Antiviral activity of borage (*Echium amoenum*)

Mohsen Abolhassani

Hybridoma Lab, Department of Immunology, Pasteur Institute of Iran, Tehran, Iran

Submitted: 19 April 2009
Accepted: 20 July 2009

Arch Med Sci 2010; 6, 3: 366-369
DOI: 10.5114/aoms.2010.14256

Corresponding author:
Mohsen Abolhassani
Department of Immunology
Pasteur Institute of Iran
Tehran, Iran
Phone/fax: (+98-21) 6649 2596
E-mail:
mabolhassani@yahoo.com

Abstract

Introduction: Borage (*Echium amoenum*) is a large annual plant of the *Boraginaceae* family that grows in most parts of Europe and in northern parts of Iran. The flower of borage is used as a medicinal herb in various countries as an antifebrile and antidepressant, for treatment of stress, circulatory heart diseases and pulmonary complaints, as a poultice for inflammatory swellings, as a diuretic, laxative, emollient and demulcent, and recently as a possible cancer protective factor. The Iranian borage is used in traditional medicine for infectious diseases and influenza and as an antifebrile. In this report, an aqueous extract of dried borage (*Echium amoenum*) flowers was tested *in vitro* for its antiviral activity.

Material and methods: Bacteriophage 3C and its specific host, *Staphylococcus aureus* 8327 were used Aqueous extract of *E. amoenum* dried flower was prepared and anti-viral activity was determined by agar overlay method and the burst size was determined by one-step growth experiment. Antibacterial activity was determined by disc diffusion, agar-well diffusion and minimum inhibitory concentration methods.

Results: The extract showed concentration-dependent antiviral activity against free bacteriophage 3C and reduced the yield of phage from the host *Staphylococcus aureus* 8327. Antiviral activity of the extract is heat resistant. Autoclaving the extract at 110°C for 1 h did not eliminate its antiviral activity and the effect was similar to the extract that was filter sterilized. However, the activity of the freeze-dried extract was diminished during 90 days of storage at 4°C and the activity of the working solution was diminished in a one-week period at 4°C.

Conclusions: These results may provide a justification for the traditional use of the Iranian borage flower for infectious diseases and antifebrile activity.

Key words: borage, *Echium amoenum* (Fish & C.A. Mey), bacteriophage, antiviral activity, *Staphylococcus aureus*.

Introduction

Borage is a large, hairy annual herb that is a member of the *Boraginaceae* family [1]. It grows in most parts of Europe and in the Mediterranean region and also in northern parts of Iran. The flowers are bright blue and star-shaped and the fruit consists of four brownish-black nutlets. Borage flourishes in ordinary soil and may be propagated by division of rootstocks and by putting shoots in sandy soil in a cold frame in summer and autumn or from seeds sown in good light soil from the middle of March to May [2].

The flowers and the leaves of borage are used medicinally in France as an antifebrile and antidepressant, for treatment of stress, circulatory heart

diseases and pulmonary complaints, as a poultice for inflammatory swellings [3, 4], as a diuretic (due to potassium nitrate), laxative, emollient and demulcent (due to the mucilage), and recently as a possible cancer protective factor [5].

The plant constituents have been isolated by different investigators and contain γ-linolenic acid (GLA), α-linolenic acid (ALA), Δ-fatty acyl desaturase, Δ8-sphingolipid desaturase [6], pyrrolizidine alkaloids, mucilage, resin, potassium nitrate and calcium salt combined with mineral acids.

In this report, an aqueous extract of dried borage (*Echium amoenum*) flowers was tested *in vitro* for its antiviral activity.

Material and methods

Plant and extract

The borage found in Iran is *Echium amoenum* (Fish & C.A. Mey), which is different from the borage grown in Europe, *Borago officinalis* L. (*Boraginaceae*). Dried borage flower used in this report was collected from Ardebil province, north of Iran in mid August.

Cold aqueous extract (pH 5.8) of *E. amoenum* dried flower (5%, w/v) was used throughout the experiments. Extraction was also done at pH 7.0 and 8.5, but pH 5.6 was the best for higher extraction and antibacterial activity. The dried flower (15 g) was steeped for 6 h at 4°C in 300 ml distilled water with constant stirring. The material was centrifuged and the supernatant was filter-sterilized and then freeze-dried.

