reducing the oxidative stress and improving renal function. Ethanolic extract of *Centella* has a high antioxidant capacity and can remove free radicals. This might be due to the presence of various antioxidant compounds in the extract. Some researchers believe that increased antioxidants in the extract are the main cause of the decrease in urea and creatinine levels in the groups receiving isoniazid (Ghosh et al., 2017). A similar mechanism was observed in the present study. Studies have noted that AA reduces kidney damage following doxorubicin induced toxicity in vital organs. The tissue is protects by modulation of Nrf2 translocation as this translocation indirectly generates phase II antioxidant enzymes. As an obvious fact, AA has antioxidant and radical scavenge functions (Kamble and Patil, 2018). The results of a study examining the effects of *Centella* on inflammatory cytokines in the kidney of diabetic rats indicated its modulating effects on inflammatory cytokines through reducing TNF-α and INF-γ and increasing IL-4 and IL-10. It might be part of the *Centella's* protective mechanism (Masola et al., 2018). Hence the improvement of the observed tissue indices in the *Centella* groups can be attributed to the presence of antioxidants and anti-inflammatory agents in the extract.

In the present study, 7 consecutive days of gentamicin injection resulted in significant tissue damages to the cortex and medulla in the gentamicin group compared to the sham group. The same findings are obtained in other studies (Sayed-Ahmed and Nagi, 2007; Yaman and Balikci, 2010). These damages are probably caused by inflammation, glomerular congestion and tubular fibrosis (Lakshmi and Sudhakar, 2010). On the other hand, *Centella* treatment significantly reduced tissue damage in the treated group in comparison to the gentamicin group. *Centella* has the potential to enhance cellular regeneration capacity (Ruszymah et al., 2012). AA has also been documented to reduce the tissue damage caused by lipopolysaccharide in rats since it inhibits Notch signaling (an important pathway in inflammation). Studies have suggested that AA also improves microcirculation and reverses fibrosis in humans with varicose veins. *Centella* showed positive protective effects on the renal function improvement in the adriamycin-induced nephropathy in rat model (Wang et al., 2013). In addition, Meng et al., with the ligation of the left ureter and the development of renal fibrosis during seven consecutive days, showed that AA reduced tissue damages by inhibiting the TGF-β/Smad pathway (the pathway leading to renal fibrosis) (Meng et al., 2015; Yuyun et al., 2018). In the present study, *Centella* might also improve tissue damage through regulating the fore mentioned pathways, a subject to be further studied in the future.

Conclusion

The results of this study showed that treatment with *Centella* hydroalcoholic extract in a gentamicin-induced nephrotoxicity model could improve renal function and prevent further damages due to the presence of multiple active substances with antioxidant potential and through free radical scavenging activity and reducing inflammation. Accordingly, the *Centella* extract would be a promising treatment for gentamicin nephrotoxicity.

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Conflict of interest

No Conflict of interest is declared.

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