

## Brief communication (Original)

# Post-antibiotic and post-antibiotic sub-MIC effects of gentamicin, sophoraflavanone G, and their combination against a clinical isolate of *Staphylococcus aureus*

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**Background:** Post-antibiotic effect (PAE) defines the potential of a drug to delay re-growth of a bacterial population after short-term exposure and removal of a drug. Determination of the PAE is recommended in pre-clinical evaluation of all new antimicrobial agents, because it influences optimal antimicrobial dosing intervals.

**Objective:** Evaluate the PAE and PA-SME of gentamicin and sophoraflavanone G against *Staphylococcus aureus* (*S. aureus*) itself and in combination.

**Methods:** A spectrophotometric method was used to determine the PAE and PA-SME.

**Results:** Sophoraflavanone G and gentamicin, showed considerable PAE and PA-SME at the tested concentrations against *S. aureus*. The increased duration of PAE caused by sophoraflavanone G and gentamicin was dose-dependent. In addition, sophoraflavanone G at sub-MIC concentrations enhanced the PAE and PA-SME of gentamicin in a dose-dependent manner. The highest enhancing effect was observed for gentamicin at the synergistic MIC and 1/2 the synergistic MIC levels against *S. aureus* 0.03 µg/mL (30 ng/mL) of sophoraflavanone G (with PAE=55 minutes). It enhanced the post-antibiotic sub-MIC effect (PA-SME) duration of gentamicin at concentrations of 4 µg/mL from 15 minutes to 80 minutes (a six-fold increase).

**Conclusion:** Sophoraflavanone G is promising for the preparation of an effective therapeutic formulation against gentamicin-resistant *S. aureus*.

**Keywords:** Gentamicin, post-antibiotic effect, sophoraflavanone G, *Staphylococcus aureus*

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The emergence of bacterial resistance to antibiotics is a major health problem, leading to treatment failure for many drugs [1-3]. There has been increasing interest in the use of compounds that diminish or eliminate antimicrobial resistance [3, 4]. These compounds are co-administered with antimicrobial agents to increase the drug effectiveness. Plant-based natural products may enhance antimicrobial potency for combating infectious diseases [5-8] as well as other diseases [9].

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Prenylated flavonoids found in the dried roots of *Sophora* spp (*Fabaceae*) are used in Asian traditional medicine. They have been shown to have antimicrobial and anti-inflammatory properties [10, 11]. Sophoraflavanone G a prenylated flavonoids from *Sophora* spp is reported to have various effects on eukaryotic cells, including inhibition of tyrosinase [12] and antioxidant activity [13]. It also has antibacterial activity against Gram-positive and Gram-negative bacteria in addition to antifungal activity [14, 15]. Furthermore, it enhances antimicrobial activities of ampicillin (β-lactamase) and gentamicin (aminoglycoside) in a synergistic manner [14, 16].

Gentamicin sulfate is a water-soluble aminoglycoside, which is used in treatment of susceptible bacterial infections caused by Gram-

negative bacteria, including *Pseudomonas*, *Proteus*, and *Serratia*, and Gram-positive bacteria, including *Staphylococcus aureus*. However, resistant clinical strains of these bacteria have been found to include methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) strains. Sophoraflavanone G, from *Sophora pachycarpa*, has potent bactericidal activity and enhances the antibacterial activity of gentamicin at sub-minimum inhibitory concentrations (sub-MIC) against *S. aureus*. In fact, 0.03 µg/mL of sophoraflavanone G decreased the MIC of gentamicin for *S. aureus* from 32 to 8 µg/mL, corresponding to a four-fold decrease [12].

Post-antibiotic effect (PAE) defines the potential of a drug to delay re-growth of a bacterial population after short-term exposure and removal of a drug. Determination of the PAE is recommended in pre-clinical evaluation of all new antimicrobial agents because it influences optimal antimicrobial dosing intervals. There is no investigation on the effect of sophoraflavanone G on the PA-SME duration of gentamicin at concentrations of  $\leq$  sub-synergistic MIC against *S. aureus*. In this study, we investigated the PAE as well as the post-antibiotic sub-MIC effect (PA-SME) in suppression of re-growth induced by sub-MIC drug concentrations in *S. aureus* that were caused by gentamicin, sophoraflavanone G, and their combinations.

