STUDY OF THE ANTIBACTERIAL THERAPEUTIC EFFICACY OF JULIFLORINE, JULIFLORICINE AND A BENZENE INSOLUBLE ALKALOIDAL FRACTION OF *PROSOPIS JULIFLORA*

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SUMMARY: The antimicrobial alkaloids, juliflorine, julifloricine and benzene insoluble alkaloidal fraction of Prosopis juliflora*, have been studied for their therapeutic efficacy after topical application in produced superficial skin infection. Infection was produced by rubbing freshly isolated* Staphylococcus *aureus from human clinical specimen onto 9 cm^2 shaved skin. Various concentrations of juliflorine, julifloricine, benzene insoluble alkaloidal fraction and gentamicin (standard antibiotic) prepared in petroleum jell were applied onto infected areas. Juliflorine was found to be effective on Staphylococcal skin infection. Juliforine in 0.5%, 1%, and 2.5% concentrations were found to heal 25%, 50% and 100% lesions in two weeks and microbiological efficacy was found to be 16.66%, 33.33%, 58.33% and 91.66% with 0.1%, 0.5%, 1% and 2.5% concentrations of juliflorine. Julifloricine was found to be less effective than juliflorine and the benzene insoluble alkaloidal mixture was found comparatively more effective than juliflorine. Healing was slightly faster with the mixture. Both juliflorine and the mixture were found effective at 2.5% concentration, but these were also found toxic. Gentamicin was found more superior to the alkaloids in artificially produced skin infection.*

The study demonstrated that juliflorine and benzene insoluble alkaloidal fractions were effective in Staphylococcal skin infections. Generally dose related clinical and microbiological efficacy was noted but much less effective than standard antibiotic gentamicin and needs the development of more effective and non-toxic derivatives.

Key Words: Prosopis juliflora, staphylococcus aureus.

INTRODUCTION

The use of indigenous medicinal herbs for the treatment of diseases are actively practised in both Unani and Ayurvedic system of Indo-Pakistan subcontinent. Drugs derived from medicinal plants have served through the ages as the mainstay for the treatment of various diseases and human ailments. Throughout the world some 70% of the people rely on the traditional herbal remedies to cure a wide

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variety of ailments from minor infection to asthma, dysentery, malaria etc. Scientific analysis of medical plants has led to the discoveries of many important drugs (1,2).

In view of these facts, systemic investigations of medicinal plants were initiated in order to isolate the active principles of various plant materials to bring up new medically important substances, which could be used as effective therapeutic agents.

In large number of medicinal plants, the therapeutic value is due to the presence of alkaloids, which in certain respect ranks among the most interesting of the naturally occurring substances.

The genus *Prosopis* (mesquite) are known to possess medicinal value (3). *Prosopis juliflora* a shrub grows abundantly in Sindh and Punjab provinces of Pakistan (4). Juliflorine and julifloricine, the main alkaloids of *Prosopis juliflora*, have been isolated for the first time by Ahmad *et. al.* (5) and the antibacterial and antifungal activities were reported by Khan K.A. and Ahmad *et al.* (6-9). From *P. juliflora*, a benzene insoluble alkaloidal fraction (containing 2 major and 3 minor alkaloids) have also been isolated and reported to possess antibacterial and antifungal activities (10,11).

The present work deals with the study of the therapeutic efficacy of these alkaloids against artificially produced Staphyloccocal skin infection on rabbits. Efficacy of these alkaloids were also compared with an antibacterial antibiotic, the gentamicin.

MATERIALS AND METHODS

Rabbits (Lipus capensis-local) with body weight 1.5-2 Kg were used in this study. Animals were kept in animal house at 25-30°C. 1%, 2.5% and 5% solution of juliflorine, julifloricine and a benzene insoluble alkaloidal fraction were prepared in PEG-400. 0.1 ml of each concentration in triplicate was applied locally on either side of the shaved surface of the shoulder for seven successive days. The applied areas of the skin was examined daily in comparison with the control animals and clinical response if any was recorded.

Ointments of the alkaloids in the following concentrations were prepared in petroleum jelly.

1. Juliflorine 0.1%, 0.5%, 1% and 2.5%.
2. Julifloricine 0.1%, 0.5%, 1% and 2.5%.
3. Benzene insoluble alkaloidal fraction 0.1%, 0.5 %, and 1%.
4. Gentamicin 0.1% and 0.5%.

The above preparations were on the infected skin of the rabbits.

Infective material: In order to study the effectiveness of the alkaloids *in vivo* dermal infection using Staphylococcus aureus (clinical isolates) were produced in rabbits. The inocula of the culture was prepared as following.

To prepare *Staphylococcus* aureus inoculum, the culture was grown in Tryptone soya broth (Oxoid). Growth was adjusted to approximately 10^7-10^8 CFU per ml by comparing with Mc Farland Index (12).

