IJPSR (2019), Volume 10, Issue 7 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 November 2018; received in revised form, 05 March 2019; accepted, 11 March 2019; published 01 July 2019

A COMPREHENSIVE REVIEW ON ACNE, ITS PATHOGENESIS, TREATMENT, *IN-VITRO* AND *IN-VIVO* MODELS FOR INDUCTION AND EVALUATION METHODS

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Keywords:

Acne, Epidemiology, Treatment, Novel technology, Induction of acne

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ABSTRACT: Acne is a common inflammatory disorder which is common among the adolescent age groups. Even though it is not a life-threatening disease but it affects the patient's self-esteem. Additionally, they have also gone into depression and suicidal ideation. Based on the severity it can be classified into several types. Various factors are responsible for the occurrence of acne. The main factors are discussed in this article. According to the Global Burden of Disease study about 85% of the populations are being affected when we consider the epidemiology. Among this, the Americans are most prone to it. A variety of medications are available in the market for the treatment of acne like retinoid derivatives, benzoyl peroxide, and antibiotics *etc*. Among which the topical treatment is most preferred. Other treatments are carried out only if the existing treatment doesn't give the desired results. Nanotechnology has also been incorporated for the existing drug in order to target the disease. Acne can be induced in the animal. Existing works of literature gives information on how to induce acne in an animal by using bacteria as well as chemical. Different *in-vitro* and *in-vivo* studies exist to determine several aspects of the drug. In this article, we have focused on all the above topics in brief.

INTRODUCTION: Acne is otherwise called as acne vulgaris. It is a chronic inflammatory disease. It occurs on the parts like: face, neck, upper chest, upper back, *etc*. According to studies, acne is the most prevalent disease in the world. It affects the patient both psychologically as well as psychosocially. The available topical treatments are having few side effects.

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DOI: 10.13040/IJPSR.0975-8232.10(7).3155-77

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(7).3155-77

In order to avoid these side effects, researchers have formulated drug by incorporating nanotechnology in their formulation and they have also gone for combination therapy in order to decrease the concentration of the drug. In this article, we have discussed on acne, its pathophysiology, epidemiology, its treatment lines and finally the induction of acne in an animal model.

Acne: "Acme" is a Greek word of acne which means "Prime of life". Acne is a chronic inflammatory state which occurs in the skin (Acne vulgaris). The inflammation of skin occurs due to the oil secreted by the sebaceous gland or which is otherwise called as oil glands of the skin.

Acne is seldom life-threatening condition, but it affects the self-esteem of an individual. People with the age group ranging from 12-24 are more prone to acne and about 85 % of the population are being affected [1].

TABLE 1A: THE SEVERITY OF ACNE CAN BE CLASSIFIED INTO

Grade I	Grade II	Grade III & IV
No inflammation	Pustules and papules	Deeper inflamed nodules
Mild	Moderate	Severe

TABLE 1B: TYPES OF ACNE ARE AS FOLLOWS

Acne vulgaris	Nodular acne	Acne cosmetic
Comedonal acne	Acne rosacea	Excoriated acne
Cystic acne	Acne mechanica	Acne look-alike conditions

