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TIME-KILL CURVE STUDIES OF ACNANO AGAINST STAPHYLOCOCCUS AUREUS & STAPHYLOCOCCUS EPIDERMIDIS

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ABSTRACT

Acnano is a polyherbal nano-emulsion indicated for the treatment of mild to moderate as well as chronic acne. It contains Tea tree oil, Rosemary oil & Mentha oil along with Citrus limon. This study was undertaken to determine the antimicrobial efficacy of Acnano against acne causing microbes such as Staphylococcus aureus & S. epidermidis by time kill curve studies. A rapid killing time was achieved by Acnano. Bacterial count was 2 Log10 cfu/mL after 6 h of study in both the organisms. No deviation in pattern of bacterial inhibition was found and there was no regrowth reported even after exposure for longer time under influence of Acnano. In conclusion, Acnano possesses good antimicrobial activity against S. aureus & S. epidermidis.

Key Words : *Acnano, time kill curve, S. aureus, S. epidermidis*

Introduction

Acne is a chronic disease of the pilosebaceous follicle that affects mainly adolescents and is characterized by the intermittent formation of discrete papular or pustular lesions often resulting in considerable scarring[1,2].

Staphylococcus aureus & *Staphylococcus epidermidis* are two major microbes related to acne[3, 4]. To fight these microbes, a lot of drugs such as topical retinoids[5], Adapalene & benzoyl peroxide[6], salicylic acid[7], isotretinoin[8], Japanese & Chinese traditional medicines[9] etc. are available in the Indian market but nano-technology based herbal emulsions for acne are not available.

Acnano is a patent protected drug and is a blend of essential oils and lemon which is effective against mild, moderate as well as chronic acne. It is an aromatic, clear, transparent light golden yellow viscous herbal nano-emulsion which is a research product of Venus Medicine Research Centre, Baddi, India.

This study was undertaken to determine the antimicrobial efficacy of Acnano against two acne causing microbes i.e. *Staphylococcus aureus* & *Staphylococcus epidermidis* by time kill curve studies.

Material and Methods

Acnano (Batch No. RND09H01, Mfg August 2009) was procured from Venus Medicine Research Centre, Baddi (India). It was kept at room temperature away from direct light & moisture. Adapalene was purchased from Enaltec Labs Pvt. Ltd., Navi Mumbai - 400614, India. One famous commercial brand Code A was procured from Local market Panchkula (Haryana).

Selection of microorganism :

Both the microorganisms *Staphylococcus aureus (MTCC No. 737)* & *Staphylococcus epidermidis*

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(MTCC No. 435) used in the present study were procured from IMTECH (Institute of Microbial Technology), Sector 39-A, Chandigarh – 160036, India.

Media and Reagents

Media and reagents used in study were Sterile water, Phosphate buffer, Dey/Engley neutralizing broth, Nutrient broth, Mueller hinton broth, Mannitol salt agar, Barium chloride and Sulphuric acid. All media were purchased from Hi Media, India and prepared as per manufacturer's instructions.

Preparation of Inoculum

The inoculum suspension were enumerated in duplicate by standard microbiological procedures at the initiation and completion of testing appropriate dilution were prepared and enumerated by standard microbiological procedure. To prepare the inoculum suspension from an agar plate, the microbial growth was washed from the agar surface with buffered phosphate diluent to make 10 fold dilutions from 10^{-1} to 10^{-10} of the organism. An inoculum of 1.0 ml of each dilution was plated on the appropriate media. Plates were incubated at 37°C for 48 h. Count of surviving organisms were recorded. Average triplicate plate (3 plates from each dilution) counts were multiplied by the dilution factor to arrive at cfu/ml. Select which inoculum suspension that carrying minimum of 10^6cfu/ml microbial population.

Time Kill Curve studies:

For each strain, time kill curve studies were performed in MH broth in glass flasks with an inoculum of 5×10^6 to 1×10^7 CFU/ ml in the presence of Acnano. A flask of inoculated MH broth with no sample served as a control . The surviving bacteria were counted after 0, 3 and 6 hrs of incubation at 37 ^{0}C by subculturing 50 µl serial dilutions (in 0.9 % NaCl) in to MH plates with a spiral plater.