Artificial Infection: The shoulder, hips and back of rabbits were first shaved with razor and the area was disinfected with alcohol. The 0.1 ml of bacterial inoculum was applied and rubbed on the skin in area of 9 cm^2 with glass rod. The infected rabbits were kept at room temperature for 3-7 days. The infection was confirmed by microscopy and culture.

Therapeutic trials: The alkaloidal formulation was applied locally with spatula on the infected area of the skin of two rabbits with bacterial infection. Two rabbits were also kept as control and compared with those under treatment.

Bacteriological Evaluation: At the end of each week treatment the 6 clinical samples from each animal were collected by sterile swab and inoculated on Mannitol salt agar (Oxoid) plates. The effectiveness of alkaloids in reducing the number of possitive swab sample per treated group is expressed as percentage of that in the untreated control group.

Effectiveness = 100 - (T x 100)/K %

Where T = Average number of possitive swab samples in the particular group tested.

K = Average number of possitive swab samples in the control group.

Clinical Evaluation: The degree of local change in each animal was noted on 3rd and 11th days after infection. The effectiveness of the alkaloids where assessed on 11th, 18th and 25th day after infection and expressed as percentage of the degree of infection in the untreated group.

Table 1: Study of the dermal toxicity of Juliflorine, Julifloricine and a Benzene insoluble alkaloidal fraction of Prosopis juliflora in rabbits.

Concentrations in PEG-400		Days						
		1ST	2ND	3RD	4TH	5TH	6TH	7TH
Juliflorine	PEG-400	No effect	No effect	No effect	Slight Hardness	Slight Hardness	Hardness	Hardness
	1%	No effect	No effect	No effect	Slight Hardness	Slight Hardness	Hardness	Hardness
	2.5%	No effect	No effect	Slight Hardness with irriation	Hardness with irritation	Hardness with irritation	Toughness of skin with itching	Troughness of skin with itching
	5%	No effect	Hardness of skin with inflammation	More severe irritation with inflammation	Tough crusted inflamed skin	Irritation with bleeding skin	Tough crust with bleeding	Laceration with bleeding
Julifloricine	1%	No effect	No effect	No effect	Slight Hardness	Slight Hardness	Hardness of skin	Hardness of skin
	2.5%	No effect	No effect	No effect	Slight Hardness	Slight Hardness	Hardness of skin	Hardness of skin
	5%	No effect	No effect	Slight Hardness	Hardness	Hardness	Slight toughness	Slight toughness
Benzene insoluble alkaloidal fraction	1%	No effect	No effect	Slight Hardness of skin	Slight Hardness of skin	Hardness with reddening	Hardness with reddening	Inflammation
	2.5%	No effect	Slight Hardness	Hardness reddening inflammation	Hardness reddening inflammation	Hardness reddening inflammation	Hardness reddening inflammation	Hardness reddening inflammation
	5%	No effect	Hardness	Reddening	Degeneration of skin tissue	Severe degeneration suppuration with secondary infection	Severe degeneration suppuration with secondary infection	Severe degeneration suppuration with secondary infection

PEG = Polyethylene Glycol

RESULTS AND DISCUSSION

It is a well known fact that intensive use of antibacteries often followed the development of resistant strains (13). This propensity of drug resistance requires the search for new, effective and safe drugs.

All these alkaloids were applied locally in rabbits. 1% juliflorine in PEG-400 did not show any sig-

Table 2: The chemotherapeutic efficacy after topical application of ointment of Juliflorine, Julifloricine, a Benzene insoluble alkaloidal fraction and Gentamicin in bacterial model of Staphyloccus aureus in rabbits.

Eficacy							
Compounds		Clinical / healing %		Cultural %		Reinfection	Remark
		1 week	2 week	1 week	2 week		
Control		0	0	0	0		
Juliflorine	0.1 %	0	0	0	16.66		
	0.5 %	0	25	16.66	13.33	+	
	1.0 %	25	50	50	58.33	+	a
	2.5 %	62.5	100	66.66	91.66	+	a
Julifloricine	0.1 %	0	0	0	0		
	0.5 %	0	0	0	0		
	1.0 %	0	25	16.66	25	+	
	2.5 %	25	37.5	33.33	50	+	
Benzene insoluble alkaloidal fraction	0.1 %	0	12.5	0	16.66		
	0.5 %	25	25	33.33	41.66	+	
	1.0 %	25	50	58.33	75	-	a
	2.5 %	87.5	100	91.66	100	-	b
Gentamicin	0.1 %	100	*	100	*	-	
	0.5 %	100	*	100	*	-	

* = Treatment discountinued.

a = Slight hardening of the skin.