Bacteriophage and bacterium

Bacteriophage 3C and its specific host, *Staphylococcus aureus* 8327, were obtained from Tehran University of Medical Sciences, Faculty of Health, Iran. Nutrient broth (NB) containing 5 g peptone (Difco), 5 g NaCl and 3 g beef extract in 1 l of distilled water (pH 7.5) was used as the culture medium for the study of phage-infected or non-infected bacteria.

Anti-bacterial activity of the extract was determined by agar-well diffusion, and the minimum inhibitory concentration (MIC) methods [7]. In the agar-well diffusion method, 9 mm diameter wells were prepared on agar containing 0.5 ml of bacteria (2×10^{11} cells/ml). Freeze-dried extract was diluted and different concentrations were added to the wells (1.25 mg to 10 mg).

To determine minimum inhibitory concentration (MIC), 6 tubes containing 20 ml broth medium were selected, containing 1 ml of bacteria (8×10^8 bacteria, OD 600 = 1), with various concentration of extract (12.5, 6.25, 3.1, 1.5 and 0.75 mg/ml). After 24-h incubation at 37°C, the turbidity was determined [7].

Determination of burst size

The burst size was determined by a one-step growth experiment. The 10 ml log-phase *S. aureus* cells (2×10^{11} cells /ml) were infected with 0.2 ml phage 3C (4×10^4 phage/ml) in NB medium at 37°C. After 10 min, the mixture was centrifuged and the pellet was suspended in the original volume of fresh NB medium pre-warmed to 37°C. Cells were divided into 0.5 ml aliquots in small tubes and incubated for 65 min at 37°C. Every 3 min, one tube was removed and mixed with 3 ml warmed 0.7% agar and poured on a plate to assay the phage.

To determine the effect of extract on intracellular phage growth, to each tube 5 mg extract was added and after incubation at 37°C the plaques were measured. In this procedure, all the phages were adsorbed to the bacteria and no free phage was detected in the supernatant.

Antiviral activity of borage extract on free phage

In 6 tubes containing 2 ml of NB medium, 0.1 ml phage (4×10^4 phage/ml) and 0.1 ml of different concentrations of extract (0-5 mg) were added and the tubes were incubated for 24 h at two different temperatures (24°C and 37°C). Each tube was diluted with NB medium (1/10) mixed with 0.5 ml bacteria and as in the above procedure the plaques were measured. Anti-viral activity was determined by the agar overlay method and antibacterial activity by disc diffusion [8], agar-well diffusion and minimum inhibitory concentration (MIC) methods [9].

Results and discussion

To determine the antiviral effect of borage flower the aqueous extract was prepared. To get the best aqueous extraction, distilled water with three different pHs of 5.6, 7.0 and 8.5 was used and from 15 g of dried flower about 8.24, 6.84 and 7.02 g of lyophilized powder were obtained respectively. Table I shows the effect of different pH on extraction of borage. Since pH 5.6 had the best extraction and inhibitory activity it was selected for extraction. Figure 1 shows anti-bacterial activity of different concentration of extract on *Staphylococcus aureus* in the agar-well diffusion method. As seen in this figure, the 5 and 10 mg extracts had 4 mm and 10 mm of average inhibition zone respectively.

The burst size experiment showed that the latent period of phage was 35-38 min and the phage plaque number was about 70-80 (Figure 2). After 35 min, the number of phage increased and reached about 440 plaques at 60 min. To study the effect of extract on intracellular bacteriophage growth, first the minimum inhibitory concentration of extract on phage host was determined. The minimum inhibitory concentration of extract on

Table I. Effect of different pHs on extraction of borage and its anti-bacterial activity

pH	Extract lyophilized powder [g]	Mean inhibition zone on agar-well diffusion
5.6	8.249	10
7.0	6.840	7
8.5	7.024	6

