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ORIGINAL ARTICLE

Evaluation of Anti-inflammatory and Antimicrobial Activity of AHPL/AYCAP/0413 Capsule

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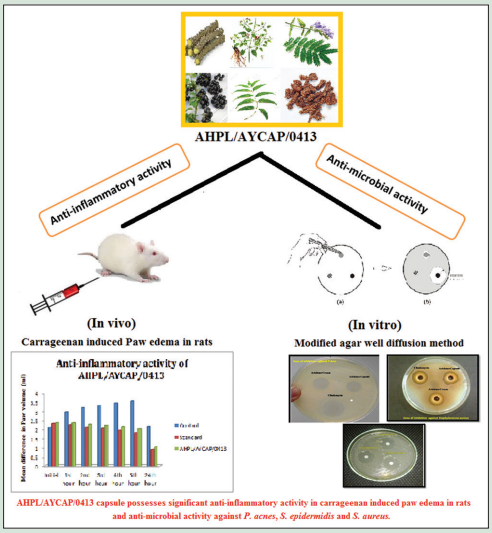
ABSTRACT

Background: Conventional therapeutic agents used for treatment of Acne are associated with various adverse effects necessitating development of safe and effective alternative therapeutic agents. In this context, a polyherbal formulation AHPL/AYCAP/0413 was developed for treatment of Acne. **Objectives:** To evaluate Anti-inflammatory and antimicrobial activity of AHPL/AYCAP/0413. **Material and Methods:** 1) Anti-inflammatory activity: Anti-inflammatory activity of AHPL/AYCAP/0413 in comparison with Diclofenac was assessed in carrageenan induced rat Paw edema model. 2) Anti-microbial activity for *P. acne*: Propionibacterium acnes were incubated under anaerobic conditions. Aliquots of molten BHI with glucose agar were used as the agar base. Formulation and clindamycin (10 µg/ml) were introduced in to the Agar wells randomly. 3) Anti-microbial activity for *Staphylococcus epidermidis* and *Staphylococcus aureus*: *Staphylococcus epidermidis* and *Staphylococcus aureus* were incubated under aerobic conditions at 37°C. TSB with glucose agar was used as the agar base. 0.5ml 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). **Result:** Significant reduction in rat paw edema (51% inhibition) was observed with formulation AHPL/AYCAP/0413 which was also comparable to that of Diclofenac (58% inhibition). Zone of inhibition for formulation was 18.33 mm, 19.20 mm and 26.30 mm for *P. acnes*, S. epidermidis and S. aureus respectively. This activity was also comparable to that of Clindamycin. **Conclusion:** AHPL/AYCAP/0413 capsule possesses significant Anti-inflammatory and Anti-microbial activities which further justifies its role in the management of Acne vulgaris.

Key words: Acne vulgaris, AHPL/AYCAP/0413 capsule, alternative system of medicine, herbal

SUMMARY

- Anti-inflammatory and antimicrobial activities of polyherbal formulation AHPL/AYCAP/0413 were evaluated
- AHPL/AYCAP/0413 contains Guduchi extract (*Tinospora cordifolia*), Manjishtha extract (*Rubia cordifolia*), Sariva extract (*Hemidesmus indicus*), Nimba extract (*Azardirachta indica*), Khadira extract (*Acacia catechu*) and Kakmachi extract (*Solanum nigrum*)
- Anti-inflammatory activity of AHPL/AYCAP/0413 in comparison with Diclofenac was assessed in carrageenan induced rat Paw edema model. Significant reduction in rat paw edema (51% inhibition) was observed with formulation AHPL/AYCAP/0413 which was also comparable to that of Diclofenac (58% inhibition)
- Anti-microbial activity of AHPL/AYCAP/0413 was assessed against *Propionibacterium acnes, Staphylococcus epidermidis* and *Staphylococcus aureus*. Zone of inhibition for formulation was 18.33 mm, 19.20 mm and 26.30 mm for *P. acnes*, *S. epidermidis* and *S. aureus* respectively indicating 68.42%, 85.71% and 81.17% activity. This activity was also comparable to that of Clindamycin
- Therefore it is evident that, AHPL/AYCAP/0413 capsule possesses significant Anti-inflammatory and Anti-microbial activities which further justifies its role in the management of Acne vulgaris.



AHPL/AYCAP/0413 capsule possesses significant anti-inflammatory activity in carrageenan induced paw edema in rats and anti-microbial activity against *P. acnes*, *S. epidermidis* and *S. aureus*.

Abbreviations Used: mg: Milligram, kg: Kilogram, w/v: Weight by volume, ml: Milliliters, h: Hour, BHI: Brain Heart Infusion, CFU: Colony forming units, µg: Microgram, A.I.: Activity index, P.I.: Percent inhibition, TSB: Trypticsoy Broth, mm: millimeters, P. acnes: Propionibacterium acnes, S. epidermidis: Staphylococcus epidermidis, S. aureus: Staphylococcus aureus.

