



ISSN : 0974-8520



AYUJI

An International Quarterly Journal of Research in Ayurveda

Website : www.ayujournal.org

Volume 38 / Issue 1-2 / January-June 2017

Publication of
INSTITUTE FOR POST GRADUATE TEACHING AND RESEARCH IN AYURVEDA
Gujarat Ayurved University, Jamnagar, India

Medknow

 Wolters Kluwer

Evaluation of anti-inflammatory and antimicrobial activity of AHPL/AYTOP/0213 cream

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Abstract

Background: Acne vulgaris is almost a widespread disease occurring in all races. *Propionibacterium acnes* initiate acne and inflammatory mediators aggravate it. Conventional therapies for acne include comedolytic, anti-inflammatory, and anti-biotic agents. Due to adverse effects of these therapies, people are searching for alternative options. In this context, a polyherbal formulation AHPL/AYTOP/0213 cream was developed for the treatment of Acne. **Objective:** The objective of this study is to study anti-inflammatory and antimicrobial activities of AHPL/AYTOP/0213 cream. **Materials and Methods:** Skin irritation study was conducted on AHPL/AYTOP/0213 cream as per OECD guidelines. (1) Anti-inflammatory activity: Anti-inflammatory activity of AHPL/AYTOP/0213 cream in comparison with diclofenac sodium cream was assessed in carrageenan-induced rat paw edema model. (2) Antimicrobial activity for *P. acnes*: *P. acnes* were incubated under anaerobic conditions. Aliquots of molten brain–heart infusion with glucose agar were used as the agar base. Formulation and clindamycin (10 µg/ml) were introduced in to the Agar wells randomly. (3) Antimicrobial activity for *Staphylococcus epidermidis* and *Staphylococcus aureus*: bacteria were incubated under aerobic conditions at 37°C. Tryptic soy broth with glucose agar was used as the agar base. A volume of 0.5 ml of formulation and clindamycin (10 µg/ml) were introduced in to the wells randomly. The antibacterial activity was evaluated by measuring zones of inhibition (in mm). **Results:** AHPL/AYTOP/0213 cream is nonirritant. Significant reduction in rat paw edema (43%) was observed with AHPL/AYTOP/0213 which was also comparable to diclofenac sodium cream (56.09%). Zone of inhibition for formulation was 20.68 mm, 28.20 mm, and 21.40 mm for *P. acnes*, *S. epidermidis* and *S. aureus*, respectively, which was comparable to clindamycin. The minimum inhibitory concentration of formulation AHPL/AYTOP/0213 obtained in anti-microbial study was 2.5 mg/mL. **Conclusion:** AHPL/AYTOP/0213 cream is nonirritant and possesses significant anti-inflammatory and antimicrobial activities, which further justifies its role in the management of acne vulgaris.

Keywords: Acne vulgaris, AHPL/AYTOP/0213, antiandrogenic, antibacterial, herbal

Introduction

Acne vulgaris is one of the commonly encountered skin disorders. It is considered as an adolescent disorder which is related to the pilosebaceous follicle of the skin and characterized by formation of open and closed comedones, papules, pustules, nodules and cysts. Acne affects both males and females although males tend to have more with the onset of puberty. It affects around 9.4% of the total global population and is the eighth most prevalent disease worldwide.^[1] Several factors such as disturbed hormonal (androgen) balance, excess sebum production and hyper keratinization are involved in the pathophysiology of acne. Varieties of inflammatory mediators are also involved in the pathogenesis of acne which is produced as a result of immuno stimulation caused by colonization of

Propionibacterium acnes in the duct of the sebaceous follicle. Various noninflammatory lesions such as comedones and inflammatory lesions such as papules and cysts are produced as a result of this process leading to the development of acne vulgaris.^[2]

In modern medicine, several treatments are available for acne vulgaris, but treatment must comply with type and severity of the lesions. Treatment mainly includes prolonged use of