One-way Analysis of Variance (ANOVA) was used to determine statistical difference between Acnano, standard & Code A brand, in all organisms under study. $p<0.05$ were considered statistically significant. A kill curve (log10 cfu/ml vs. time) was drawn for each product and linear regression analysis for each set of data was made. For each strain, time-kill bacterial kill studies were performed in Acnano, Adapalene and Code A brand with an inoculum of $1x10^6$ to $5x10^6$ cfu/ml. A flask of inoculated MH broth with no drug sample served as a control. The surviving bacteria were counted after 0, 2, 4, 6, 8 and 10 hours incubation at 37°C by sub culturing 1 ml serial dilutions in to D/E neutralizing broth.

Determining Time-Kill Endpoints

A bactericidal effect is defined as a 3 log decrease in the cfu/ml or a 99.9% kill over a specified time [10]. The definition of kill for this study has been used as per National Committee for Clinical Laboratory Standards (1992) together with modifications based on a suggestion by Handwerger and Tomasz that a kill can be determined at 6 h[11]. A constant logarithmic rate of kill has been assumed during a time-kill. A 90% kill at 6 h is equivalent to a 99.9% kill at 24 h. In this study the kill measurement was determined by the actual reduction in viable counts at 6 h for each isolate.

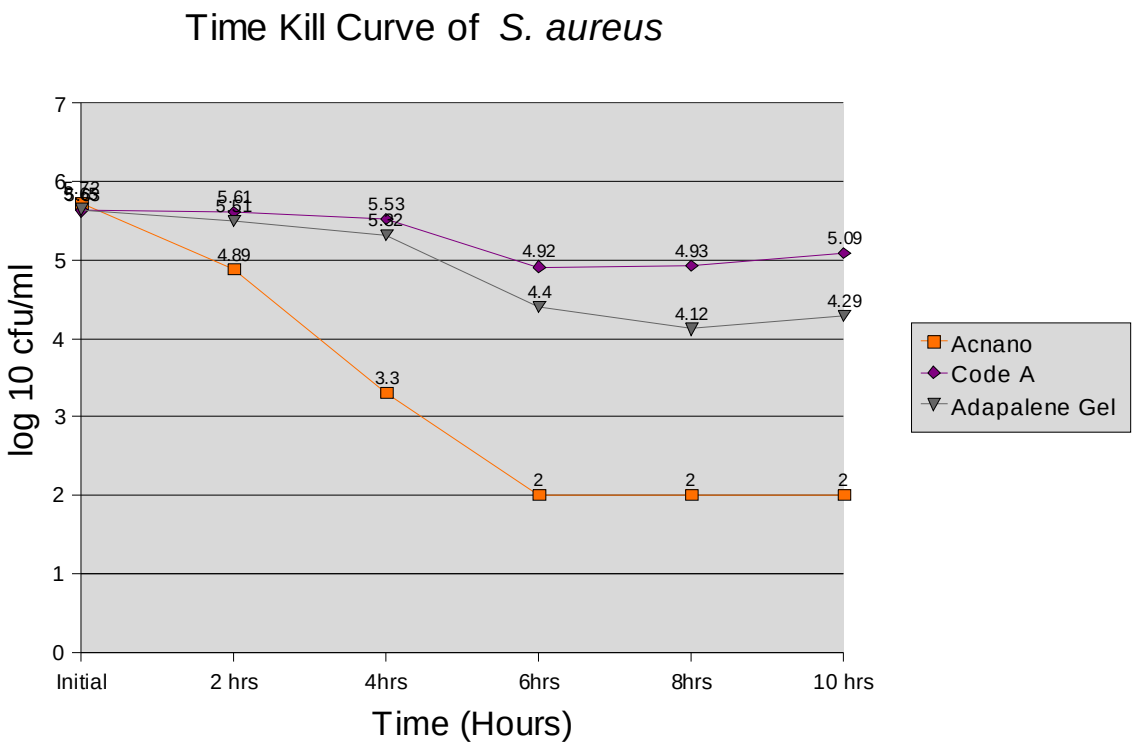

Fig. 1 showing Time kill Curve of S. Aureus.

