SHORT COMMUNICATION

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*

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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 aurous* 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. aurous* (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

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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 10^8 CFU/ml. This microbial suspension was diluted to 10^5 CFU/ml (1 mm of 10^n CFU/ml *S aurous* suspension was added to 9 ml nutrient agar and made 10^{n1} 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 10^4 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 after suitable dilution with methanol. The drug content was calculated according to the standard calibration curve. To find the mean percent, calculating the average of three determinations was done. To evaluate the *in-vitro* drug diffusion a cellulose membrane was fixed at one end of the cylinder. Stick of nicotinamide was taken on the cellulose membrane on the cylinder. Samples were withdrawn at specified intervals over a period of 160 min and analyzed for drug diffusion by measuring the absorbance at 263 nm.

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.

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

Ingredient (mg%)	Formulation code					
	A1	A2	A3	A4	A5	A6
Stearic acid	3.5	4.2	4.2	5.2	6.2	6.8
NaOH*	0.7	0.5	0.7	0.83	1.02	1.35
1-2 propan-diol	84.8	84.1	84.1	83.04	82.02	81.1
Purified water	7	7	7	6.9	6.8	6.8
Nicotinamide	4	4	4	4	4	4

*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

Formulation code	Medicated stick#				
	Transparency	Surface feature	pH*	Spread ability	Drug content%
A1	Transparent	Solid	9	++	96.56
A2	Transparent	Semi solid	7.8	0	96.39
A3	Transparent	Solid	8.2	++	96.39
A4	Transparent	Solid	8.6	+	95.1
A5	Transparent	Solid	9.0	+	94.8
A6	Transparent	Solid	10.5	+	93.3

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

Table 3: *In vitro* drug release of nicotinamide in pH 6.8 phosphate buffer

Time (min)	% drug released A3			Average drug release%	
	1+	2	3	Mean	Mean ± SD
00	0	0	0	0	0
5	42.6955	49.8045	23.14573	38.54858	38.54858±1.62
10	53.84479	93.89218	46.79502	64.844	64.844±0.82
20	92.92062	105.9775	66.40403	88.43404	88.43404±1.70
30	91.72393	113.4419	92.62441	99.26343	99.26343±0.47
40	74.93483	116.1671	105.9182	99.00671	99.00671±1.81
50	90.72867	108.7855	93.18128	97.56517	97.56517±0.82
60	70.30213	109.6031	107.3164	95.74052	95.74052±1.86
80	89.15284	73.3827	105.314	89.28318	89.28318±1.34
100	99.40166	95.27844	86.74763	93.80924	93.80924±0.54
120	84.4372	81.83057	77.24526	81.17101	81.17101±0.30
130	88.90403	90.24289	75.8827	85.00987	85.00987±0.66
140	77.6955	77.74289	76.91351	77.45063	77.45063±0.03
150	81.9372	73.76185	75.1718	76.95695	76.95695±0.36
160	69.65047	95.68128	88.61967	84.65047	84.65047±1.13

SD = Standard deviation

Table 4: Comparison of zone of inhibition between nicotinamide and mupirocine

Anti-biotic	Zone of inhibition (mm)	*P* value
Nicotinamide	15.4 (±3)	0.003
Mupirocin	19.6 (±4)	

Values have expressed as mean ± standard deviation; mm = Millimeter

DISCUSSION

In this study, we produced stick of nicotinamide and we evaluated this product in eradication of *S. aurous*. The best materials were chosen, which were compatible with skin acne. As it is mentioned in the text, the absorbance and releasing of the nicotinamide were examined in the laboratory. The hardness of this stick was a challenging point and some ingredients were used as hardeners, which are explained above.

Sticks have some good properties such as being portable, easy to use and change the dosage of administration for external use and applied directly to the affected site. [14]

Soft sticks are made of some ingredients as the base, which are different in melting points. Sometimes some of them with a high melting point are added as a hardener. Nicotinamide provide potent anti-inflammatory and anti-bacterial activities without the risk of inducing bacterial resistance while it has lightening effect. These materials have no side-effects on the skin and don't cause the sebaceous gland clogged. We used mupirocin as standard because it is wide anti-microbial affect and it is affected on *S. aurous*. It is noted in some articles. [15]

In addition some of the studies have compared clindamycin phosphate 1% and nicotinamide 4% gel in the treatment of acne and the result show that they are both similar in efficacy.[15,16]

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. After that we evaluated the antimicrobial effect of this product in the laboratory (using in 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.

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