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Access this article online

Quick Response Code:



Website:
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DOI:
10.4103/ayu.AYU_150_17

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How to cite this article: Nipanikar SU, Chitlange SS, Nagore DH, Buzruk D. Evaluation of anti-inflammatory and antimicrobial activity of AHPL/AYTOP/0213 cream. Ayu 2017;38:82-7.

oral and or topical antibiotics (doxycycline, clindamycin and erythromycin), comedolytic (retinoid) and antiinflammatory agents. Although these medicines are better treatment options for acne management, the side effects of these medications such as increased skin dryness, scaling, erythema, burning, stinging, itching and bacterial resistance are noticeable. Hence, people are looking for alternative treatment options for acne vulgaris.^[3,4]

AHPL/AYTOP/0213 cream is a polyherbal formulation developed by Ari Healthcare Private Limited, in the form of cream for effective management of acne vulgaris, hyperpigmentation and various skin disorders. *Daruharidra* extract (*Berberis aristata* DC., Family- Berberidaceae),^[5] *Lodhra* extract (*Symplocos racemosa* Roxb., Family- Symplocaceae),^[6] *Yashtimadhuka* extract (*Glycyrrhiza glabra* Linn. Family- Leguminosae),^[7,8] *Jatiphala* extract (*Myristica fragrans* Houtt, Family- Myristicaceae),^[9] *Manjishtha* extract (*Rubia cordifolia* Linn., Family- Rubiaceae),^[10] *Vacha* extract (*Acarus calamus* Linn., Family- Araceae),^[11] *Dhanyaka* extract (*Coriandrum sativum* Linn., Family- Umbelliferae)^[12] and *Shalmali* extract (*Salmalia malabarica* Schott. and Endl. Family- Bombacaceae)^[13] have been used in AHPL/AYTOP/0213 cream.

Thus, the present study was planned to evaluate antiinflammatory (*in vivo*) potential of AHPL/AYTOP/0213 cream in comparison with standard diclofenac sodium cream in carrageenan-induced rat paw edema and antimicrobial activity in comparison with standard clindamycin against *P. acnes*, *Staphylococcus epidermidis* and nonitalic *Staphylococcus aureus*. Skin irritation study was also conducted on AHPL/AYTOP/0213 cream as per OECD guidelines.

Materials and Methods

Acute dermal toxicity study

This study was conducted to assess the primary skin irritant effect of AHPL/AYTOP/0213 cream in albino rats. The details are as follows:

Wistar albino rat species of age/weight 180-250 g of either sex, preferably females, were used in this study.

Methodology

Ethics committee approval

A total of six animals were approved by the Institutional Animal Ethics Committee (IAEC) in the meeting held on December 31, 2013. The protocol no. approved was DYPIPSR/IAEC/13-14/P-01. Skin irritation study of AHPL/AYTOP/0213 cream was performed as per OECD guideline no. 402.

Procedure

The back of the albino rats was shaved to remove the fur carefully 24 h before application of the sample. AHPL/AYTOP/0213 cream was applied on the skin patches of albino rats and the site of the application was examined at 24, 48 and 72 h for changes in any dermal reactions. The irritation index was calculated to

assess the irritation potential of AHPL/AYTOP/0213 Cream through draize scoring criteria as follows:

Erythema and escher formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (beef redness) to Escher formation	4
Preventing grading of erythema	4

Edema formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Anti-inflammatory activity of AHPL/AYTOP/0213 cream

This study was conducted to evaluate anti-inflammatory activity of AHPL/AYTOP/0213 cream in Wistar albino rats and the details of the study are given below:

Ethics committee approval

IAEC has approved the study. The protocol no. was DYPIPSR/IAEC/13-14/P-01.

