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Studies on inhibitory effect of Eucalyptus oil on sebaceous glands for the management of acne

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Acne is the most common disorder virtually seen to affect teenagers and young adults between age of 14-30. It is characterized by inflamed specialized sebaceous follicles which are present at face, back and chest. Some serious factors responsible for generation of acne are abnormal follicular keratinization and desquamation, excessive secretion of sebum, and proliferation of *Propionibacterium acnes* in follicles. Other factors aggravating or worsening the acne conditions are secondary infections caused by some pathogenic strains of bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, etc. There are various topical and systemic therapies available in market to treat or control the acne but maximum of them have the side effects like itching, redness, skin peeling, stinging and photosensitivity. Again, the development of resistance of available antibiotics for *P. acne* and other bacterial strains has necessitated the search for new antimicrobial agents. Thus, the current work was designed to gain attention towards the alternate pathway for controlling the acne condition by decreasing the production of sebum from sebaceous glands. The eucalyptus oil, obtained from *Eucalyptus globulus* Labill. (Myrtaceae) was chosen and its biocide action on various bacterial strains was established using agar-well diffusion technique to prove its efficacy in controlling the secondary infection condition i.e. worsening of acnes. The *in vivo* rat sebaceous gland model was chosen to show the effectiveness of eucalyptus oil in decreasing the sebum production by reducing the size of sebaceous glands to control the spread of acne. The results were found to be promising for eucalyptus oil in controlling the sebum protection and thus establishing the other pathway for the management of acne.

Keywords: Acne, Antibacterial, *Eucalyptus globulus*, Eucalyptus oil, Sebaceous glands, Sebum.

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Introduction

Acne is the most common condition which affects maximally all individuals between 14-30 years of age[1]. It's most common form is *Acne vulgaris*, which is caused by the inflammation of sebaceous glands situated at face, chest and back[2]. It is characterized by formation of either of following: Comedone, papule, pustule, nodule, sebaceous cyst, scarring, etc.[3] Etiology of acne generally has four basic reasons: (i) abnormal follicular keratinization and desquamation, (ii) excessive secretion of sebum, (iii) proliferation of *Propionibacterium acnes* in follicle followed by secondary infections, and (iv) subsequent production of inflammation[4-6]. During immunological responses, both humoral and cell-mediated pathways are involved[7,8]. Acne is not thought to be contagious diseases. Among the bacterial strains, only those species that can colonize a normal skin as resident flora can be a cause of acne. Therefore, only three species of microorganisms can be responsible for the development/worsening the condition of acne; these are: *Propionibacterium*, *Staphylococcus* and *Escherichia* species[9].

Sebum, the lipid-rich secretion of sebaceous glands, has a central role in the pathogenesis of acne and provides a growth medium for *P. acnes*. People with acne have higher rate of sebum production than unaffected individuals. Moreover, the severity of acne is generally proportional to the amount of sebum production[10]. Enlargement of the sebaceous glands and increased production of sebum is stimulated by the increase in production of adrenal and gonadal androgens that precedes the clinical onset of puberty. The first signs of *Acne vulgaris* commonly occur at the time of puberty[11,12]. Other factors aggravating or worsening the acne conditions are secondary infections caused by some pathogenic strains of bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, etc.[13] There are various

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topical and systemic therapies available in market to treat or control the acne but maximum of them have the side effects like itching, redness, skin peeling, stinging and photosensitivity[14]. Again, the development of resistance of available antibiotics for *P. acne* and other bacterial strains has necessitated the search for new antimicrobial agents[15]. Medicinal plants have been used as a source of acne remedies since ancient times and they have shown great promises in the treatment of infectious diseases[16-18]. One of these medicinal plants is *Eucalyptus globulus* Labill. belonging family Myrtaceae. Traditionally, this plant claims the anti-inflammatory, analgesic and antimicrobial property[19-22]. The study had been conducted on isolation of some component like Eucalyptone G[23]. The eucalyptus oil is known to has effect on *Propionibacterium acnes*, the main causative agent for development of acne in human skin flora[24]. But the development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents[25]. Thus, the current work was designed to show the action of eucalyptus oil by alternate pathway for controlling the acne condition, i. e. by decreasing the production of sebum from sebaceous glands. The biocide action of Eucalyptus oil was established using agar-well diffusion technique on various bacterial strains to prove its efficacy in controlling the secondary infection condition i.e. worsening of acne. The *in vivo* rat sebaceous gland model was chosen to show the effectiveness of eucalyptus oil in controlling the sebum production to control the spread of acne.