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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]

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Several factors such as disturbed hormonal (androgen) balance, excess sebum production, and hyperkeratinization are involved in the pathophysiology of acne. Varieties of inflammatory mediators are also involved in the pathogenesis of acne which are produced as a result of Immunostimulation 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 oral and/or topical antibiotics (doxycycline, clindamycin, erythromycin), comedolytic (retinoid) and anti-inflammatory 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/AYCAP/0413 capsule is a polyherbal formulation developed for the treatment of acne vulgaris. It contains Guduchi extract (*Tinospora cordifolia*), Manjishtha extract (*Rubia cordifolia*), Sariva extract (*Hemidesmus indicus*), Nimba extract (*Azadirachta indica*), Khadira extract (*Acacia catechu*), and Kakmachi extract (*Solanum nigrum*). Most of these ingredients possess antimicrobial and anti-inflammatory activities.[5-17]

Thus, the present study was planned to evaluate anti-inflammatory *(in vivo)* potential of AHPL/AYCAP/0413 in comparison with standard diclofenac sodium in carrageenan induced rat paw edema and antimicrobial activity in comparison with standard clindamycin against *P. acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.

MATERIALS AND METHODS

Anti-inflammatory activity of AHPL/AYCAP/0413 against carrageenan-induced paw edema in rats[18,19]

Wistar rats of either sex weighing 150–250 g were taken and divided into three groups with six animals in each group. Group 1 animals were starved overnight and termed as control. Group 2 animals were orally administered with diclofenac sodium (15.42 mg/kg/day) as the standard drug. Group 3 animals were orally administered with AHPL/AYCAP/0413 (197.33 mg/kg/day). The test and standard drugs were orally administered with feeding needle, 30 min before carrageenan injection. 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, 5, and eventually 24 h after drug administration.

Antimicrobial activity of AHPL/AYCAP/0413 in comparison with standard drug clindamycin against *Propionibacterium acnes*

The antibacterial activity of AHPL/AYCAP/0413 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/AYCAP/0413 and standard clindamycin (10 µg/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 ± 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 zone of inhibition of formulation and standard were calculated by Formula 1.

The zone of inhibition for formulation and standard were calculated by formula:

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

P.I. = A.I × 100s

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

Antimicrobial activity of AHPL/AYCAP/0413 in comparison with standard clindamycin against *Staphylococcus epidermidis* and *Staphylococcus aureus*

The antimicrobial activity of AHPL/AYCAP/0413 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 µg/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/AYCAP/0413 and standard were calculated by formula 1.

RESULTS

Anti-inflammatory activity of AHPL/AYCAP/0413

Significant reduction in paw edema was observed in AHPL/AYCAP/0413 and Diclofenac groups as compared to control group. The percentage inhibition of paw edema for AHPL/AYCAP/0413 group was found to be

Table 1: Anti-inflammatory activity of AHPL/AYCAP/0413 capsule

Groups	Initial	Mean difference in Paw volume (ml) and (% in hibition)					
		1st hour	2nd hour	3rd hour	4th hour	5th hour	24th hour
Control	2.1	2.95	3.18	3.29	3.41	3.54	2.15
Standard	2.32	2.25	2.11	2.05	1.95	1.80	0.90
(% Inhibition)	-	23.72	33.64	37.68	42.81	49.15	58.13
Test	2.36	2.35	2.28	2.21	2.14	2.02	1.05
(% Inhibition)	-	20.33	28.30	32.82	37.24	42.93	51.16

51%, while percentage inhibition of paw edema observed for standard Diclofenac sodium group was 58%. The details are presented in Table 1.

Antimicrobial activity of AHPL/AYCAP/0413 capsule

Antimicrobial activity against Propionibacterium acnes (*in vitro*)

The zones of inhibition for AHPL/AYCAP/0413 and standard clindamycin were 18.33 mm and 26.80 mm, respectively. The A.I. for AHPL/AYCAP/0413 was 0.68 and percentage inhibition was found to be 68.42 [Figure 1].

Antimicrobial activity against Staphylococcus aureus

The zones of inhibition for AHPL/AYCAP/0413 and standard clindamycin were 19.20 mm, 22.40 mm, respectively. The A.I. for AHPL/AYCAP/0413 was 0.85, and percentage inhibition was found to be 85.71 [Figure 2].

Antimicrobial activity against Staphylococcus epidermidis

The zones of inhibition for AHPL/AYCAP/0413 and standard clindamycin were 26.30 mm, and 32.40 mm respectively. The A.I. for AHPL/AYCAP/0413 was 0.81 and percentage inhibition was found to be 81.17 [Figure 3].

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 noninflammatory microscopic lesions. When *P. acnes* grow in these follicles, cytokines are released in response to immunostimulant reaction. This leads to 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.