Anti-inflammatory activity of AHPL/AYTOP/0213 cream against carrageenan induced paw edema in rats

Eighteen Wistar rats of either sex 6-8 weeks age and weighing 150-250 g were taken and divided into three groups with six animals in each group. Animals from Group 1 were applied topically with specified quantity of 1 g of diclofenac sodium cream as standard drug. Animals from Group 2 were applied topically with specified quantity of 1 g of AHPL/AYTOP/0213cream as a test drug. Group III was taken as a control. The test and standard drugs were applied to the plantar surface of the left hind paw by gently rubbing 5 times with the index finger 30 min before carrageenan injection (Irish moss). After 30 min, 1% w/v of 0.05 ml carrageenan was injected subcutaneously. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to lateral malleolus mark. The paw volume was measured plethysmographically immediately after injection at 1, 2, 3, 4, and 5 h eventually 24 h after drug application^[14, 15]

Antimicrobial activity of AHPL/AYTOP/0213 cream

Antimicrobial activity of AHPL/AYTOP/0213 cream in comparison with standard clindamycin against *Propionibacterium acnes*

The antibacterial activity of AHPL/AYTOP/0213 cream in comparison with standard clindamycin was determined by modified agar well diffusion method. *P. acnes* were incubated in brain-heart infusion (BHI) medium with 1% glucose for 48 h under anaerobic conditions and adjusted to yield approximately

1.0×10^8 CFU/ml. Aliquot of molten BHI with glucose agar was used as the agar base. Prepared inoculum was added to the molten agar, mixed and poured over the surface of the agar base and left to solidify. A sterile 8 mm borer was used to cut wells of equidistance in each of plates; 0.5 ml of solutions of AHPL/AYTOP/0213 and standard clindamycin (10 μ g/ml) were introduced into the wells randomly. The plates were then incubated at 37°C for 48 h under anaerobic conditions in an anaerobic jar (Hi-media) with gas pack and indicator strip and the jar was kept in an incubator for 48 h at 37°C \pm 1°C. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiosis. Citric acid releases carbon dioxide and sodium borohydride releases hydrogen when they come in contact with oxygen. An indicator strip of methylene blue, when introduced into the jar, changes in color from white to blue in the absence of anaerobiosis. The zones of inhibition of formulation and standard were calculated by formula 1. [16,17]

Formula 1

$$AI = \frac{\text{Zone of inhibition of formulation}}{\text{Zone of inhibition obtained for standard}}$$

P.I. = Activity index \times 100s

where,

A. I. - Activity index; P.I. - Percent inhibition

Antimicrobial activity of AHPL/AYTOP/0213 cream in comparison with standard clindamycin against *Staphylococcus epidermidis* and *Staphylococcus aureus*

The antimicrobial activity of AHPL/AYTOP/0213 in comparison with standard clindamycin was determined by modified agar well-diffusion method. *S. epidermidis* and *S. aureus* were incubated separately in tryptic soy broth (TSB) with 1% glucose for 24 h under aerobic conditions at 37°C and adjusted to yield approximately 1.0×10^8 CFU/ml. TSB with glucose agar was used as the agar base in both the cases. Prepared inoculums were added to the molten agar, mixed, and poured over the surface of the agar base and left to solidify. A sterile 8 mm borer was used to cut wells of equidistance in each of plates; 0.5 ml of solutions of formulation and standard clindamycin (10 μ g/ml) were introduced into the wells randomly. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition (in mm). Three

experiments were performed separately in both the cases. The zone of inhibition for AHPL/AYTOP/0213 and standard were calculated by formula 1. [16,17]

Results

Acute dermal toxicity study

Rats skin where AHPL/AYTOP/0213 cream was applied showed no erythema or edema. The primary skin irritation index of the cream was calculated as 0.00.

Anti-inflammatory activity of AHPL/AYTOP/0213 cream

Significant reduction in rat paw edema was observed in AHPL/AYTOP/0213 and diclofenac sodium cream groups as compared to control group. The percentage inhibition of rat paw edema for AHPL/AYTOP/0213 was found to be 43%, while percentage inhibition of rat paw edema for standard diclofenac sodium cream was 56.09%. The details are presented in Table 1.