Materials and Methods

Procuring and authentification of material

The fresh leaves of *E. globulus* was purchased from the local market of Indore (MP), India and authenticated at Government Agricultural College, Indore. A voucher specimen number SCOPE/Phcog/08a/09 was also retained in Department of Pharmacognosy, SCOPE, Indore for future reference. The essential oil of leaves was collected by hydrodistillation method using a Clevenger-type apparatus. The oil was separated from the water by decantation and was dried by filtration over anhydrous sodium sulfate. The physical properties of oil are: colourless, aromatic, weight, 0.92 per ml, refractive index, 1.47 and solubility, miscible with ethanol, chloroform, ether and glacial acetic acids. The oil was established as good quality oil according to Indian Pharmacopoeia[26]. The oil is stored in amber colored bottle and kept away from sunlight throughout the experiment to avoid rancidity and environmental degradation. The 1 ml of oil was dissolved in 1 ml of DMSO (Dimethyl sulphoxide) and from it 10, 20, 30, 40, 50, 60, 70, 80 and 90 µl were poured in well to account for the antimicrobial susceptibility testing.

Test organisms

Two Gram positive bacteria (*S. aureus* 2079 and *S. aureus* 5021), and two Gram negative bacterial strains (*P. aeruginosa* and *E. coli*) were used to test the activity. The microbial strains were obtained from the NCIM, National Chemical Laboratory, Pune, India. Bacterial strains were maintained on agar slants stocks and subsequently sub-cultured into newly prepared nutrient agar slants.

Antimicrobial susceptibility testing

Sterile agar media (at 35°C) was poured into sterile petri dishes, which then been inoculated with the test organisms. The plates were allowed to gel for an hour. Wells (7 mm diam.) were made with the aid of flamed borer on the surface of the agar plates. Various concentrations as listed above of *E. globulus* oil were delivered in each well. These were incubated at 37°C for 24 hours. The presence of zones of inhibition was recorded which showed the presence of antimicrobial action. From the inhibition zones seen, antimicrobial activity was expressed in terms of average diameter of the zones of inhibition measured in triplicates. The gentamycin was used as a standard for antibacterial activity[27,28].

Evaluation of anti-acne activity using male Sprague-Dawley rats

Dermal toxicity test

The acute dermal toxicity study was carried out in adult male sprague-dawley by "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No.434. Test procedure with a dose of 10 ml was adopted. The test substance was applied uniformly over a shaven area which is approximately 10% of the total body surface area. The animals were loosely held in contact with the skin by cotton gauze and held in place with a non-irritating tape. Then the animals were observed continuously for 4, 24 and 72 h for the signs of edema, erythema, skin rashes and finally for mortality after 24 h till 14 days[29].

Irritation test

Rats were selected in a group of three for the experiment. The dose selected for test substance (0.05, 0.5 and 5 ml) was evenly applied to an area of

about 6 cm of skin of left lateral surface of the rats under a gauze patch. Right untreated shaven surface was considered as control area which was also covered with gauze patch. The gauze patches of both left and right surfaces of the animal were loosely held in contact with the skin by cotton gauze and held in place with a non irritating tape. At the end of the 4 h exposure period, residual test substance was removed, using water without altering the integrity of the epidermis. Results were observed and tabulated[29].

Anti-acne activity

Groups of 5 adult male Sprague-Dawley rats weighing 180-220g were shaved in the interscapular area. Twenty-four hours later, the test sample or the standard (cyproterone acetate) is applied locally to the shaved area at increasing doses (0.05, 0.5 and 5 ml/cm^2) in 20: l ethanol. The treatment is continued for three weeks. Controls received ethanol only. The animals are sacrificed 24 h after the last administration. Pieces of skin from interscapular region was excised and processed for evaluation by light microscopy. The volume density of the smooth endoplasmic reticulum vesicles is measured[30].

