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# Comparison between Alpha and Silver Sulfadiazine ointments in treatment of *Pseudomonas* infections in 3rd degree burns

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## KEYWORDS

Burn infection;  
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Wound infection;  
Rats;  
3rd Degree burn;  
*Pseudomonas aeruginosa*

**Abstract** The goal of this study was to evaluate the efficacy of Alpha ointment in the treatment of burn wounds and compare its results with silver sulfadiazine (SS). Similar burn ulcers were produced on anterior surface of thigh of 60 rats. The wounds were infected with *Pseudomonas aeruginosa* and dressing and debridement was performed daily. The first group of rats received topical SS, the second group received Alpha ointment and the third (control group) received no medication. Wound healing, contraction, culture, and scar formation were evaluated at the end of the second and 10th week. Alpha ointment was equally effective as SS, considering wound healing and contraction. Wound infection was significantly less common in Alpha ointment group compared to the other two groups ( $p < 0.05$ ). Alpha ointment is a less expensive drug with an acceptable result compared to SS. Therefore, we recommend it as an alternative to SS, especially in patients with low economical backgrounds or in those who show adverse reactions to SS.

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## Introduction

3rd Degree burns, which are the most severe form of burn, usually need dressing with appropriate medications in order to prevent infection.<sup>1–3</sup> This

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topical treatment should reduce bacterial proliferation, most commonly caused by *Pseudomonas* species, and prevent resistant sepsis.<sup>4,5</sup> Several groups of topical medications and antibiotics have been used in the treatment of wound infection of burn ulcers, e.g. mefenamide, silver sulfadiazine, neomycine, polymyxine B, etc.<sup>4,6,7</sup> Silver sulfadiazine, one of the most commonly used topical antibiotics, is effective in the treatment of common bacterial wound infections, including *Pseudomonas* and *Proteus* infections.<sup>8,9</sup> It significantly reduces the time needed for the treatment of deep burn wounds.<sup>10</sup> However, it still carries a high risk of resistance and may cause transient leukopenia secondary to bone marrow suppression.<sup>2</sup>

Concerns on problems such as high cost of treatment, increasing resistance to antibiotics, and inability to restore initial appearance of skin resulted in researches on newer agents for the treatment of burn wounds.<sup>2</sup> Alpha is an ointment with active ingredient of Lawsonia, available in *Lawsonia inermis* plant (from henna Lythraceae *intermis*). This ointment used in 30-g packages in Iran is cheaper (approximately one-fourth the cost of SS) than other topical antibiotics and has very few acceptable adverse reactions.<sup>11,12</sup> The aim of this study was to examine the efficacy of Alpha ointment as a bactericidal agent, its ability of wound healing and compare it with silver sulfadiazine ointment in the treatment of burn wounds in rats as an experimental model.

## Methods

In a randomized clinical trial, 60 rats with equal mean weights were divided into three equal groups. A standard 3rd degree burn wound was produced on the anterior surface of thigh of the rats under general anesthesia with ether. The burn wound was induced after shaving the area with a hot plate with same size (1 × 1.5 cm) and at an equal temperature (heated up to 156 °F or 69 °C and placed for 3 s on the skin).<sup>13</sup> Each rat was placed in an isolated cage to inhibit transmission of infection. The wounds were examined after 24 h and if necrotic tissue was observed, it was removed. Debridement procedure in the standard way was done for all the animals. Then the wounds were inoculated with *Pseudomonas aeruginosa*. All of the rats were infected at the same time with the same samples. Weight of the rats were measured in the beginning of the study and on one, two and 10 weeks thereafter.

This study was approved by the ethical committee of Shiraz University of Medical Sciences and the

Principles of laboratory animal care (NIH publication No. 86-23, revised 1985) were followed.

Exotoxin-A producing *P. aeruginosa* species were obtained from the infected wounds of patients who were admitted to the burn department of Ghotb-el-din Hospital, a burn care-center affiliated to Shiraz University of Medical Sciences. Cultures of these infected wounds were documented as positive with *P. aeruginosa*. The species were cultured in blood agar medium and incubated at 37 °C for 48 h. Some laboratory tests such as TSI, oxidase and citrate were performed to confirm the bacterial species. To determine the exotoxin-A producing properties of *P. aeruginosa* they were cultured in liquid medium and then centrifuged and the supernatant was concentrated 20 times. Then the Ouchterlony test was performed to confirm the exotoxin producing properties of the species. The number of bacteria in each tube was adjusted to 0.5 McFarland (equal to  $1.5 \times 10^8$  bacteria/cm<sup>3</sup>). Each rat received injection of the bacterial preparation in volume equal to  $15 \times 10^6$  bacteria. The bacterial species were injected subcutaneously into the burn wound. Twenty-four hours after the injection, wounds of the first group were dressed with silver sulfadiazine ointment (Sina Daru, Tehran, Iran). In the second group the ulcers were dressed with Alpha ointment (Sina Daru, Tehran, Iran), and in the third group, no ointment was utilized. The dressings were changed daily and debridement was performed if needed in all the groups in a similar manner.

Clinical evaluation of the wound, including size, existence of satellite lesions, contraction of the wounds, and crust formation, was performed on the second and 10th week. The wounds were classified according to their characteristics (grade 1: clean; grade 2: color change; grade 3: color change and nodules around the wound; and grade 4: clear pussy discharge). In addition to the clinical evaluation, the wounds were cultured in blood agar medium and placed in a 37 °C incubator. They were checked after 48 h for the presence of *P. aeruginosa* using different laboratory tests.

The obtained data were analyzed by SPSS Win 10.0 software. Student *t*-test, Chi-square, and ANOVA test were used to compare the study groups and the significance level was determined as less than 0.05.