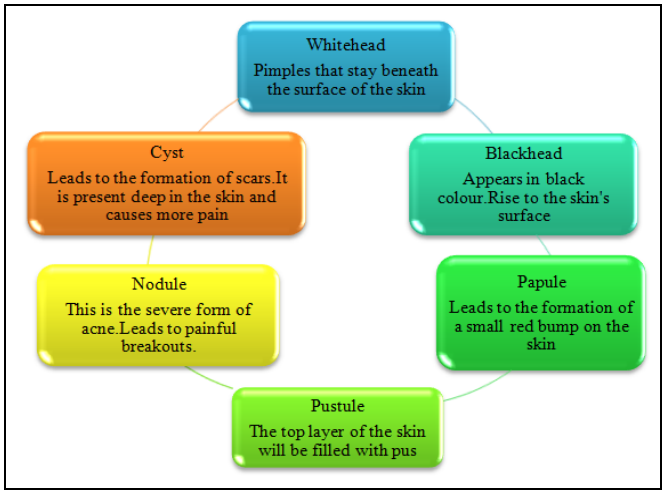
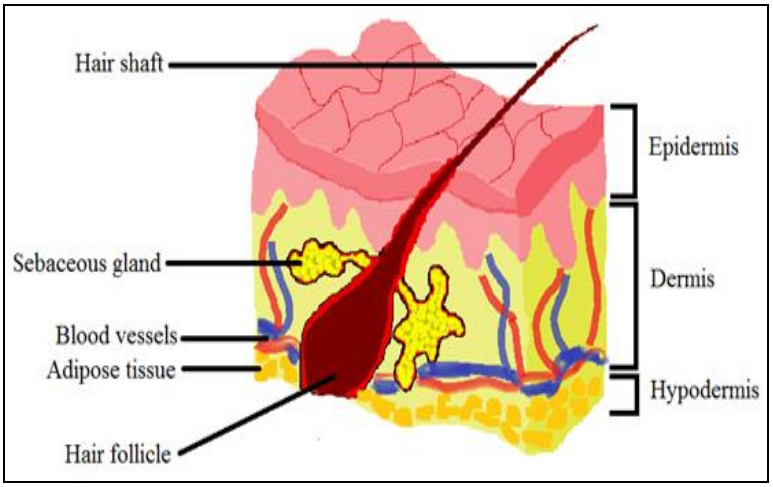
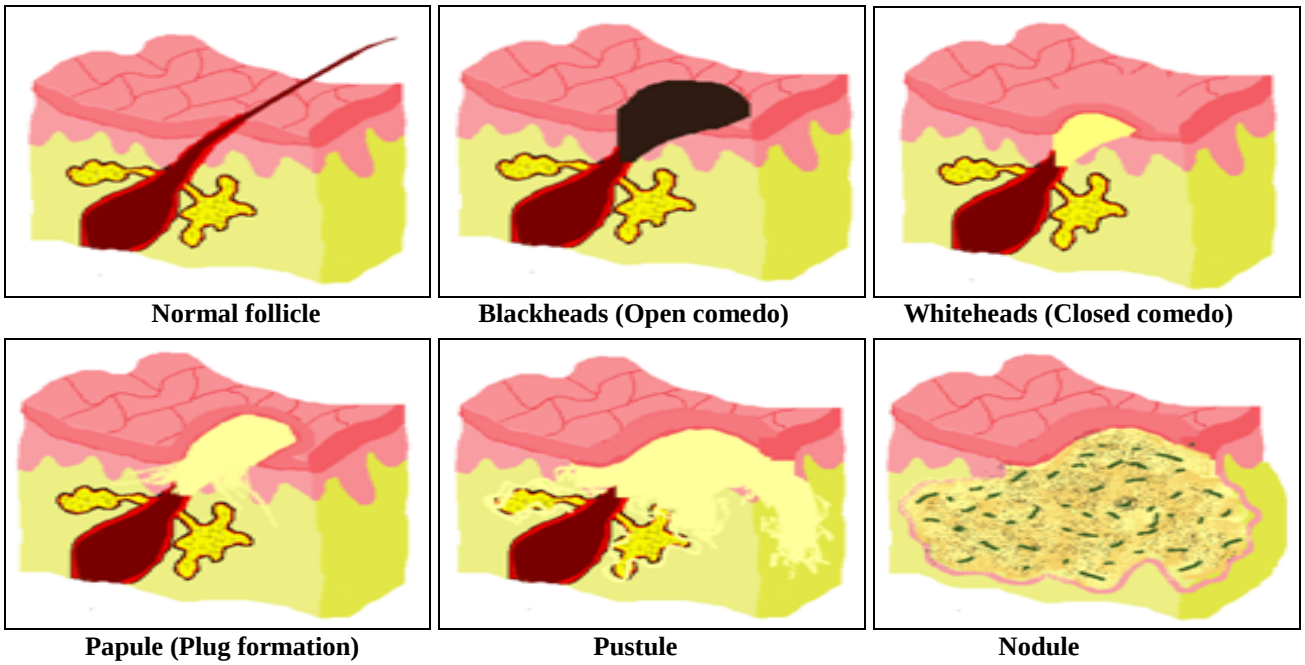


