ANTI-STAPHYLOCOCCAL ACTIVITY OF EW1, A TRADITIONAL HERBAL REMEDY FOR CARBUNCLES

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ABSTRACT: A powder (EW1) made from a mixture of herbs used for the treatment of carbuncles by traditional medicine practitioners in China was investigated for anti-staphylococcal activity by agar diffusion, time-kill studies and M.I.C. determinations performed on 17 clinical isolates and a reference strain ATCC 29213. It was found that EW1 had little demonstrable *in vitro* activity against the clinical isolates tested but inhibited the growth of the ATCC strain at 10 mg/l and retarded its growth in broth culture by an average of 1.5 log reduction in colony count. *(JUMMEC 2000; 1: 48-50)*

KEYWORDS: Traditional medicine, anti-staphylococcal activity.

Introduction

Although modern, evidence-based medicine is regarded as the most advanced form of curative practice, in many populations, traditional, alternative or complementary medicine still commands a large following. The World Health Organization has estimated that over 80% of the world's inhabitants rely mainly on traditional medicine for their primary health care needs (1). Even in developed countries, the increasing public acceptance of alternative medicine has resulted in medical schools offering courses in this specialty and insurance companies and managed care organizations giving benefits to those who seek treatment from alternative medicine practitioners.

The most widely practiced alternative therapy is herbal medicine. Recognizing the vast potential of plants as natural resources for drugs, many international and local government agencies are encouraging basic science research and clinical studies on medicinal plants. A convenient starting point for researchers is the investigation of biological activities of herbal preparations that have been used by traditional practitioners. Unfortunately, scientific investigations on these preparations are complicated by the fact that herbs are frequently used in combination, their active principles are often unknown, the composition of herbal mixtures is not always disclosed and is often altered to suit the constitution of the consumer.

EW1 is a powder prepared from a mixture of herbs that have been used by traditional healers in China for the treatment of carbuncles. The composition of the mixture is a well-kept trade secret. It is usually applied as a paste onto the skin, and has been reported by the users to be effective in reducing swelling, redness, purulent discharge and hastening re-epithelialization. As carbuncles are usually caused by staphylococci, an investigation was carried out to test the *in vitro* activity of this herbal mixture on control and clinical strains of staphylococci.

Materials and method

Bacterial strains

The 18 bacterial strains tested consisted of a control *Staphylococcus aureus* (ATCC strain 29213), three clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), 4 isolates of methicillin-sensitive *Staphylococcus aureus* (MSSA) and 10 isolates of coagulase-negative staphylococci (CNS), of which 5 were methicillin-resistant and 5 methicillin-sensitive. The clinical isolates were from wound swabs of in-patients of the University of Malaya Medical Centre, Kuala Lumpur.

Preparation of stock solution

The EW1 powder was transported from Shanghai, China, at room temperature and kept at 4°C for several weeks until used for testing. Different conventional solvents were used to dissolve the powder including chloroform, dimethylformamide, dimethylsulfoxide, glacial acetic acid, absolute methanol, absolute ethanol, saturated sodium bicarbonate, 7% sodium bicarbonate, 2N NaOH, water, 0.1N HCl and a combination of glacial acetic acid and 7% sodium bicarbonate. Of these 12 solvents, only HCl was able to bring the powder into solution without heating. Hence, a stock solution of 10mg/ml was made in 0.1N HCl by dissolving 10 mg in 900 µl of sterile millipore-filtered water and 100 µl

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of 1N HCl, filter-sterilized using a 0.22 μm membrane filter and stored at –20°C.

Determination of Minimal Inhibitory Concentrations (M.I.C.) by agar dilution

The EW1 stock solution was diluted in sterile distilled water to give 3.125 to 800 mg/l. Two ml of each dilution was added to 18 ml of molten Mueller-Hinton agar to make a series of agar plates containing 0.3 to 80 mg/l of EW1. The 18 staphylococcal strains to be tested were incubated in Nutrient Broth (NB, Oxoid) overnight and then diluted 1:100 to approximately 10^5 organisms/ml. Using a micropipettor, 100 μl of each diluted culture was delivered onto each of the EW1-containing agar plates. The plates were then incubated overnight at 37°C and read for growth of bacteria after 18 hours. The M.I.C. was the lowest concentration of EW1 that totally inhibited the growth of bacteria.

Direct application of EW1 onto a bacterial lawn

As EW1 is usually applied as a thick patch directly onto the skin, the concentration of the active principle at the site of application may be higher than those used in the M.I.C. determination. To investigate the inhibitory effect of EW1 at higher concentrations, overnight NB cultures of the 18 staphylococcal strains were inoculated onto Mueller-Hinton agar plates with sterile cotton wool swabs and allowed to dry on the bench. The stock EW1 solution was diluted to 1000 mg/l and 20 μl volumes of the diluted solution were pipetted onto the inoculated agar plates. In addition, one gram of EW1 powder was made into a paste with a few drops of water and 0.1N HCL and directly applied onto the surface of the inoculated agar plates in circular patches of about 7 mm diameter. All plates were incubated overnight at 37 °C and then read for growth inhibition zones.

