

Topical Nanoemulsion of Rifampicin with Benzoic Acid and Salicylic Acid: Activity Against *Staphylococcus aureus*, *Stap. epidermis* and *Candida albicans*

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(Received: May 23, 2018; Accepted: July 5, 2018; Published: January 17, 2019)

Abstract

The aim of this study was to formulate a topical nanoemulsion which can be used to treat both bacterial and fungal infections of the skin. Eight self-emulsifying drug delivery systems composed of different combinations of rifampicin (1%), benzoic acid (6%) and salicylic acid (3%) were formulated and designated as F1, F2, F3, F4, F5, F6, F7 and F8. Nanoemulsions of the eight formulations were prepared using oleic acid, Tween 80 and water by sonication method. Antibacterial and antifungal effects were observed against *Staphylococcus aureus*, *Stap. epidermis* and *Candida albicans* by the Kirby-Bauer method. The nanoemulsion containing only rifampicin showed strong activity against *S. aureus* and *S. epidermis*, but not against *C. albicans*. The nanoemulsion containing both benzoic acid and salicylic acid showed better activity in *C. albicans*, but not against *S. aureus* and *S. epidermis*. However, the nanoemulsions consisting of all three active ingredients showed significant results against *S. aureus*, *S. epidermis* and *C. albicans*.

Key words: Nanoemulsion, rifampicin, benzoic acid, salicylic acid, *S. aureus*, *S. epidermis*, *C. albicans*

Introduction

Skin microflora consists of varied microorganisms which are usually either commensal or mutualistic to the host. An improved understanding of the skin microflora helps us acquire insight into the involvement of microorganisms in causing skin disorders and enables us to devise novel therapeutic approaches for cure. Some of the microorganisms which normally reside on the skin are *Staphylococcus* spp., *Propionibacterium* spp., *Corynebacterium* spp., *Malassezia* spp., *Candida* spp., *Debaryomyces* and *Cryptococcus* spp. (Grice *et al.*, 2011). These microbes can become pathogenic if they are able to adhere to the host, invade and multiply. Some common bacterial and fungal skin infections are acne, impetigo, folliculitis, dermatophytosis, candidal skin infections, nail fungus and seborrheic dermatitis (Chiller *et al.*, 2001; Bryant, 2014).

Since, rifampicin is a poorly water-soluble drug; penetration of the bacterial cell wall with conventional topical formulation is relatively difficult. In our previous studies, two novel drug delivery systems were formulated to topically deliver rifampicin in targeting acne (Begum *et al.*, 2015(a); Begum *et al.*, 2015(b)). Rifampicin was selected as the drug of choice because in an assay, the bacterial flora present in acne lesions has displayed highest susceptibility to it (Zaluga *et al.*, 1996). It is a wide spectrum antibiotic active at low concentrations against mycobacteria and gram-positive organisms (Wehrli, 1983). These new and improved delivery systems control the release of the active entity to the specific site of action for reduced toxicity and increased efficacy (Biju *et al.*, 2006). The first study was on rifampicin niosome which was a vesicular formulation encapsulating the drug within

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unilamellar vesicles. Self-emulsifying drug delivery system of rifampicin (SEDD-R) was the focus of our second study which was prepared using oleic acid and different surfactants. Drug content, entrapment efficiency; *in vitro* drug release, pH stability and antibiotic sensitivity studies were performed for both delivery systems. The results for SEDD-R were comparatively better than that for rifampicin niosomes (Begum *et al.*, 2015a; Begum *et al.*, 2015b). Hence, nanoemulsion was selected as the mode of delivery for this study.

Superficial dermatological infections can range from both bacterial to fungal in origin. So, we wanted to incorporate an antifungal component with the rifampicin nanoemulsion for wide spectrum of activity. Some widely used topical antifungal preparations are creams, lotions and ointments containing azoles, allylamines, polyenes, benzoic acid and salicylic acid (Poojary, 2017). Whitfield's ointment is the oldest antifungal preparation containing benzoic acid and salicylic acid, which is currently replaced by newer preparations (Russell *et al.*, 1992). Although, it might be used for some superficial bacterial infections, it is far more effective as an antifungal agent. Currently, no commercial product(s) are available in the form of nanoemulsion which can target both bacterial and fungal skin infections. Therefore, our study has been devised to develop a novel antibacterial and antifungal preparation containing rifampicin together with benzoic acid and salicylic acid which can resolve most mild to moderate skin infections.