Antimicrobial activity of AHPL/AYTOP/0213 cream

Antimicrobial activity against *Propionibacterium acnes* (in vitro)

The zones of inhibition for AHPL/AYTOP/0213 cream and standard clindamycin were 20.68 mm and 26.80 mm, respectively. The A.I. for AHPL/AYTOP/0213 cream was 0.77 and percentage inhibition was found to be 77.19 [Figure 1].

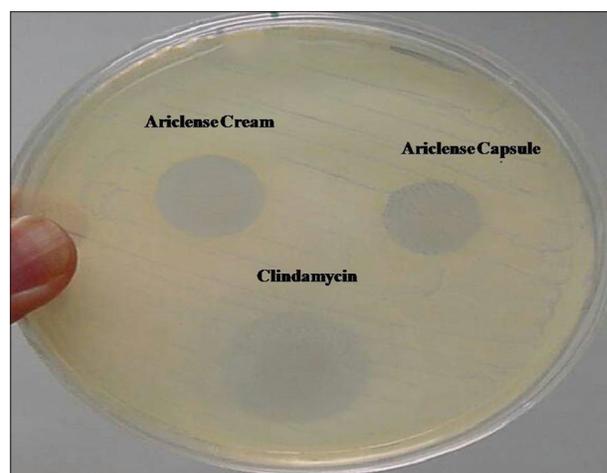


Figure 1: Zone of inhibition against *Propionibacterium acne* (at column width)

Table 1: Anti-inflammatory activity of AHPL/AYTOP/0213 cream

Groups	Mean difference in paw volume (ml) (percentage inhibition)						
	Initial	1 st h	2 nd h	3 rd h	4 th h	5 th h	24 th h
Control	2.63 \pm 0.55	2.81 \pm 0.83	2.91 \pm 0.59	3.15 \pm 0.92	3.38 \pm 0.68	3.55 \pm 1.05	2.05 \pm 1.00
Standard	2.32 \pm 0.79	2.19 \pm 0.93	2.14 \pm 0.75	2.06 \pm 1.02	1.94 \pm 0.65	1.81 \pm 0.82	0.90 \pm 0.88
Percentage inhibition	-	22.06	26.46	34.60	42.60	49.01	56.09
Test AHPL/AYTOP/0213	2.36 \pm 0.78	2.33 \pm 0.70	2.29 \pm 1.09	2.22 \pm 0.68	2.15 \pm 0.89	2.06 \pm 0.97	1.15 \pm 1.32
Percentage inhibition	-	17.08	21.30	29.52	39.04	41.97	43.90

The minimum inhibitory concentration of formulation AHPL/AYTOP/0213 obtained in antimicrobial study was 2.5 mg/mL. The details are presented in Table 2.

Antimicrobial activity against *Staphylococcus aureus*

The zones of inhibition for AHPL/AYTOP/0213 and standard clindamycin were 21.40 mm, 22.40 mm respectively. The A.I. for AHPL/AYTOP/0213 was 0.95 and percentage inhibition was found to be 95.33 [Figure 2]. The details are presented in Table 3.

Antimicrobial activity against *Staphylococcus epidermidis*

The zones of inhibition for AHPL/AYTOP/0213 and standard clindamycin were 28.20 mm and 32.40 mm, respectively. The A.I. for AHPL/AYTOP/0213 was 0.87 and percentage inhibition was found to be 87.03 [Figure 3]. The details are presented in Table 4.