## Materials and methods

### Sophoraflavanone G preparation

*Sophora pachycarpa* plants were collected in May 2005 north of Khorasan, Mashhad Province, Iran, and identified in the Department of Pharmacognosy, Faculty of Pharmacy, Mashhad University of Medical Science, Mashhad (Iran). The method for the extraction and identification of sophoraflavanone G was described previously [14]. The roots were air-dried at room temperature and crushed to powder. An acetone extract of the powder was prepared by macerating 6.5 g of the powder for 24 hours with three changes of solution at room temperature. The acetone was evaporated and the brownish, viscous residue (4.3% yields) was subjected to column chromatography (300 g silica gel). Fractions were eluted with petroleum ether: acetone (95:5) and assayed for antimicrobial activity. Fractions containing bactericidal activity were subjected to preparative thin-layer chromatography (TLC) on silica gel, using petroleum ether/acetone (50:50) as the solvent. The

active fraction was identified as sophoraflavanone G, using NMR spectroscopic methods ( $^1\text{H}$  and  $^{13}\text{C}$  NMR, DEPT,  $^1\text{H}$ - $^1\text{H}$  COSY, HSQC and HMBC).

### Antibacterial susceptibility

Susceptibility tests were carried out by the standard broth dilution method in accordance with NCCLS guidelines (CLSI). The MIC values of gentamicin and sophoraflavanone G for the clinical isolate *S. aureus* strain used were 32 and 0.05 µg/mL, respectively.

### Determination of PAE and PA-SME assays

The PA-SME and PAE of gentamicin and sophoraflavanone G were separately determined at the MIC, 1/2 MIC and 1/10 MIC concentrations, which were 32, 16, and 3.2 µg/mL, for gentamicin, and 0.05, 0.025, and 0.005 µg/mL for sophoraflavanone G. The test strain was incubated in the presence of these three concentrations of gentamicin and sophoraflavanone G for two hours before removal of the tested compounds by centrifugation of cultures, followed by two washes with 10 mL of drug-free pre-warmed (37°C) Mueller-Hinton broth (MHB).

The pellets were re-suspended in 50 mL of pre-warmed MHB to determine the PAE and PA-SME MIC values for each of the concentrations of gentamicin and sophoraflavanone G. Drug-free bacterial cultures in MHB inoculated under the same conditions were used as controls. All cultures were incubated at 37°C for 24 hours. The optical densities of cultures were measured at 600 nm at intervals until the end of the experiment. To evaluate the PA-SME of gentamicin in combination with sophoraflavanone G, the test strain was incubated in the presence of gentamicin at the synergistic MIC concentration (8 µg/mL) and sub-synergistic MIC concentrations (4 and 0.8 µg/mL) with or without sophoraflavanone G at sub-MIC concentrations (0.03 µg/mL). The PAE and PA-SME periods were calculated as the time necessary for the antibiotic-treated cultures to reach 50% of the  $\text{OD}_{\text{max}}$  of the control culture, minus the time taken for the control culture to reach the same point [16].

## Results

We have previously shown that that sophoraflavanone G at sub-MIC concentration (0.03 µg/mL) can decrease the MIC of gentamicin for *S. aureus* from 32 to 8 µg/mL (a four-fold decrease). In

the current study, we have shown it also affects the PAE and PA-SME of gentamicin. The effects of gentamicin and sophoraflavanone G at different concentrations (MIC, 1/2 MIC, and 1/10 MIC) on the reduction of growth of *S. aureus* after a short time exposure are demonstrated in **Fig. 1** and **2**. The PAE induced by gentamicin was concentration-dependent and increased when the bacteria were exposed to higher drug concentrations (**Fig. 1**). The duration of the PAE of sophoraflavanone G for *S. aureus* was also dose-dependent (**Fig. 2**). The PAE of sophoraflavanone G for *S. aureus* at concentrations equal to MIC, 1/2 MIC and 1/10 MIC were 20, 55, and 115 minutes, respectively.

