Preparation and pharmaceutical evaluation of nicotinamide stick for eradication of Staphylococcus epidermidis

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Background: Staphylococcus epidermidis is a part of the skin’s normal flora that can cause acne. This study was designed to evaluate the efficacy of nicotinamide as a stick in eradication of staphylococcus. Materials and Methods: For evaluating of Anti-microbial effect on S. epidermidis used well plate method. We chose five plates for nicotinamide and five for mupirocin. The zones of inhibition were measured and compared. Results: The results showed nicotinamide stick had anti-microbial effects, but in comparison to mupirocin it was significantly less \((P = 0.003)\). Conclusion: Nicotinamide stick was made and evaluated. This study showed that nicotinamide had anti-microbial effect on staphylococcus.

Key words: Medicated sticks, nicotinamide, Staphylococcus epidermidis

INTRODUCTION

Staphylococcus epidermidis is a part of the normal flora of skin which is one of the germs that can cause acne.\(^1\)

Nicotinamide is one of the B vitamins with moisturizing, lightening, anti-irritation and anti-biotic effects without the side effects of antibiotics.\(^2-6\) The other names for nicotinamide are vitamin pp, bionic and niacin. It can be administered for mild to moderate acne.\(^7\) Some of its side-effects are dry skin, irritation, peeling, itching and redness.\(^8-10\) Different form of drug used for acne treatment such as gel, lotion, cream that is exist in the market, but the new form of products are sticks.\(^11, 12\)

Some of the studies showed that the nicotinamide gel is effective in decreasing the severity of acne.\(^13\) Many patients express difficulty in application of ointments, creams, gels as local forms of drugs. It results in non-compliance and inert therapy. Recent advances in novel drug delivery systems help to enhance efficacy and safety of drug components by using the new formulations and drug forms. An advantage of this drug delivery system includes patient compliance, convenience for efficient treatment include application without fingertip, immediate onset of action, reduced dosage regimen and economic issues.\(^12\)

Hence, we decided to conduct this study to prepare the nicotinamide stick for eradication of Staphylococcus aureus and evaluate its pharmaceutical aspects.

MATERIALS AND METHODS

This study was conducted from January 2011 to January 2012 in the laboratory of the Department of Pharmaceutics of School of Pharmacy and Pharmaceutical Sciences of Isfahan University of medical sciences, Isfahan, Iran.

Stearic acid (Merck, Germany), 1,2 propan-diol (Merck, Germany), NaOH (Merck, Germany), nicotinamide (Loba chemie Pvt. Ltd., Mumbai) and purified water were used to make sticks. S. aureus (ATCC 1112), nutrient agar (Merck, Germany) and nutrient-broth (Merck, Germany) were used for microbial test.

Preparation of nicotinamide stick: Medicated sticks of nicotinamide were prepared by heating and congealing according to the formulae given in Table 1. Stearic acid was heated and melted (oily phase). The NaOH solution was made and added to propylene glycol (aqueous phase). Added the aqueous phase slowly to the oily phase, stirring constantly then Nicotinamide solution was added to them. The warm mixture was poured into the stick mould and cooled to get the desired shape of a stick. Various concentrations of
stearic acid (3.50-6.80% w/w) were used as shown in Table 1.

Preparation of inoculum and seed layer: *S. aurous* was purchased from Persian type culture collection and sub cultured on nutrient agar at 37°C for 24 h. The inoculum of *staphylococcus* was prepared by suspension of colonies in nutrient-broth and its concentration was measured in 580 nm by ultraviolet spectrophotometer for 10⁶ CFU/ml. This microbial suspension was diluted to 10⁴ CFU/ml (1 mm of *S. aurous* suspension was added to 9 ml nutrient agar and made 10⁶ CFU/ml suspension concentration.

For doing microbial test palates prepared with two layers, base and seed layer. For preparing base layer poured nutrient agar on to the plate and after it became hard poured with 10⁴ CFU/ml *staphylococcus* suspensions and made the seed layer. After the seed layer became hard too, we dig wells whit holding down the Pasteur pipette with 9 mm diameters in the seed layer. We created three wells in each plate and put 20 μg nicotinamide stick and mupirocin in it separately.

**Evaluation of sticks**

Evaluation of sticks includes evaluation of weight, thickness, length, pH, spreading, drug content, drug diffusion and microbial test.

For weight, thickness and length three sticks were selected randomly and average of them calculate and compared with the individual. Spread ability should be in a way that can easily be drawn on the skin and so the spread ability was evaluated and ranked according to this grading: No spread ability (0), low spread ability (+), average spread ability (++), high spread ability (+++). To ascertain the drug content uniformity, the stick equivalent to 20 mg of nicotinamide was extracted with methanol and liquid was filtered. The nicotinamide content was determined by measuring absorbance at 263 nm by ultraviolet spectrophotometer for 10⁸ CFU/ml. This microbial suspension was diluted to 10⁶ CFU/ml *S. aurous* suspension was added to 9 ml nutrient agar and made 10⁶ CFU/ml suspension concentration.