Time-kill studies

Two tests were carried out to compare the growth of *S. aureus* ATCC 29213 in NB with that in EW1 and in the solvent HCl. In the first test, the ATCC strain was incubated in NB overnight. Ten μl of this broth culture was used to inoculate each of three tubes containing a) 1 ml NB with 100 mg/l of EW1, b) 1 ml of NB with HCl (final concentration of 0.001N), and c) 1ml of NB. The inoculated tubes were then incubated in a 37°C water-bath. At 0, 2, 4, 5 and 6 hr after incubation, 20 μl was removed from each tube and diluted from 10^{-1} to 10^{-8}. Twenty μl of each dilution was then spotted on to a Nutrient Agar plate and incubated overnight. The number of colonies grown on the agar plate was counted to obtain the number of cfu/ml. In the second test, the whole procedure was repeated without the HCl control tube.

Results

In the agar dilution test, the ATCC control did not grow on agar containing ≥ 10 mg/l of EW1 but there was no demonstrable growth inhibition for any of the clinical strains even up to 80 mg/l (Table 1).

Table 1. Minimal Inhibitory Concentration of EW1 for staphylococci

Bacteria	No.of strains	MIC (mg/l)
S. aureus ATCC 29213	1	10
Methicillin-sensitive *S. aureus*	3	>80
Methicillin-resistant *S. aureus*	4	>80
Methicillin-sensitive CNS	5	>80
Methicillin-resistant CNS	5	>80

CNS, coagulase-negative staphylococci

When EW1 was applied as a paste, there was a clear zone of growth inhibition of 10 mm diameter around the ATCC control but not around any of the clinical strains. When applied as a 1000 mg/l solution, 3 clinical strains besides the ATCC control were partially inhibited as shown by the presence of tiny colonies within the zone of inhibition formed in the area where EW1 was dropped onto the staphylococcal lawn.

In the first time-kill study, the colony count of ATCC 29213 in NB grew from $1.5x10^3$ at 0 hour to $3.5x10^6$ at 6 hours of incubation. The corresponding counts in HCl and EW1 at 6 hours were $8.5x10^6$ and $2.0x10^5$ respectively. These counts indicated that, at the final concentration of 0.001N, the HCl solvent did not interfere with the growth of staphylococci while EW1 reduced the colony count by more than 1 log. This reduction in viable count was again seen in the second study where the count of ATCC 29213 rose from $5x10^2$ at 0 hour to $1x10^7$ in NB and $1.5x10^5$ in EW1 at 6 hours. Pooling together the results of both studies, the mean colony count in the absence of EW1 was $7.3x10^6$ cfu/ml while that in the presence of EW1 was $1.75x10^5$ cfu/ml, making a mean reduction of 1.5 log (Fig.1).

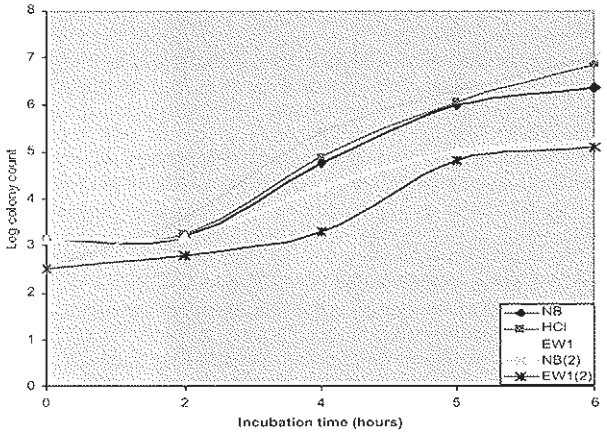

Figure 1. The effect of EW1 on the growth of *S. aureus* ATCC 29213

Discussion

Herbal medicine has been used for thousands of years by many different cultures. Its curative ability is established by the collective experience from large numbers of users. Scientific documentation of efficacy is scarce. The evaluation of herbal remedies may require strategies that are different from those used to evaluate drugs used in modern medicine. The biological activity of herbs may vary according to factors such as the age of the plants, the time of year in which the plants are collected and the geographical area from which the plants are collected. In addition, herbs are often used in combination with other plants, minerals or animal parts, all of which may vary in biological activity according to how and where they are obtained. Hence the therapeutic activity of herbal preparations may be less predictable or reproducible than that of synthesized drugs.

The results of the preliminary studies on EW1 described in this report show that this herbal preparation does have some anti-staphylococcal activity as indicated by the M.I.C. of 10 mg/l against ATCC 29213. However, this M.I.C. is considerably less than that of commonly used anti-staphylococcal antibiotics such as cloxacillin and vancomycin, both of which have a M.I.C. of 0.5-2.0 mg/l for ATCC 29213. This weak inhibitory activity was also seen in the time-kill studies where the mean reduction in colony count after 6 hours of incubation was less than 100-fold. With clinical isolates, the results were even less impressive. None of the 17 clinical strains tested were inhibited when EW1 was applied as a paste to simulate clinical usage, and only 3 strains of methicillin-sensitive *S aureus* were partially inhibited at a very high concentration of 1000 mg/l. These results suggest that the reported therapeutic effect of EW1 on carbuncles may not be solely due to its anti-staphylococcal activity.

For acceptance as an evidence-based therapeutic agent, further studies on EW1 are required to establish its *in vitro* and *in vivo* efficacy.

Acknowledgement

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Reference

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