## Results

Results of wound grading among the different groups of rats at the end of the second week are shown in Table 1. Alpha ointment significantly

**Table 1** Grading of the wounds, scar formation, positive culture and contracture of the studied rats in the three groups after two and 10 weeks

		SS	Alpha	p-Value*	Control	p-Value**
Second week	Grade 1, n (%)	4 (20)	4 (20)	NS	1 (5)	<0.05
	Grade 2, n (%)	7 (35)	12 (60)	<0.05	4 (20)	<0.05
	Grade 3, n (%)	5 (25)	1 (5)	<0.05	0 (0)	<0.05
	Grade 4, n (%)	4 (20)	3 (15)	NS	15 (75)	<0.05
	Positive culture	5 (25)	4 (20)	NS	8 (40)	<0.05
10th Week	Grade 1, n (%)	11 (55)	14 (70)	NS	9 (45)	<0.05
	Grade 2, n (%)	1 (5)	2 (10)	NS	2 (10)	NS
	Grade 3, n (%)	0 (0)	0 (0)	NS	0 (0)	NS
	Grade 4, n (%)	8 (40)	4 (20)	<0.05	9 (45)	<0.05
	Scar formation	6 (30)	8 (39)	NS	5 (26)	<0.05
	Contracture	15 (75)	20 (100)	<0.05	19 (95)	NS

The data are presented in percentage. SS: silver sulfadiazine; NS: not significant; \*Comparison between SS group and Alpha group; \*\*Comparison between the rats receiving Alpha ointment and control group.

Classification of the wounds: grade 1: clean; grade 2: color change; grade 3: color change and nodules around the wound; and grade 4: clear pussy discharge.

decreased the rate of wound infection, positive culture and scar formation compared to control group ( $p < 0.05$ ) (Table 1). A decrease in weight was observed in the first week and an increase in the second week. Weight was similar in all the three groups. Compared to SS group, Alpha ointment group revealed similar results concerning wound infection, positive culture and scar formation. However, contraction was significantly less common in the SS group compared to the control and Alpha ointment groups ( $p < 0.05$ ) (Table 1).

Among the studied rats, two rats in the second group and one in the third group died. Grading of the wounds, scar formation, positive culture rate and contracture rate of the studied rats in all the three groups after two and 10 weeks are shown in Table 1.

## Discussion

In this study Alpha ointment group revealed similar therapeutic and prevention results compared to silver sulfadiazine. A wide variety of substances have been reported to be useful in the treatment of infected burn wounds, e.g. topical phenytoin,<sup>14</sup> honey,<sup>15,16</sup> proflavine,<sup>17</sup> etc. Among the different topical medications, mefenamide is one of the most commonly studied ointments that had some advantages over the other drugs. Unfortunately, this drug was excluded from the market because of its frequent side effects. Silver sulfadiazine is a topical antibacterial ointment that is used widely for the prevention and treatment of burn wounds<sup>17</sup> and must be used every 24 h to be effective.<sup>18</sup> This ointment can penetrate into scars and has

antimicrobial effects.<sup>19</sup> It has been reported that this ointment is effective in treating *Pseudomonas* infections.<sup>20</sup>

Henna leaf (*L. inermis*) is commonly known as Hanna in Iran and Mehndi in India. It is a very popular natural dye to color hand and hair and is an integral part of indigenous culture. It does not permanently dye hair and hands when used right on the very outer layer of the skin, but it might result in a permanent tattoo-like effect in deeper uses. Henna is also known for its medicinal values. Alpha ointment is a new pharmacological preparation in Iran that in traditional medicine has been used in skin diseases, boils, burning sensation in feet and burns.<sup>21</sup> Although it has been used in some burn centers its efficacy in treating wound infection or repair has not been systematically studied. Additionally, there have been only a few studies on Alpha drug and its efficacy in the treatment and prevention of wound infection in burn patients.<sup>21</sup> We observed that this ointment (and its effective component *L. inermis*) effectively stopped bacterial growth and induced healing of burn wounds comparable to SS. In a study performed by Habbal et al. the antimicrobial activity of henna's fresh and dry leaves and seeds obtained from Oman against three standard bacterial strains namely *Staphylococcus aureus*, *Escherichia coli* and *P. aeruginosa* was evaluated. They observed that all fresh and dry leaves and seeds of the Omani henna demonstrated antibacterial activity against all the three standard strains.<sup>22</sup> On each episode of debridement and dressing, approximately 5 g of Alpha ointment is needed for every 1% of 3rd degree burned body surface area. As for SS, 10 g is needed for the same percent, degree and body surface

area. Fifty grams of Alpha ointment costs 2500 Rials (equivalent to 0.28\$). However, 50 g of SS costs 5000 Rials (equivalent to 0.56\$). Therefore, the cost of Alpha ointment is approximately one-fourth of SS and is economically beneficial, especially in our setting and also in other developing countries with a low socio-economical status.

Catania and King presented the results of an in vivo evaluation of silver sulfadiazine dry foam. Using burned guinea pigs infected with *P. aeruginosa*, they applied SS on the infected wound and concluded that SS had significant anti-*P. aeruginosa* properties.<sup>20</sup> In our study we proved that Alpha ointment has anti-*P. aeruginosa* properties similar to SS and therefore, may be used as an alternative drug with similar results.

In the third group in which no ointments were used, the results of wound healing and contraction were similar to the other two groups. Therefore, it can be concluded that daily debridement and change of dressing with irrigation is equally effective in the treatment of burn wound and preventing scar and functional disabilities.

Considering these results, it can be concluded that Alpha ointment may be a suitable alternative for SS. Additionally this ointment is more economical. It is suggested that further studies are to be done with larger sample sizes on human cases for a longer study period to thoroughly evaluate the efficacy of Alpha ointment on burn wounds in human.

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