Materials and Methods

Organisms: *S. aureus* (AC1) and *S. epidermis* (AC3) were obtained from laboratory stock isolated from acne patients and identified in previous study (Khan *et al.*, 2015) used as test organisms. *S. aureus* (ATCC 25923) and *S. epidermis* (ATCC 12228) were obtained from laboratory stock used as reference strains. *C. albicans* strain was isolated from clinical patient and collected from the Microbiology Lab at Bangabandhu Sheikh Mujib Medical University (BSMMU).

Drugs and chemicals were collected from the respective sources. All chemicals were of analytical grade. Instruments used were analytical balance (Boeco, Germany), ultrasonic bath (Sonoswiss, Switzerland), vortex mixer VM-2000 (Digisystem, Taiwan), incubator (JSGI series, Korea), autoclave sterilizer (JSAC-40, Korea) and laminar flow cabinet (Esco, Singapore).

Formulation and preparation of different nanoemulsions: Nanoemulsions were prepared by sonication method with some modifications (Begum *et al.*, 2015b). Eight nanoemulsions consisting of different combinations of rifampicin (1%), benzoic acid (6%) and salicylic acid (3%) were formulated using oleic acid, Tween 80 and water. They were designated as F1, F2, F3, F4, F5, F6, F7 and F8. The exact measurements of all the constituents used in preparing the different nanoemulsions have been outlined in table 1.

Preparation of F1 nanoemulsion: F1 was prepared using 1% of rifampicin along with the excipients including oleic acid, Tween 80 and distilled water. Rifampicin was measured and transferred to oleic acid in 20 ml screw cap vial. Tween 80 was measured and taken in 100 ml glass bottle. The distilled water was taken in 100 ml glass bottle. All three containers were placed in ultrasonic water bath and heated at 70°C. This was continued until rifampicin dissolved completely to leave a clear solution. Once all three containers attained the same temperature (70°C), rifampicin dissolved in oleic acid was added to Tween 80 in the 100 ml glass bottle. This was then followed by gradual addition of distilled water to this mixture along with occasional shaking using vortex. This was continued until cloudy nanoemulsion was formed. The nanoemulsion was then cooled to room temperature and stored between 4°C to 10°C.

Preparation of F2 nanoemulsion: F2 was prepared using 6% of benzoic acid along with the same excipients. In this case, benzoic acid was added to Tween 80 instead of oleic acid. All three containers were placed in ultrasonic bath to attain the same temperature. The oleic acid was added to

Tween 80 once benzoic acid has dissolved in it. Rest of the procedure was the same as before.

Preparation of F3 nanoemulsion: F3 containing 3% salicylic acid was prepared in the exact same manner as F2 excepting that, salicylic acid was used instead of benzoic acid. The same steps were followed.

Preparation of F4 nanoemulsion: In case of F4, the measured amount of benzoic acid and salicylic acid was added to Tween 80. Then oleic acid was added to Tween 80 once these active ingredients have dissolved in it. Rest of the procedure was the same as before.

Preparation of F5 nanoemulsion: In F5, rifampicin was added to the container containing oleic acid. Benzoic acid was added to Tween 80. Once all the drugs have dissolved, the same steps were followed.

Preparation of F6 nanoemulsion: The procedure followed for preparing F6 was the same as for F5. Only that here salicylic acid was added to Tween 80 instead of benzoic acid.

Preparation of F7 nanoemulsion: In F7, rifampicin was added to the container containing oleic acid. Benzoic acid and salicylic acid were added to Tween 80. Once all the drugs have dissolved, the same steps were followed.

Preparation of F8 nanoemulsion: F8 which contains no drug in the formulation was used as control in the study. Once all the containers attained the same temperature, the same procedure was followed.

Antibacterial activity test: Mueller Hinton agar was used for determining the effectiveness of the different nanoemulsions against the bacterial strains by Kirby-Bauer method (Bauer *et al.*, 1966). A cell suspension of each of the bacterial samples was prepared in sterile saline. Approximate cell density of 5×10^6 cfu/ml was present in the suspension which was determined by optical density (OD). AC1, AC3, *S. aureus* (ATCC 25923) and *S. epidermis* (ATCC 12228) strains were each spread uniformly on the surface of the medium in different plates using sterile swab. Holes were made using the cork borer in the

agar plate. 20 μ L of each of the nanoemulsion was added to each hole using sterilized pipettes. The agar plates were then incubated at 37°C for 24 hours. Diameter of the zone of inhibition was recorded in millimeters and average of duplicate tests was taken.