Results and Discussion

Various bacteria with major role of *P. acnes* and *S. epidermidis* cause pathogenesis of acne. Both of these microbes, and others potentially related to acne pathogenesis, are present on normal skin. With either inflammatory mediators or excess sebum production, these microbes can overgrow and further worsen the acnes condition[15].

A study illustrated the potential pathogenicity of *P. acnes* strains and their synergy with *S. aureus, E. coli and Pseudomonas aerugniosa.* The data suggested that *P. acnes* can cause abscess or enhance the growth of abscesses through synergy with other bacterial species in a mixed infection[13]. Our study has supported this fact and showed the effect of Eucalyptus oil on some pathogenic bacteria involved in acne. Antimicrobial activity of *E. globulus* oil was checked against clinically important bacterial strains. The oil was generally active against all the microbial strains studied except *P. aeruginosa* which was the most resistant bacterial strain studied. The oil was highly active against Gram positive bacteria *Staphylococcus aureus* 5021 (25.5 mm zone of inhibition at highest dose). Thus, it is highly controllable in defending the secondary infections in case of acnes. All the results are tabulated in Table 1.

During the dermal toxicity test and irritation test, no signs of any allergy like edema or erythema were shown by the oil and thus, it was considered to be safe for the application as tabulated in Table 2.

The oil was also found to control the sebum production by reducing size of sebaceous glands as

Table 1 — Antimicrobial activities of Eucalyptus oil

Microbial strain	Zone of inhibition (mm) produced by different concentrations of oil dissolved in DMSO										Antibiotic
Dose (v/v) ► Organisms ▼	10	20	30	40	50	60	70	80	90	DMSO	Gentamycin (30 µg/ml)
Staphylococcus aureus 2079	-	1.5	3.5	5.5	6	8.5	9.3	10.3	11.3	-	14
Staphylococcus aureus 5021	-	4	7	10.5	14.3	19.3	21.5	25.3	25.5	-	32
Pseudomonas aeruginosa	-	0	0	0	0	0	0	0	0	-	0
Escherichia. coli	-	1.5	2.5	2.5	3.0	4.0	4.0	4.0	5.3	-	0

* Diameter of the well is 7 mm, DMSO was taken as a control and the results shown are the mean of three replicates.

Table 2 — Dermal toxicity/skin irritation tests on Sprague-Dawley rats

S. No.	Dose (ml/cm$^{2)}$	Erythema				Edema			
		0h* 4h	24h	48h	72h	0h* 4h	24h	48h	72h
1.	0.05	- -	-	-	-	- -	-	-	-
2.	0.5	- -	-	-	-	- -	-	-	-
3.	5	- -	-	-	-	- -	-	-	-

* Immediately after the removal of patch; Absent: - ; mean n=5 for dermal toxicity test and n=3 for skin irritation test

shown in histopathological slides of skin (Plate 1). The toxic group showed the huge enlargement in the size of sebaceous glands under the influence of testosterone hormone. The level of this hormone is increased in body at the time of puberty in males thus, causing more sebum production. The oil was found to

