Lack of Nephroprotective Efficacy of *Althaea Officinalis* Flower Extract Against Gentamicin Renal Toxicity in Male Rats

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Original Article

ABSTRACT

Background: Gentamicin (GM) is used as antibiotic for Gram-negative infections, but its administration is limited due to a side-effect of nephrotoxicity. It was attempted to investigate the effect of *Althaea officinalis* flower extract (AOFE) against nephrotoxicity induced by GM in male rats.

Methods: 30-year-old male Wistar rats were divided into five groups. Group 1 as a negative control group received AOFE 250 mg/kg/day. Groups 2-5 received saline, AOFE 50 mg/kg/day, AOFE 250 mg/kg/day, and AOFE 500 mg/kg/day for 9 days, respectively, and GM (100 mg/kg/day) was added from the 3rd day on. At the end of the experiment, blood samples were obtained, animals were sacrificed, and the kidneys were removed immediately.

Results: Gentamicin (in group 2) significantly increased serum levels of blood urea nitrogen and creatinine as well as the pathological damage score ($P < 0.05$) when compared with group 1. Low dose of AOFE did not decrease the nephrotoxicity induced by GM while the high dose of AOFE aggravated renal toxicity ($P < 0.05$).

Conclusions: Although AOFE acts as an antioxidant, at the doses used in the current study did not ameliorate nephrotoxicity induced by GM.

Keywords: Gentamicin, *Althaea officinalis*, nephrotoxicity, rat

INTRODUCTION

Gentamicin (GM) is one of the amino glycoside drugs used in Gram-negative infections.[1-3] Nephrotoxicity is specified by renal dysfunction, which is distinguished by increasing serum levels of blood urea nitrogen (BUN) and creatinine (Cr).[4,5] Researchers have tested different compounds for preventing or treating damages induced by GM. Compounds such as lycopene,[6] metformin, garlic,[7,8] Vitamin E, probucol,[9] and erythropoietin[10] could prevent renal damage induced by GM. Furthermore, several studies have suggested that supplementations of herbal extracts such as *Ginkgo biloba*,[11]

Bauhinia variegata,[12] *Pongamia pinnata* flower,[13] and grape seed[14] may attenuate GM-induced nephrotoxicity. *Althaea officinalis* (marshmallow, marshmallow, or a common marshmallow), the member of *Malvaceae* family, is well-known for its medicinal properties.[15,16] It is demonstrated that *A. officinalis* has potential therapeutic benefits in lipomia, inflammation, gastric ulcer, and platelet aggregation.[17] The pharmacological and antioxidant activities of *A. officinalis* refer to various compounds such as polysaccharides and flavonoids present in the plant.[16,18] In the present study, we attempted to investigate the effect of *A. officinalis* flower extract (AOFE) as an antioxidant against nephrotoxicity-induced by GM in male rats.

METHODS

Adult male Wistar rats (Animal Centre, Isfahan University of Medical Sciences) were used in this study. Animals were housed in standard conditions with free access to food and water. This research was approved in advance by the Isfahan University of Medical Sciences Ethics Committee.

Preparation of extract

Dried violet flowers of *A. officinalis* were selected and powdered. Preparation of the extract was fulfilled in two steps; first, 600 ml ethanol 70% was added to 150 g prepared powder and the total mixture was shaken for 24 h at the temperature of 23-25°C. Then, it was filtrated by Whatman paper (70 mm). After filtration, the removed extract was incubated at the temperature of 4°C. Then, 600 ml ethanol 96% was added to the material remained from the first step and again the total mixture was shaken for 24 h at the temperature of 23-25°C. The extract obtained after filtration in this step was mixed with the yield of the first step. Then, the total extract was incubated at the temperature of 50°C for 48 h and finally 100% dried extract was obtained.

Study design

Thirty animals (192.4 ± 4.6 g) were divided into five groups.

- Group 1 (n = 6) as negative control group received AOFE 250 mg/kg/day for 9 days, and saline was added from day 3 on
- Groups 2 (n = 5) as positive control group received saline during the study and GM (100 mg/kg/day) was added from day 3 on
- Group 3 (n = 6) received AOFE 50 mg/kg/day for 9 days, and GM (100 mg/kg/day) was added from day 3 on. Groups 4 (n = 7) and 5 (n = 6) had the same regimen of group 3 except AOFE dose which were 250 mg/kg/day and 500 mg/kg/day, respectively. All administrations were done intraperitoneally. At the end of the experiment, animals were anesthetized by ketamine (75 mg/kg). Blood samples were obtained via heart puncture, and the serum was kept at −20°C to measure the serum levels of BUN and Cr. Finally, the animals were killed. The kidneys were removed and weighed immediately. Left kidney was fixed in formalin and staining was performed to detect the tissue damage.

Pathological investigation

The left kidney was fixed in 10% neutral formalin and embedded in paraffin. After slicing, hematoxylin and eosin staining was performed to examine tissue damage including tubular atrophy, cast, debris, and necrotic materials in the tubular lumen. Intensity of tubular lesion was scored from 1 to 4, while zero score was assigned to normal tissue without damage.

Statistical analysis

Data were reported as mean ± standard error of the mean. The two groups were compared with regard to the serum levels of BUN and Cr, and kidney weight (KW) by independent Student's t-test. The parameters were analyzed by one-way ANOVA followed by least significant difference test among the groups. The kidney tissue damage score (KTDS) was compared using Kruskal–Wallis or Mann–Whitney tests. P <0.05 were considered as significant.