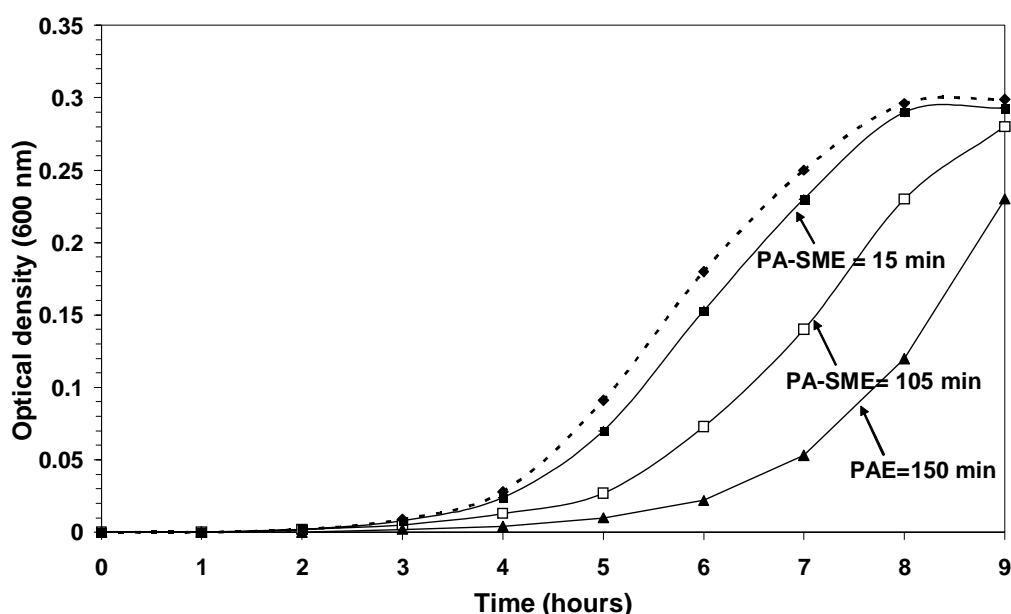
**Figure 3** shows the effect of a sub-inhibitory concentration (0.03  $\mu\text{g/mL}$  of sophoraflavanone G on the PA-SME of gentamicin at three different concentrations (synergistic MIC, 1/2 synergistic MIC, and 1/10 synergistic MIC).

The PA-SME durations of gentamicin at synergistic MIC (8  $\mu\text{g/mL}$ ), 1/2 synergistic MIC (4  $\mu\text{g/mL}$ ) and 1/10 synergistic MIC (0.8  $\mu\text{g/mL}$ ) in the absence and presence of sophoraflavanone G (at 0.03  $\mu\text{g/mL}$  concentration) were also compared. The results are shown in **Fig. 4**. The PA-SME duration for gentamicin at synergistic MIC (8  $\mu\text{g/mL}$ ) and 1/2 synergistic MIC (4  $\mu\text{g/mL}$ ) concentrations was not long.