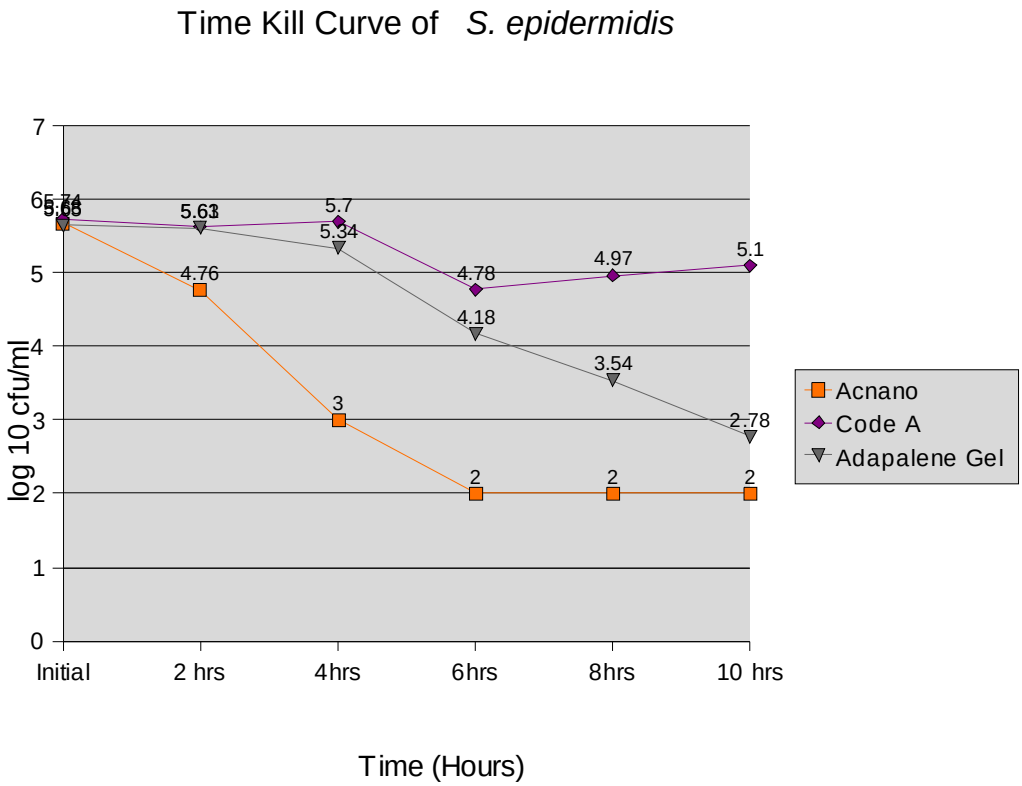

Fig. 2 showing Time kill Curve of S. Epidermidis

Results & Discussion

Acnano is a research product of Venus Medicine Research Centre, Baddi (India). It is a synergistic blend of essential oils and citrus. Tea tree oil (*Melaleuca alternifolia* oil) contains terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole etc. as major constituents[12]. It has been reported to be active against *Staphylococcus epidermidis*[13] and *Staphylococcus aureus*[14, 15, 16].

Rosemary oil *(Rosmarinus officinalis* oil*)* contains p-cymene, linalool, gamma-terpinene, beta-pinene, alpha-pinene, eucalyptol etc. as major components[17]. It is also effective against *Staphylococcus aureus* [14, 15, 16] and *Staphylococcus epidermidis*[18].

Mentha arvensis oil (mint oil) contains menthol as major constituent[19] and is active against *Staphylococccus aureus*[20].

Citrus limon juice mainly contains citric acid and ascorbic acid and is a potent antimicrobial agent[21].

S. aureus time-kill curve analysis demonstrated a non significant change in colony count in each time point. 5.72 to 2.00 log10 cfu/ml of colony count was recorded in case of Acnano whereas in standard adapalene (0.1%) 5.65 to 4.29 log10 cfu/ml and in commercial brand Code A, 5.63 to 5.0909 log 10 cfu/ml of colony count were recorded. (Fig. 1).

In *S. epidermidis* also, there was a non-significant change in colony count in every time point. In Acnano, 5.68 to 2.00 log10 cfu/ml of colony count was recorded whereas in Adapalene, 5.65 to 2.778 log10 cfu/ml and in commercial brand Code A, 5.74 to 5.0969 log 10 cfu/ml of colony count were recorded. (Fig. 2).

There was no regrowth of these organisms in the culture media under study even after exposure for longer time under influence of Acnano.

Addition of Acnano to a culture led to a significant reduction in the microbial population over a period of time which proves the potent antimicrobial property of Acnano.

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