RESULTS

Gentamicin itself induced nephrotoxicity, which was confirmed by increasing in the serum levels of BUN and Cr as well as elevating in KTDS and KW (P < 0.05) [Table 1]. Administration of various doses of AOFE accompanied with GM did not attenuate the serum levels of BUN

and Cr; rather it increased the values ($P < 0.05$). High dose of AOFE aggravated renal damage induced by GM in comparison with other groups ($P < 0.05$) [Figure 1]. Sample images from group 1 treated with AOFE alone and group 5 treated with GM plus high dose of AOFE are demonstrated in Figure 2.

DISCUSSION

The aim of this study was to investigate whether AOFE could ameliorate nephrotoxicity induced by GM in the male rat. We observed that AOFE administration did not ameliorate nephrotoxicity induced by GM; rather it intensified renal failure. GM induces renal dysfunction, which is characterized by increase in levels of Cr, uric acid, and BUN.[19-21] In addition, it is accompanied with tissue alterations such as glomerular congestion, disruption of glomerular capillaries, vacuolar degeneration of tubular epithelial cells, and hyaline cast formation.[20,21] Our findings are in agreement with the results of these studies. Furthermore, the present study indicated that GM enhanced normalized KW probably due to edema caused by tubular necrosis.[22] Useful properties of *A. officinalis* flower were documented in the literature,[17] but we did not obtain positive results in the administered doses. It is demonstrated that administration of 50 mg/kg dose of *A. officinalis* flower result in a significant increase in serum HDL cholesterol level.[17] Also, antiinflammatory and antiulcerogenic effects of the extract were observed at doses of 50, 100, and 250 mg/kg.[17] In contrast, we observed that doses of 50 and 250 mg/kg of AOFE aggravated the increased levels of BUN and Cr induced by GM. In addition, AOFE at the dose of 500 mg/kg aggravated both renal dysfunction and tissue damage. It has reported that increasing the dose of AOFE to 500 mg/kg significantly decreased stool water content.[17] It is also reported that high doses of some antioxidants provide a harmful effect on survival of the subjects.[23,24] Therefore, it is possible that AOFE at the doses lower than 50 mg/kg may ameliorate nephrotoxicity induced by GM.

Table 1: BUN and Cr serum levels, KTDS, and KW g/100 g body weight in the experiment groups

Group	BUN (mg/dl)	Cr (mg/dl)	KTDS	KW (g/100g BW)
AOFE 250	29.33±1.28	0.7±0.08	0.16±0.16	0.67±0.02
GM	47.58±7.98*	1.30±0.17*	1.6±0.24*	0.79±0.04*

Group AOFE 250 received AOFE 250 mg/kg/day for 9 days and accompanied with saline from day 3 on. The GM group received saline for 9 days and GM 100 mg/kg/day was added from day 3 on. * indicates significant difference ($P<0.05$) from group AOFE 250. BUN=Blood urea nitrogen, Cr=Creatinine, KTDS=Kidney tissue damage score, KW=Kidney weight, AOFE=*Althaea officinalis* flower extract, GM=Gentamicin

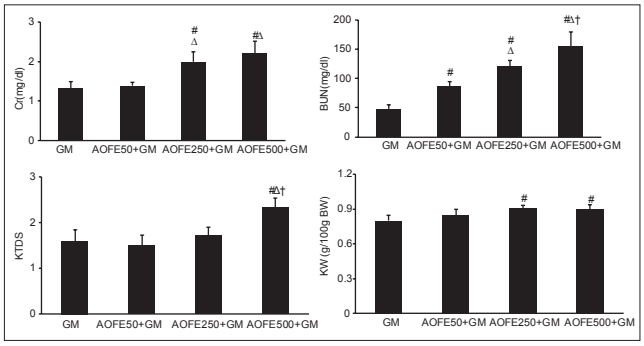

Figure 1: Blood urea nitrogen (BUN) and creatinine (Cr) serum levels, kidney tissue damage score (KTDS), and kidney weight g/100g of body weight in the experiment groups. Gentamicin (GM), *Althaea officinalis* flower extract (AOFE) 50 + GM, AOFE 250 + GM, and AOFE 500 + GM groups received saline, AOFE 50 mg/kg/day, AOFE 250 mg/kg/day, and AOFE 500 mg/kg/day for 9 days, respectively, and GM (GM; 100 mg/kg/day) was added from day 3 on. #indicates significant difference from GM group. Δindicates significant difference from AOFE 50 + GM group. †indicates significant difference from AOFE 250 + GM group

CONCLUSIONS

Although AOFE acts as an antioxidant, doses of AOFE used in the current study did not ameliorate nephrotoxicity induced by GM, and it is necessary to test doses lower than 50 mg/kg.

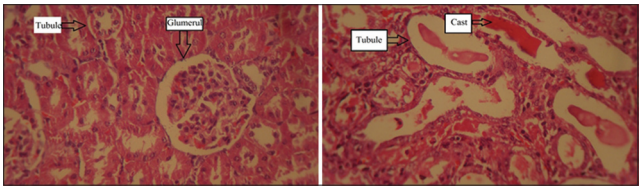

Figure 2: Sample images from group 1 treated with *Althaea officinalis* flower extract (AOFE) alone (left) and group 5 treated with GM plus high dose of AOFE (right). The tissue damage in group 5 is higher than other groups

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