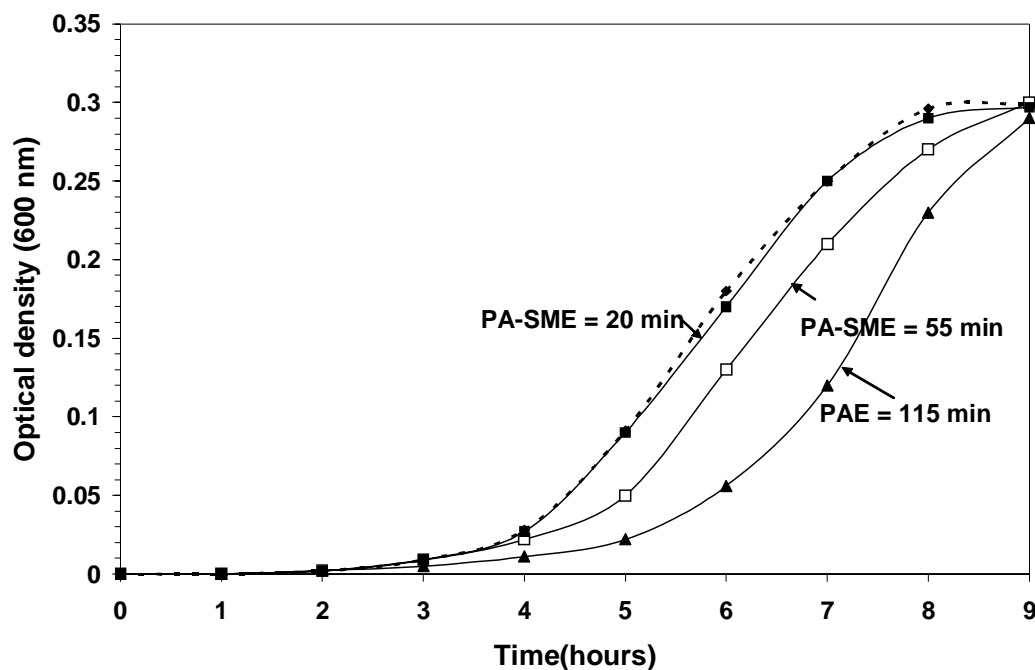
## Discussion

This study is the first investigation of the effect of sophoraflavanone G on the PA-SME duration of gentamicin at concentrations of  $\leq$  sub-synergistic MIC against *S. aureus*. The present results showed that at sub-inhibitory concentrations tested, sophoraflavanone G significantly increased the PA-SME duration of gentamicin. Sophoraflavanone G at a concentration of 0.03  $\mu\text{g/mL}$  not only enhanced the antibacterial activity of gentamicin but also increased the PA-SME duration of gentamicin at synergistic MIC levels. At this time, the reason for these enhancements is not known. It might be attributed the antibacterial activity of sophoraflavanone G to reducing the fluidity of cellular membrane [15].

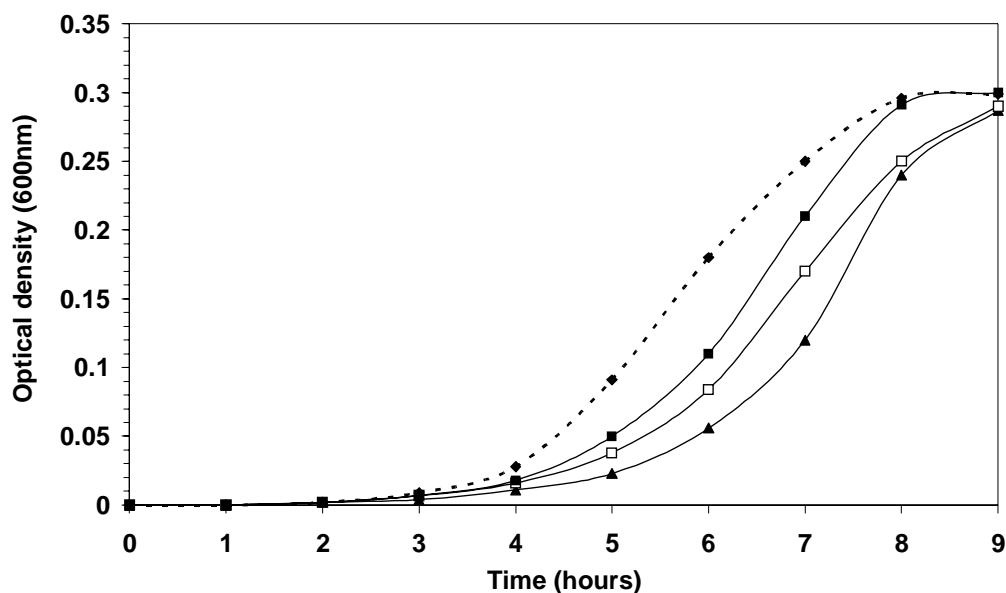
The concentration of sophoraflavanone G that was necessary to decrease the PAE of gentamicin for *S. aureus* is far below the MIC of sophoraflavanone G. The safety and toxicity of this compound have not been fully addressed, so it is important to find out the minimum level of compound that could still be effective in enhancing the efficacy of other antimicrobial agents. This is the first report of the effect of the combination of sophoraflavanone G PAE and PA-SME duration of gentamicin at concentrations of  $\leq$  sub-synergistic MIC against *S. aureus*.