Antifungal activity test: Sabouraud's dextrose agar was used for determining the antifungal activity of the nanoemulsions against *C. albicans* by the Kirby-Bauer method (Bauer *et al.*, 1966). The cell suspension having an approximate cell density of 10^5 cfu/ml was determined by optical density. Just as for bacteria, the same steps were followed. However, these plates were incubated at 30°C for 48 hours. The diameter of the zone of inhibition was recorded accordingly.

Results and Discussion

Topical preparations in the multiphase systems available in the market are ointments, creams, gels and pastes. Newer topical formulations are anhydrous gel formulations, transferosomes, microemulsion gel, liposomes and nanoparticles (Wolrabb, 2016). Nanoemulsions are colloidal dispersions consisting of an oil phase, an aqueous phase, and surfactant at the appropriate ratios. These nano-sized droplets offer huge interfacial areas allowing improved transdermal delivery of the active entities. The merits associated with nanoemulsions are, enhanced drug solubilizing capacity, increased shelf life, easy preparation and enhanced bioavailability of both hydrophilic and hydrophobic drugs (Sharma *et al.*, 2013). Combination preparations offer increased clinical efficacy through cumulative effect and improved patient compliance. Most of these combination preparations are available in the form of creams or ointments (Wolrabb, 2016). However, only a handful of combination topical preparations are available for treatment of skin infections. Hence, nanoemulsion was chosen as the delivery system for combined antibacterial and antifungal preparation.

In this study, eight nanoemulsions were prepared using different combinations of rifampicin (1%), benzoic acid (6%) and salicylic acid (3%), and designated as F1, F2, F3, F4, F5, F6, F7 and F8

(Table 1). Antibacterial and antifungal activities were determined by the size of the zone of inhibition as shown in figures 1 and 2. F1 (1% rifampicin) nanoemulsions showed significant results against AC1, AC3, *S. aureus* (ATCC 25923) and *S. epidermis* (ATCC 12228) strains, but had no effect on *C. albicans*. This reconfirms that the nanoemulsion containing rifampicin is a promising agent for targeting acne due to increased bacterial resistance to conventional topical antibiotic preparations (Begum et al., 2015b). Only F7 and F4 nanoemulsions displayed positive results against the bacterial strains and *C. albicans*. The zone of inhibition obtained for F7 against the strains *S. aureus*, *S. epidermis* and *C. albicans* were greater than 25mm. On the contrary, F4 depicted better

results against *C. albicans*, but not the bacterial strains (Figure 1). F2, F3, F5 and F6 nanoemulsions failed to show significant activities against either bacterial or fungal strains. However, F8 had no effect on any of the test organisms, proving that the excipients have no therapeutic effect (Figure 2).

The aim of the study was to design such a formulation which would act on multiple microbes. The active ingredients were encapsulated within the nano droplets dispersed in an aqueous phase. The choice of an oily phase here was very significant since it influences drug loading. These droplets are then further stabilized by the surfactant molecules (Porras et al., 2004). The excipients oleic acid and Tween 80 together with distilled water helped to formulate this nanoemulsion. Rifampicin present in

Table 1. Formulation of different nanoemulsions.

Ingredients	Formulations (mass in g)							
	F1	F2	F3	F4	F5	F6	F7	F8
Rifampicin	1.0	-	-	1.0	1.0	-	1.0	-
Benzoic acid	-	6.0	-	6.0	-	6.0	6.0	-
Salicylic acid	-	-	3.0	-	3.0	3.0	3.0	-
Oleic acid	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Tween 80	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Distilled water	49.0	44.0	47.0	43.0	46.0	41.0	40.0	50.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