Discussion

Pathogenesis of acne involves increased production of androgen hormones that stimulate excessive sebum secretion on face, neck and back of the chest. Accumulation of sebum, epithelial cells and keratin in the sebaceous follicle leads to formation of non-inflammatory microscopic lesions. When *P. acnes* grow in these follicles, cytokines are released in response to immunostimulant reaction. This leads to the development of inflammatory acne lesions such as papules, pustules, nodules and cysts.^[2]

Currently, various oral and topical antibiotic agents such as doxycycline, clindamycin and topical comedolytic agents such as retinoids and even oral contraceptive pills are effectively utilized in the treatment of acne. However, associated adverse events such as increased skin dryness, scaling and photosensitivity limit their widespread and long-term use.^[5] The major problem affecting antibiotic therapy of acne has also been the increasing bacterial resistance to standard drugs. Moreover, tetracycline, doxycycline, may lead to

adverse effects such as gastric disturbances, tinnitus, vertigo and discoloration of the teeth. While the use of retinoids has to be done with caution, they are said to be teratogens. Cheilitis, dry skin and mucous membranes, elevated liver transaminase levels, hypertriglyceridemia and decreased night vision are common adverse effects associated with retinoids. There are reported cases of depression that started with the use of isotretinoin.^[2] Therefore, increasing trend for the use of alternative treatments for acne is observed since the last 10 years.

Table 2: Antimicrobial activity against *Propionibacterium acnes* (in vitro)

Name of sample	<i>Propionibacterium acnes</i>		
	Zone of inhibition (mm)	Activity index	Percentage inhibition
AHPL/AYTOP/0213 cream	20.68	0.7719	77.19
Clindamycin	26.80	-	-

Table 3: Antimicrobial activity against *Staphylococcus aureus*

Name of sample	<i>Staphylococcus aureus</i>		
	Zone of inhibition (mm)	Activity index	Percentage inhibition
AHPL/AYTOP/0213 cream	21.40	0.9533	95.33
Clindamycin	22.40	-	-

Table 4: Antimicrobial activity against *Staphylococcus epidermidis*

Name of sample	<i>Staphylococcus epidermidis</i>		
	Zone of inhibition (mm)	Activity index	Percentage inhibition
AHPL/AYTOP/0213 cream	28.20	0.8703	87.03
Clindamycin	32.40	-	-

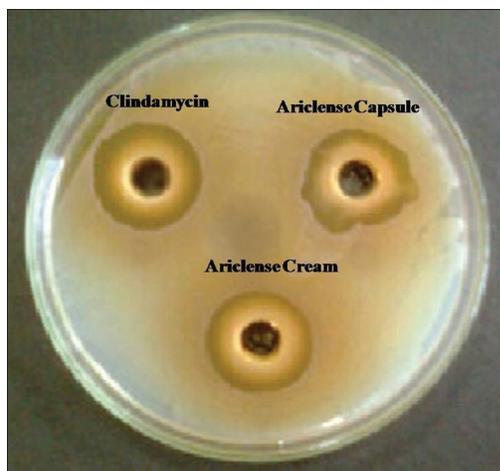


Figure 2: Zone of inhibition against *Staphylococcus aureus* (at column width)



Figure 3: Zone of inhibition against *Staphylococcus epidermidis* (at column width)

In this context, a polyherbal formulation AHPL/AYTOP/0213 cream was developed by Ari Healthcare Private Limited, for the treatment of acne vulgaris, hyperpigmentation and various skin disorders. It contains *Daruharidra* extract (*Berberis aristata*),^[18] *Lodhra* extract (*Symplocos racemosa*) *Yashtimadhuka* extract (*Glycyrrhiza glabra*),^[7,8] *Jatiphala* extract (*Myristica fragrans*),^[9] *Manjishtha* extract (*Rubia cordifolia*),^[10] *Vacha* extract (*Acorus calamus*),^[11] *Dhanyaka* extract (*Coriandrum sativum*)^[12] and *Shalmali* extract (*Salmaalial malabarica*).^[13]

Skin irritation study was conducted on AHPL/AYTOP/0213 cream as per OECD guidelines and it was found that AHPL/AYTOP/0213 cream is nonirritant and safe for use as a local application.