In this context, a polyherbal formulation AHPL/AYCAP/0413 capsule was developed by Ari Healthcare Pvt. Ltd., for the treatment of acne vulgaris, hyperpigmentation, and various skin disorders. It contains Guduchi extract (*T. cordifolia*), Manjishtha extract (*R. cordifolia*), Sariva extract (*H. indicus*), Neem extract (*A. indica*), Khadir extract (*A. catechu*), and Kakmachi extract (*S. nigrum*).

In the present study, anti-inflammatory activity *(in vivo)* of AHPL/AYCAP/0413 capsule in comparison with standard diclofenac sodium in carrageenan-induced rat paw edema model was assessed, and it was observed that AHPL/AYCAP/0413 capsule possesses significant anti-inflammatory activity. Furthermore, the anti-inflammatory activity of AHPL/AYCAP/0413 was comparable to that of diclofenac sodium.

Since most of the ingredients of AHPL/AYCAP/0413 possess anti-inflammatory activity,[5-17] it is believed that the synergistic action of these herbs could have attributed to the overall anti-inflammatory activity of formulation.

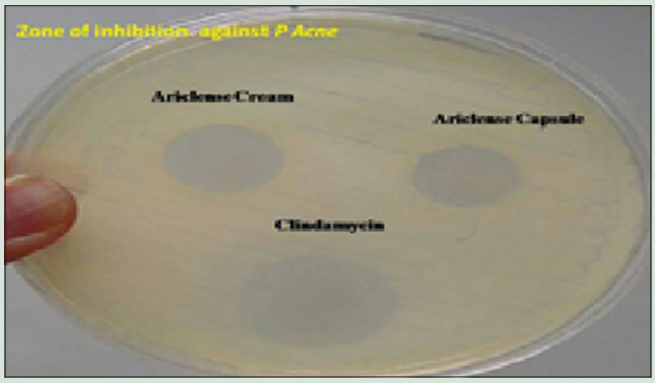

Figure 1: Zone of inhibition against *Propionibacterium acne*

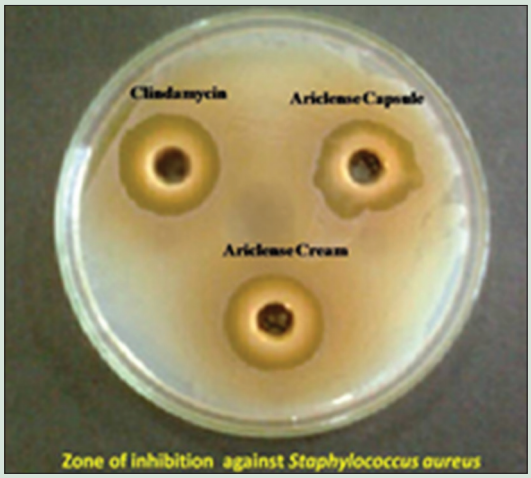

Figure 2: Zone of inhibition against *Staphylococcus aureus*

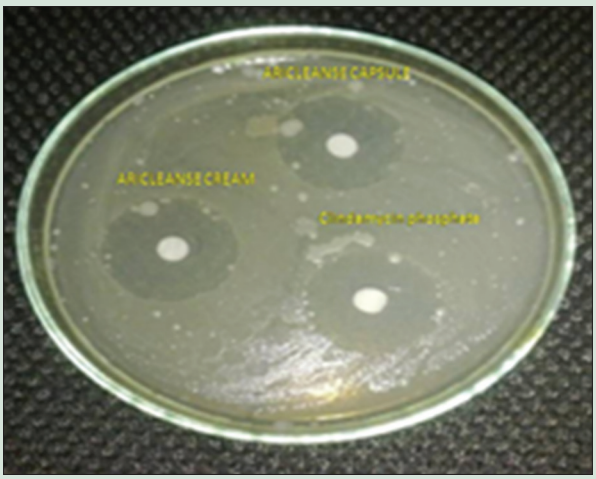

Figure 3: Zone of inhibition against *Staphylococcus epidermidis*

Another study conducted to assess antimicrobial activity of the formulation showed that this formulation also possesses antimicrobial activity against *P. acnes, S. epidermidis*, and *S. aureus* organism. The anti-microbial activity of AHPL/AYCAP/0413 was close to that of standard antibiotic, i.e., clindamycin.

Ingredients of AHPL/AYCAP/0413 such as Sariva,[5-7] Yashtimadhu,[8] Nimba,[7,9,10] and Khadir[11] possess anti-microbial activity. The synergistic effect of these plants may have contributed to the overall anti-microbial effect against *P. acnes*, *S. epidermidis* and *S. aureus* organisms.

In the view of results obtained from both the studies, it can be stated that AHPL/AYCAP/0413 capsule can be a good treatment option for effective management of acne vulgaris and various skin disorders.

CONCLUSION

According to the results of the present study, AHPL/AYCAP/0413 capsule 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/AYCAP/0413 capsule can be used as an effective treatment option for acne vulgaris and various skin disorders.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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