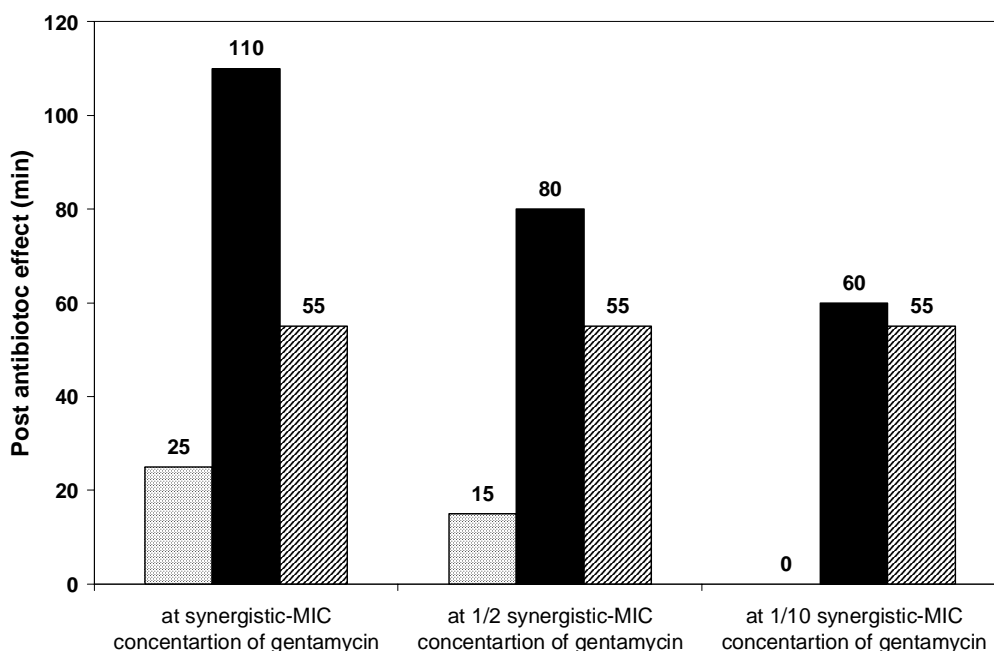
**Fig. 1** The post-antibiotic effect and post-antibiotic sub-MIC effect of gentamicin against *S. aureus* in the absence and presence of gentamicin concentrations equal to MIC, 1/2 MIC and 1/10 of MIC. Growth of *S. aureus* without gentamicin (♦), with 3.2  $\mu\text{g/mL}$  of gentamicin (■), 16  $\mu\text{g/mL}$  of gentamicin (□) and 32  $\mu\text{g/mL}$  of gentamicin (▲). The MIC of gentamicin for *S. aureus* was 32  $\mu\text{g/mL}$ .



**Fig 2.** The post-antibiotic effect and post-antibiotic sub-MIC effect of sophoraflavanone G against *S. aureus* in the absence and presence of sophoraflavanone G concentration equal to MIC, 1/2 MIC and 1/10 of MIC. Growth of *S. aureus* without sophoraflavanone G (◆), 0.005 µg/mL of sophoraflavanone G (■), 0.025 µg/mL of sophoraflavanone G (□) and 0.05 µg/mL of sophoraflavanone G (▲). The MIC of sophoraflavanone G for *S. aureus* was 0.05 µg/mL.



**Fig 3.** The effect of sub-inhibitory concentrations of sophoraflavanone G (0.03 µg/mL) on postantibiotic sub-MIC effects of gentamicin at  $\leq$  sub-synergistic MIC concentrations against *S. aureus* without any of the drugs (◆), with 0.8 µg/mL of gentamicin (■), with 4 µg/mL of gentamicin (□) and 8 µg/mL of gentamicin (▲). The MIC of sophoraflavanone G and gentamicin for *S. aureus* were 0.05 µg/mL and 32 µg/mL, respectively.



**Fig. 4** PA-SME duration in response to exposure of *S. aureus* to gentamicin and sophoraflavanone G and their combination at sub MIC concentrations. Dotted bar: PA-SME duration of gentamicin at concentrations of  $\leq$  synergistic-MIC concentration (8, 4 and 0.8  $\mu\text{g/mL}$ ). Solid bar: PA-SME duration of gentamicin at  $\leq$  synergistic-MIC concentrations (8, 4 and 0.8  $\mu\text{g/mL}$ ) in combination with sophoraflavanone G (0.03  $\mu\text{g/mL}$ ). Striped bar: PA-SME of sophoraflavanone G at sub-MIC concentration (0.03  $\mu\text{g/mL}$ ).

In conclusion, Sophoraflavanone G is promising for the preparation of an effective therapeutic formulation against gentamicin-resistant *S. aureus*.

### Acknowledgments

This work was supported by Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Iran. The views presented in this article do not necessarily reflect those of the U.S. Food and Drug Administration. The authors have no conflict of interest to report.

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