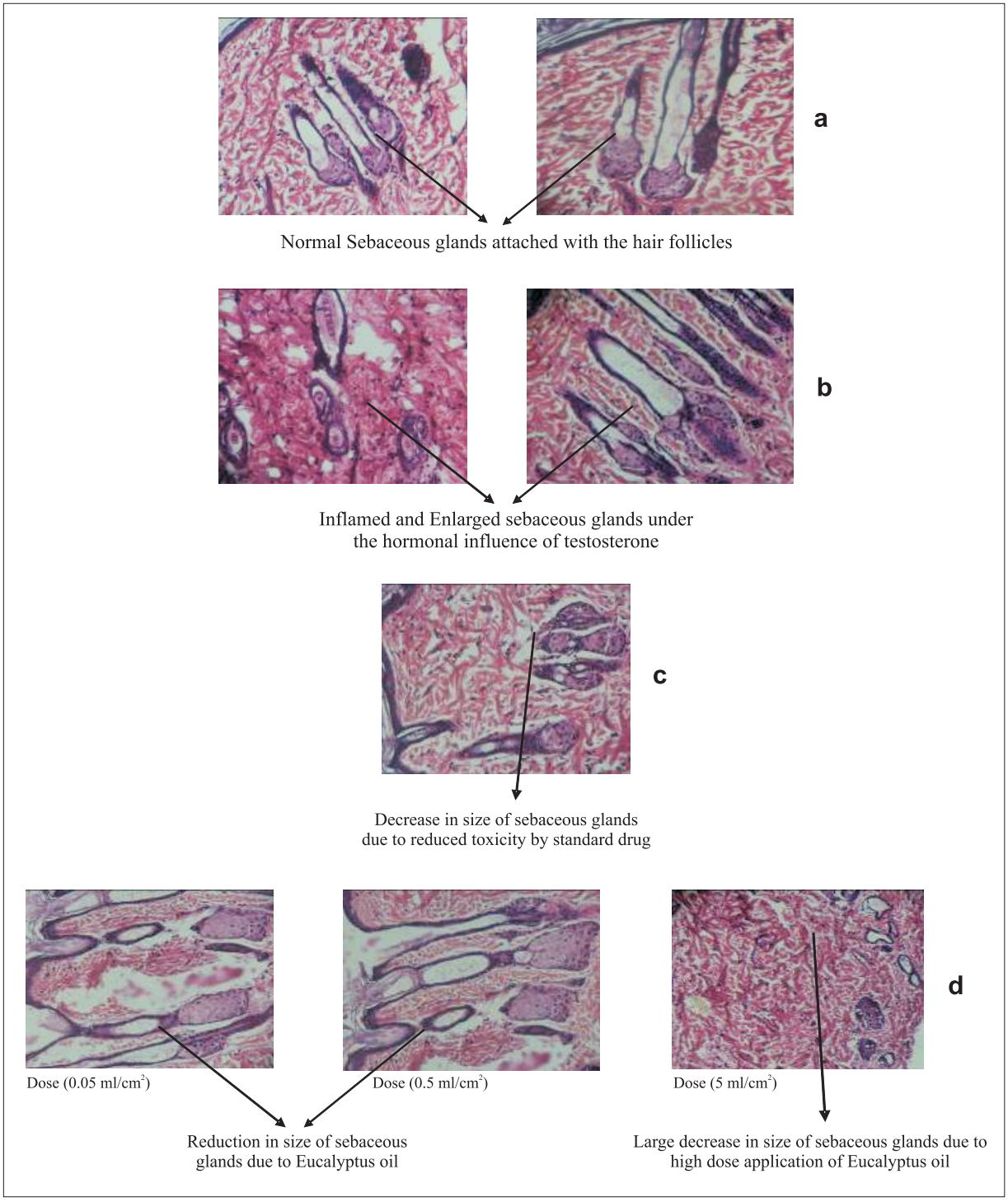

Plate 1 — Histopathological images (Photomicrograph) of sebaceous glands from the interscapular region of skin of male Sprague-Dawley rats stained with Hematoxylin and Eosin and visualized at 100X: a- Normal group treated with ethanol only; b-Toxic group treated with testosterone; c- Standard group treated with cyproterone acetate; d- Test sample of Eucalyptus oil treated with doses of 0.05, 0.5 and 5 ml/cm^2.

be very effective in decreasing the size of increased sebaceous gland and bring it to normal, thus, controlling sebum production, which is one of the factor for acne promotion.

Conclusion

The role of eucalyptus oil is very effective in controlling growth of acne. Results shown action of eucalyptus oil in controlling sebum production and thus, proved its dual action by reducing the sebum production and controlling the secondary infectious stage by other microbes establishing another pathway for the management of acne. Although eucalyptus oil has shown potency in the management of acne; probable mechanism as inhibition of sebum production and decreasing secondary infections, as justified in current study, is definitely a novel approach.

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