FIG. 1: BASED ON THE INFLAMMATORY AND NON-INFLAMMATORY CONDITION, ACNE LESIONS CAN BE CLASSIFIED INTO

Etiology:





Normal follicle | **Blackheads (Open comedo)** | **Whiteheads (Closed comedo)**

Papule (Plug formation) | **Pustule** | **Nodule**

FIG. 2: SKIN IS THE MAJOR ROUTE OF ACNE FORMATION

Main Factors which are Responsible for the Formation of Acne are as Follows:

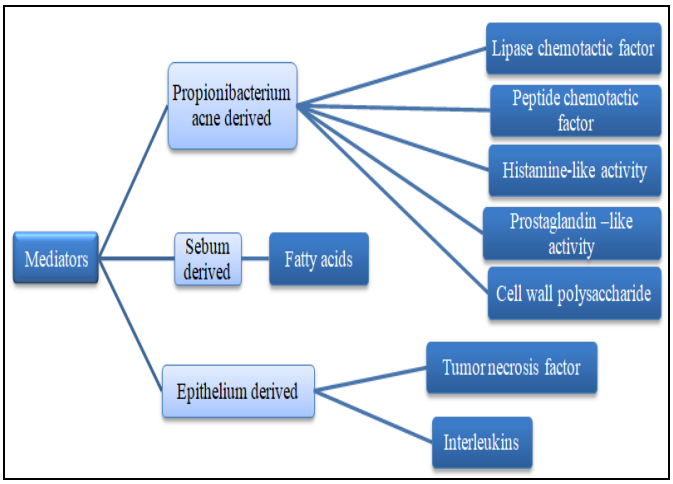
Hyperproliferation of the Follicular Epithelium: Follicular hyper-keratinization can also be called as ductal hypercornification. The hyper keratinization occurs when there is cohesion between the follicle cells and when they do not shed properly on to the skin surface then it leads to the formation of micro-comedones which thereby results in occurrence of acne. The reason behind this follicular hyper-keratinization is fatty acids. Linoleic acid is a fatty acid, when there is a decreased level of this acid then it causes hyper- keratinization. In addition the MUFAs (monounsaturated fatty acid) as well as lipoperoxides are also responsible [54].

Excess Sebum Production due to Hormones: Sebum production plays an important role in the inflammation. The sebum production is controlled by the hormones namely androgen and testosterone. To begin with, androgen hormone is considered as an initial trigger. Men with severe acne have an increased level of dehydro-epiandrosterone sulfate (DHEAS) and a decreased level of sex hormone binding globulin (SHBG) responsible for elevating the androgen level. A drastically elevated level of androgen was found in women compared to men with acne. It has been found that the adult women with significant acne are having an abnormal DHEAS, androstenedione, and SHBG [2].

In addition, the sebum also serves as a medium for the growth of *Propionibacterium acne* which thereby leads to the formation of acne. Apart from these reasons, one more mechanism is MYCN-mediated hyper activation of epidermal growth factor receptor and induction of perilipins (group of proteins that coat lipid droplets) also contributes to hyperseborrhea [54].

Role of *Propionibacterium acne* (*P. acne*): *Propionibacterium* is otherwise called as *Cutibacterium*, which is a gram-positive and anaerobic in nature responsible for inflammation of the skin. *P. acne* metabolizes the triglycerides (broken down from sebum) and thereby releases the free fatty acid which in turn stimulates the inflammatory response leading to the formation of acne. *P. acne* activates TLR2. TLR2 is toll-like receptors which are a component of the innate immune system [1]. *P. acne* activation of TLR2 on monocytes and neutrophils releases various proinflammatory mediators like cytokines, interleukins 12, 8 and tumor necrosis factor. In addition, the *P. acne* has also got a CAMP factor. CAMP factor is a Christie, Atkins, Munch-Peterson factor which is utilized by *Staphylococcus aureus* and leads to hemolysis of acne lesion [3].