The present study conducted to assess the antimicrobial activity of the AHPL/AYTOP/0213 cream showed that this formulation possess antimicrobial activity against *P. acnes*, *S. epidermidis* and *S. aureus* organism. The antimicrobial activity of AHPL/AYTOP/0213 cream was close to that of standard antibiotic i.e., clindamycin.

It is known that various ingredients of AHPL/AYTOP/0213 cream such as *Daruharidra* (*Berberis aristata*),^[18] *Lodhra* (*Symplocos racemosa*),^[6] *Yashtimadhuka* (*Glycyrrhiza glabra*),^[7] *Jatiphala* (*Myristica fragrans*)^[9] and *Manjishtha* (*Rubia cordifolia*)^[10] possess antibacterial activity against *P. acnes*. *Lodhra* (*Symplocos racemosa*)^[6] also possess antibacterial activity against *S. epidermis* which is known to be one of the acne-causing bacteria. *Dhanyaka* (*Coriandrum sativum*) is considered to be useful as an antiseptic for the prevention and treatment of skin infections with Gram-positive bacteria. It is reported to have antibacterial activity against *Streptococcus pyogenes*, *S. aureus* and methicillin-resistant *S. aureus* combined with excellent skin tolerance.^[12]

In another study, anti-inflammatory activity (*in vivo*) of AHPL/AYTOP/0213 cream in comparison with standard diclofenac sodium cream in carrageenan induced rat paw edema model was assessed, and it was observed that AHPL/AYTOP/0213 cream possess significant anti-inflammatory activity. Furthermore, the anti-inflammatory activity of AHPL/AYTOP/0213 cream was comparable to that of diclofenac sodium cream.

Most of the ingredients of AHPL/AYTOP/0213 cream possess antiinflammatory activity.^[9,11,19,20] *Rubia cordifolia* specifically shows anti-inflammatory activity by suppressing *P. acnes* induced ROS and pro-inflammatory cytokines, the two important inflammatory mediators in acne pathogenesis.^[10] *Acorus calamus* is known to inhibit the production of pro-inflammatory cytokines through multiple mechanisms and may be a novel and effective anti-inflammatory agent for the treatment of skin diseases.^[11] *Berberis aristata* also possess anti-inflammatory and analgesic activity and promotes wound healing.^[20] It is believed that the synergistic action of these herbs could have attributed to the overall antiinflammatory activity of formulation.

Glycyrrhiza glabra possesses anti-androgenic activity,^[8] hence, it may be beneficial in regulating excess sebum production, which primarily depends on androgen levels and androgen sensitivity. Excess sebum production leads to the growth of bacteria responsible for the pathogenesis of acne hence *Yashtimadhuka* is believed to be effective in Acne.

It is evident from the above discussion that the synergistic effect of these plants may have contributed to the overall antimicrobial effect against *P. acnes*, *S. epidermidis* and *S. aureus* organisms as well as anti-inflammatory activity of AHPL/AYTOP/0213 cream. It can be stated that AHPL/AYTOP/0213 cream can be a good treatment option for effective management of acne vulgaris and various skin disorders.

Conclusion

AHPL/AYTOP/0213 cream is nonirritant and safe for use as a local application. AHPL/AYTOP/0213 cream as a local application possesses significant anti-inflammatory activity in carrageenan induced paw edema in rats and antimicrobial activity against *P. acnes*, *S. epidermidis* and *S. aureus*. Thus, AHPL/AYTOP/0213 cream can be used as an effective topical treatment option for acne vulgaris and various skin disorders.

Acknowledgment

Authors sincerely thank all trial sites staff at Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune- 18, for their contribution in conduct of the present study. Authors are extremely grateful to Mr. Sanjeevan Kanjilal (Managing Director) and Dr. Anisha Kanjilal (Director) of Ari Healthcare Pvt. Ltd., for providing all the research facilities, guidance, courage and moral support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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