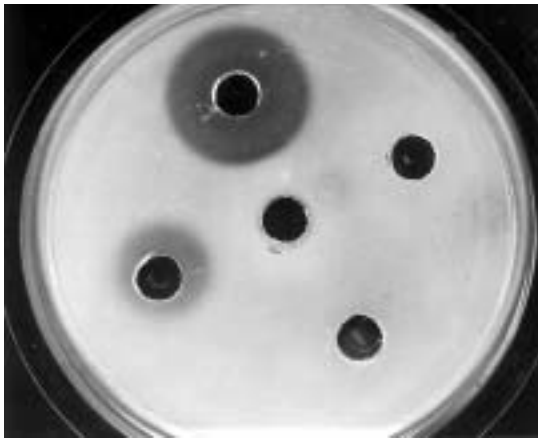

Figure 1. Anti-bacterial effect of different concentrations of borage extract on *Staphylococcus aureus*. Clockwise, 1.25 mg, 2.5 mg, 5 mg and 10 mg extract were added to the wells and the centre well is filled with distilled water. Ten and 5 mg extract with average 10 mm and 4 mm inhibition zone showed anti-bacterial activity

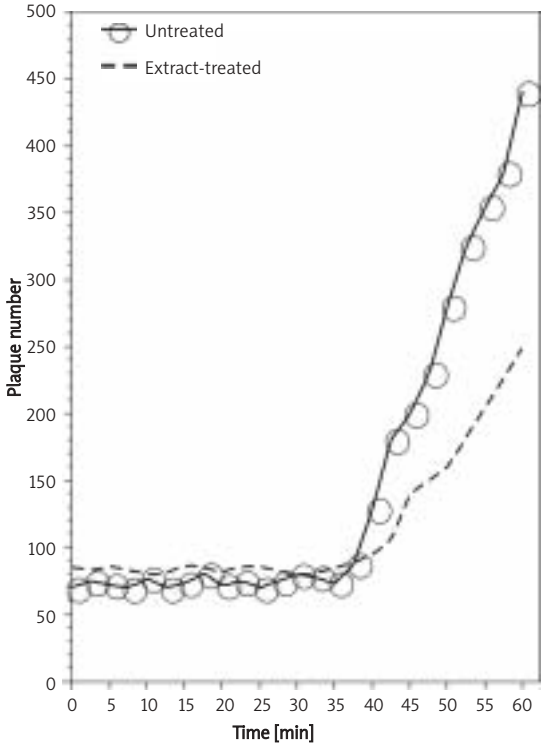

Figure 2. The effect of extract on intracellular phage growth. Phage growth was determined in the absence of extract (circle) and in the presence of extract (dotted line) for 60 min. The data represent the mean ± SD from triplicate experiments

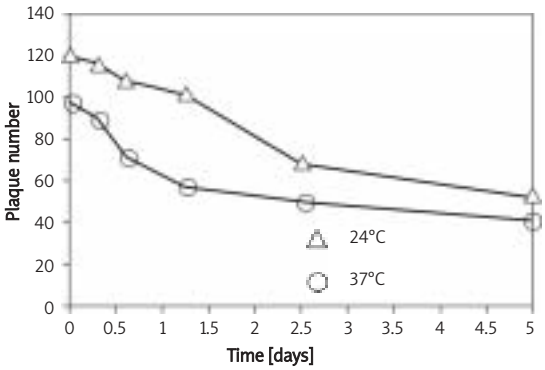

Figure 3. Antiviral effect of borage extract on free bacteriophage 3C. Phage was treated with different concentrations of extract (0 mg to 5 mg) for 24 h at two different temperatures (24°C and 37°C). After absorption to the bacteria the plaques were measured. The data represent the mean ± SD from triplicate experiments

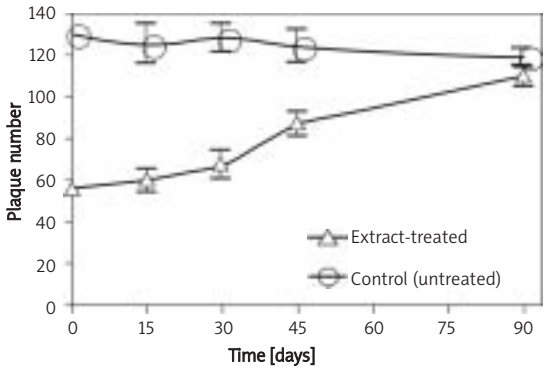

Figure 4. Stability of antiviral activity of borage extract during 90 days by agar overlay method. Each time 5 mg of freeze-dried extract was removed from the same batch and fresh stock was prepared and used for antiviral activity on free phage. The data represent the mean ± SD from triplicate experiments