Nearly 4% of nicotinamide concentration is the best concentration that is suitable with skin and affected on germs. The alkaline pH and more stearic acid cause the stick harden. The microbial test showed that nicotinamide stick had anti-microbial effects; the zone of inhibition was measured and compared with standard. The mean of zones of inhibition in a group of plates has been compared in Table 4. It shows that nicotinamide has antimicrobial effects but this effect is less than mupirocin, this difference is statistically significant (independent t-test, \( P = 0.003 \)).

**Table 1: Composition of 6 formulation of nicotinamide sticks**

<table>
<thead>
<tr>
<th>Ingredient (mg%)</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearic acid</td>
<td>3.5</td>
<td>4.2</td>
<td>4.2</td>
<td>5.2</td>
<td>6.2</td>
<td>6.8</td>
</tr>
<tr>
<td>NaOH*</td>
<td>0.7</td>
<td>0.5</td>
<td>0.7</td>
<td>0.83</td>
<td>1.02</td>
<td>1.35</td>
</tr>
<tr>
<td>1-2 propan-diol</td>
<td>84.8</td>
<td>84.1</td>
<td>84.1</td>
<td>83.04</td>
<td>82.02</td>
<td>81.1</td>
</tr>
<tr>
<td>Purified water</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>6.9</td>
<td>6.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

\*14% NaOH solution was used in this experiment but the pure NaOH is used in the table of values

**Table 2: Physical evaluation of medicated stick**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Transparency</th>
<th>Surface feature</th>
<th>pH*</th>
<th>Spread ability</th>
<th>Drug content%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Transparent</td>
<td>Solid</td>
<td>9</td>
<td>++</td>
<td>96.56</td>
</tr>
<tr>
<td>A2</td>
<td>Transparent</td>
<td>Semi solid</td>
<td>7.8</td>
<td>0</td>
<td>96.39</td>
</tr>
<tr>
<td>A3</td>
<td>Transparent</td>
<td>Solid</td>
<td>8.2</td>
<td>++</td>
<td>96.39</td>
</tr>
<tr>
<td>A4</td>
<td>Transparent</td>
<td>Solid</td>
<td>8.6</td>
<td>+</td>
<td>95.1</td>
</tr>
<tr>
<td>A5</td>
<td>Transparent</td>
<td>Solid</td>
<td>9.0</td>
<td>+</td>
<td>94.8</td>
</tr>
<tr>
<td>A6</td>
<td>Transparent</td>
<td>Solid</td>
<td>10.5</td>
<td>+</td>
<td>93.3</td>
</tr>
</tbody>
</table>

\*Each reading is an average of three determinations; *This pH is measured for 10% concentration

To evaluate the anti-bacterial effect of nicotinamide stick, well plate method was used. In this method the plate including base and seed layer were prepared so created wells. 10 plates were prepared whit three wells in each plate. The suitable amount of nicotinamide as a sample and mupirocin as blank about 20 μg put in the wells and the plates were incubated for 24 h in 37°C. The zones of inhibition was measured in mm and recorded.

Short-term stability studies on the selected formulation (A3) were carried out by storing the sticks at room temperature for a period of 3 weeks. At intervals of 1 week, the sticks were examined for drug content uniformity and any physical change.

The *in-vitro* drug release was carried out for A3 formulation in pH 6.8 phosphate buffer over a period of 160 min.

**RESULTS**

Evaluation of A1-A6 formulation of medical sticks is presented in Table 2.

Table 3 shows *in vitro* drug release of nicotinamide in pH 6.8 phosphate buffer.

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Nicotinamide stick in the treatment of acne

In this study, we produced a model of nicotinamide stick with characteristics that are mentioned and evaluated the absorption of the nicotinamide stick in lab. We evaluated the antimicrobial effect of this product in the laboratory (using eradication of S. aurous as a germ that causes acne) and compared it with mupirocin. Hence we suggest designing a study and evaluating this product in group of patients who are suffering from acne. It supposed to studied clinical efficacy of this product and compare with other products on the market. We can add salicylic acid to the formulation to increase effectiveness[17]. If the results were acceptable, this form can be added to drug list and used it to topical medication for most product.

AUTHORS’ CONTRIBUTIONS

All authors have contributed in designing and conducting the study. All authors have assisted in preparation of the first draft of the manuscript or revising it critically for important intellectual content. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

REFERENCES


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