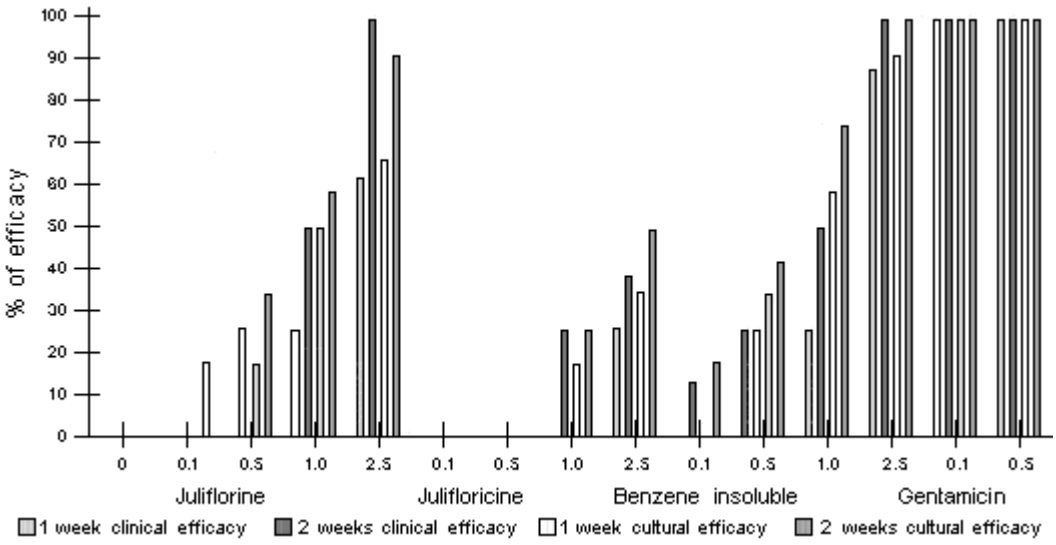
b = Skin became red and inflamed.

nificant toxicity but higher concentrations were found toxic (Table 1). Julifloricine showed no significant side effect except in 5% concentration which showed slight redness and hardening. The mixture was also applied locally and found safe at 1% concentration but 5% showed severe type of sensitivity reaction. Skin became inflammed and when it was discontinued the reaction subsided gradually to normal within 2-3 days (Table 1).

The juliflorine, julifloricine and benzene insoluble alkaloidal fraction were studied for their therapeutic efficacy in artificially produced superficial skin infection in rabbits. Infection was produced by rubbing freshly isolated culture of *S. aureus* on to 9 cm^2 shaved skin. Treatment was started after third day of infection with 0.1%, 0.5% and 2.5% of the alkaloids; and 0.5% Gentamicin prepared in petroleum jell. Each concentration was applied locally onto the infected sites of the rabbits. Clinical efficacy of the alkaloids were determined by healing of lesions; and microbiological efficacy by culture. Results for antibacterial therapeutic efficacy are shown in Table 2.

Topical application of juliflorine in concentration of 0.5%, 1% and 2.5% was found to heal 25%, 50% and 100% of the Staphylococcal infected lesions in two weeks (Figure 1). The microbiological efficacy of juliflorine to *S. aureus* infection was found to 16.66%, 33.33%, 58.33% and 91.66% in concentrations of 0.1%, 0.5%, 1% and 2.5%. In some cases reinfection was also noted after discontinuation of treatment. Julifloricine was found much less effective than juliflorine (Table 2). Benzene insoluble

Figure 1: Therapeutic efficacy of the alkaloids and gentamicin against Staphylococcal infection.



alkaloidal fration was found comparatively more effective than juliflorine. Healing was slightly faster than juliflorine and also recurrence of reinfection was nil at 1% and 2.5 % concentration. Juliflorine and benzene insoluble alkaloidal fraction were found effective at 2.5% concentration, but these concentrations were also found toxic (Table 1). The standard antibiotic, gentamicin was found much more superior to these alkaloids in artificially produced skin infections (Table 2).

The study shows that these alkaloids are effective in bacterial infections and generally dose related clinical and microbiological efficacies are noted. Juliflorine cured 25-100% lesions of Staphylococci in two weeks with 0.1-2.5% and microbiologically from 16.66-91.66% in Staphylococci infections.

Clinical examination shows that at high concentration the characteristic lesions heals or subsides. But continuous application of these alkaloids makes the skin sensitive which resulted in inflammation, induration etc. and the risk of secondary infection increases with resistant strains. In some subjects with low concentration of alkaloids, after discontinuation of treatment, reinfection was also observed. This could be their poor penetrability as *S. aureus* causes deep infection and juliflorine may not have penetrated deeper.

All these alkaloids have already been screened for mutagenicity by Ames test. They were found non-mutagenic up to 500 μg/plate (14). Although these alkaloids have shown some encouraging results but they need to undergo carefully controlled trials in order to evaluate their usefulness in daily medical practice.

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