S. aureus 8327 was 6.2 mg/ml and in all the experiment less than 6 mg/ml was used. To determine the effect of extract on intracellular phage growth, the extract (5 mg) was added at different times (every 3 min beginning in the latent period) and as above the plaque number was measured. Figure 2 (dotted line) shows that the extract did not change the phage latent period (35-38 min) or the number of plaques during the latent period. Also the extract did not prevent the release of phage progeny. However, in comparison to the control, after 35 min when the first burst occurred the number of plaques in the treated samples decreased. The reduction of extracellular phage content may be due to the inhibition of the release of progeny phage from the host cells.

To check the effect of borage aqueous extract on free phage, phage was exposed to different concentrations of extract (0 mg to 5 mg) for 24 h at 37°C at room temperature. Figure 3 shows that extract at 5 mg could inactivate almost 50% of the phage. The inhibition was more effective at 37°C than at 24°C. The inhibitory effect of extract was not due to the pH of the extract, since extract with all three pHs of 5.6, 7.0 and 8.5 had antiviral activity and the control pH did not show any effect. These data indicate that antiviral activity of the extract is due to a component of borage. It is possible that the active material affects the tail or coat proteins of the phage that are important for absorption to its host bacteria.

Antiviral activity of the extract was heat resistant. Autoclaving the extract at 110°C for 1 h did not eliminate its antiviral activity and the effect was similar to the extract that was filter sterilized. However, the activity of the freeze-dried extract was diminished during 90 days of storage at 4°C (Figure 4) and the activity of the working solution was diminished in a one-week period at 4°C.

After 5 days' exposure of the phage to extract the shape and number of plaques did not change and it was similar to the control untreated phage; this shows that the extract does not have mutagenic activity on the phage.

These results may provide a justification for the traditional use of the Iranian borage flower for infectious diseases and antifebrile activity. Borage syrup was thought not only to be good in fever, but also to be a remedy for jaundice, itch and ringworm [2]. Although several components such as linolenic acid, Δ6-fatty acyl desaturase, Δ8-sphingolipid desaturase [6], and pyrrolizidine alkaloids have been isolated and characterized, more data are needed to isolate the active antiviral component of the extract.

References

1. Zargari A. Medicinal plants. 4th ed. University Press, Tehran, Iran 1989; 510-39.
2. Grieve, M. Borage. Available at: http://www.botanical.com/botanical/mgmh/b/borage66.html.
3. Kast RE. Borago oil reduction of rheumatoid arthritis activity may be mediated by increased cAMP that suppresses tumor necroses factor-alpha. Int Immunopharmacol 2001; 1: 2197-9.
4. Kapoor R, Klimaszewski A. Efficacy of borage oil in patients with atopic eczema. Br J Dermatol 1999; 140: 685-8.
5. Gonzalez CA, Sanz JM, Marcos G, et al. Borage consumption as a possible gastric cancer protective factor. Cancer Epidemiol Biomarkers Prev 1993; 2: 157-8.
6. Sperling P, Libisch B, Zahringer U, Napier JA, Heinz E. Functional identification of a delta8-sphingolipid desaturase from Borago officinalis. Arch Biochem Biophys 2001; 388: 293-8.
7. Abolhassani, M. Antibacterial effect of Borage (Echium amoenum) on Staphylococcus aerous. Braz J Infect Dis 2004; 8: 382-5.
8. Barry AL, Coyle MB, Thornberry C, Gerlad EH, Howkinson RW. Methods of measuring zones of inhibition with the Bauer-Kirby disk susceptibility test. J Clin Microbiol 1979; 10: 885-9.
9. Braude AI. Principles of antimicrobial chemotherapy of infections. In: Braude AI (ed). Medical Microbiology and Infectious Diseases. WB Sanunders, London 1981; 220.