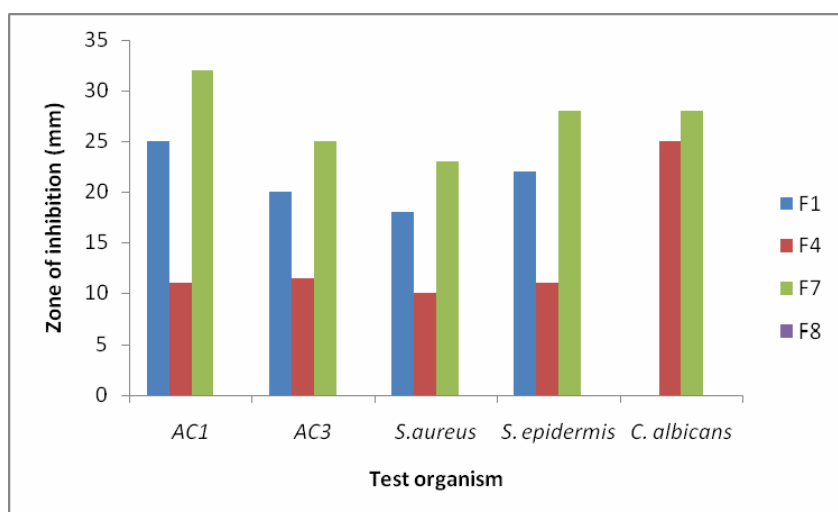


Figure 1. Zone of inhibition for the different nanoemulsion preparations for each test organism.

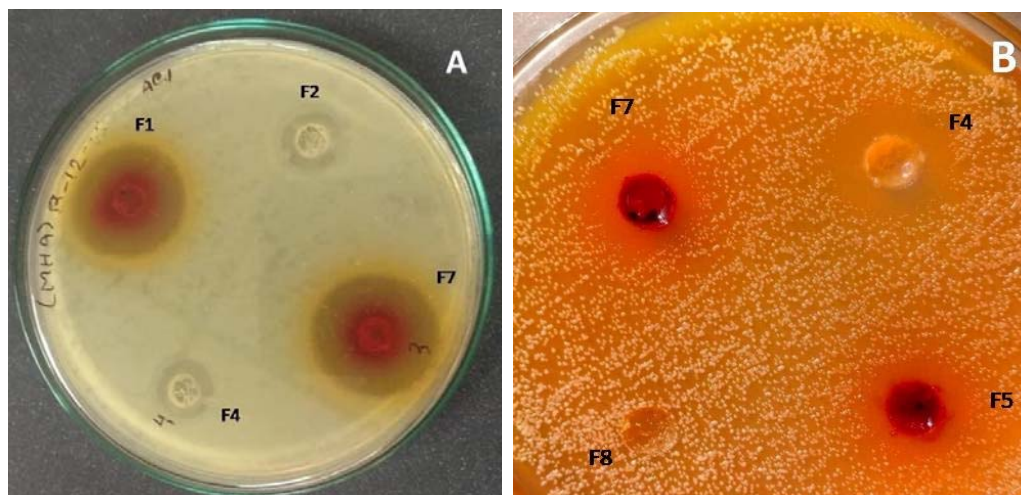


Figure 2. Inhibitory effect of the different nanoemulsions (F1, F2, F4, F5, F7 and F8) on (A) AC1 and (B) *C. albicans*.

the formulation specifically inhibits bacterial RNA polymerase by forming a stable drug-enzyme complex which in turn prevents DNA transcription (Wehrli, 1983). Therefore, F1 is effective against *S. aureus* and *S. epidermis*, but not against *C. albicans*. Benzoic acid acts as a fungistatic which causes ATP depletion in the microbial cells with the accumulation of comparatively high levels of benzoate (Warth, 1991). Salicylic acid acts as a keratolytic which is mildly anti-inflammatory and antipruritic (Furman, 2018). Hence, F4 is effective against bacteria, but mostly functional against *C. albicans*. The findings from this study confirm that the formulation F7 containing rifampicin, benzoic acid and salicylic acid can act as an effective topical remedy for both bacterial and fungal skin problems.

Conclusion

Nanoemulsion containing rifampicin along with benzoic acid and salicylic acid showed satisfactory results against *S. aureus*, *S. epidermis* and *C. albicans* and have the potential to be developed into commercial product(s). This formulation could also be tested on other microorganisms causing skin infections. However, clinical studies need to be conducted to determine its efficacy and to optimize its future applications. Therefore, it can be concluded that this formulation has potential to act as

a therapeutic agent for dermatological infections caused by both bacteria and fungi.

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