Inflammatory Mediators: Inflammation is triggered by mediators like [2].



FLOW CHART 1: REPRESENTS VARIOUS INFLAMMATORY MEDIATORS RESPONSIBLE FOR ACNE

Other Factors that is Responsible for Acne:

- **Medications:** Medicines containing lithium and steroidal drugs.
- **Due to the Cosmetics Used:** Cosmetics containing greasy materials causes acne.
- **Stress:** A study in Singapore with the adolescents reveals that there is a correlation between the stress and acne severity [3].
- **Hormonal Changes and Menstruation:** At the time of puberty there will be an increased level of androgen hormone which in turn leads to sebum production [3].
- **Hot and Humid Climatic Conditions:**
- **Type of Hair (Oily Hair):** In case, if your hair is oily in nature then daily hair wash is necessary to prevent acne formation.
- **Squeezing the Pimples:** Sometimes people use to squeeze, pinch and pick their pimples, which may further worsen the condition and can live scars on their skin [4].

- **Diet:** In few studies, it has been observed that no food including chocolates has been recognized as an underlying factor. Even the relationship between the dietary dairy products and the acne is minor. In addition to this, the relationship between acne and diet remains unclear [5].
- **Genetics:** Acne occurs due to various genes like interleukin-1 alpha, CYP1A1
- **Over Washing the Face with Cleansers:** Hard scrubbing of the skin using cleansers.
- **Environmental Factors:** Which can be due to exposure to some chemicals or dust and even pollution can affect the skin.
- Due to other conditions like Pregnancy, polycystic ovary syndrome, and acne climacterica refers to menopause allied acne.
- Acne occurs when there is a dysfunction of adaptive and innate immunity
- When there is a interaction with the neuropeptide then acne occurs

Acne Mimickers:

Erroneously Considered as Acne: [6] Sometimes because of acne mimickers (other disorders), the acne is misdiagnosed. Conditions that mimic the acne are as follows:

- Comedones presenting conditions: Nevus comedonicus.
- Conditions which comprise acne in their name: Acne agminata, cloracne.
- Appendageal abnormalities including milia.
- Benign tumors.
- Follicular component conditions including folliculitis, keratosis pilaris faceii.

Global Perspectives:

Demographics of Acne Vulgaris: According to literatures, people with white skin are more prone to acne compared to that of black skin. The reason for this is that the black skin are tough and they are less sensitive to sunlight whereas the opposite happens with white skin, they are more sensitive to sunlight and they are not having a tough skin as that of black skin.

Statistics in America:

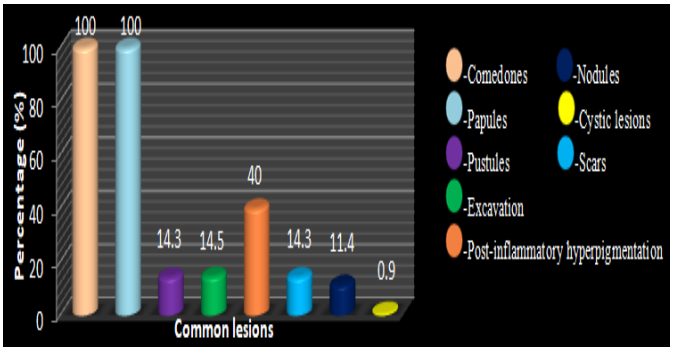


FIG. 3: GRAPH REPRESENTING THE PERCENTAGE OF DIFFERENT ACNE LESIONS

Gender:

- Women are more prone to acne compared to men starting from their teens to middle age.
- This is because of various reasons like:

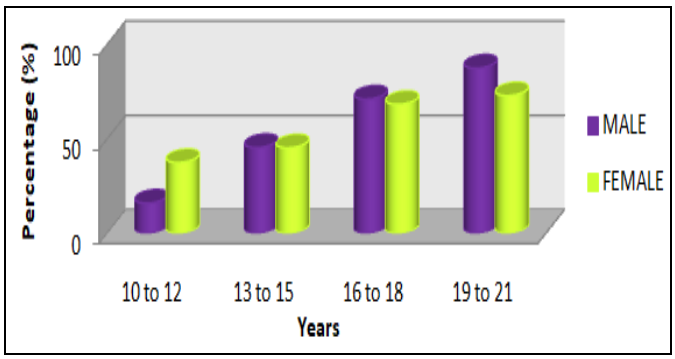


FIG. 4: GRAPH REPRESENTING ACNE WITH RESPECT TO AGE AND GENDER

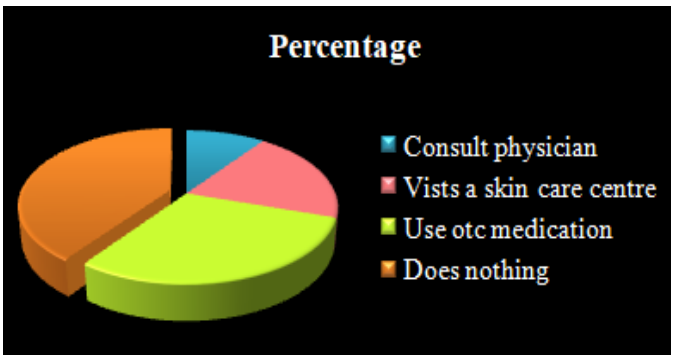


FIG. 5: A PIE CHART REPRESENTING THE PEOPLE SUFFERING FROM ACNE AND THE STEPS THEY TOOK FOR IT

- Global Burden of diseases (GBD) study states that acne vulgaris affects 85% of 12-25 years aged adults. Acne is a common disease in the US.
- 40-50 Million Americans are affected. 20 Million Americans are having severe acne.

From the pie chart it can be said that when a person is affected with acne then:

1. Only 10% consult a physician.
2. 20% visits a skin care center.
3. 30% use OTC (Over the counter) medication.
4. 40% doesn't act for it.

- In 2004, the total cost for the acne treatment including OTC and prescription medication was found to be $ 2.2 billion.

Age: Age *vs*. Acne:

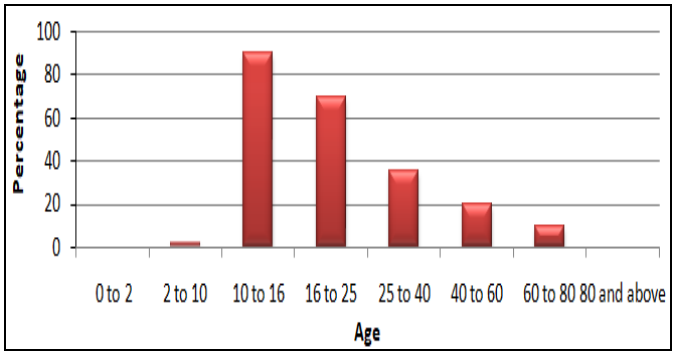
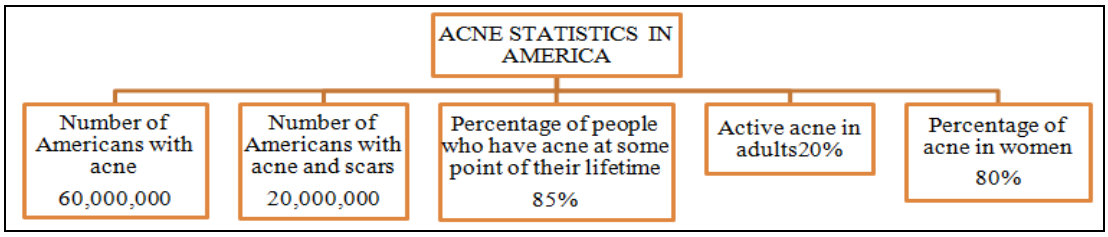


FIG. 6: A GRAPH REPRESENTING THE PERCENTAGE OF PEOPLE OF DIFFERENT AGE GROUP AFFECTED BY ACNE



FLOW CHART 2: ACNE STATISTICS IN AMERICA

Acne statistics in Jeddah: [55]

A cross-sectional study on April 2011 and September 2012

↓

In 17 yrs and above aged community

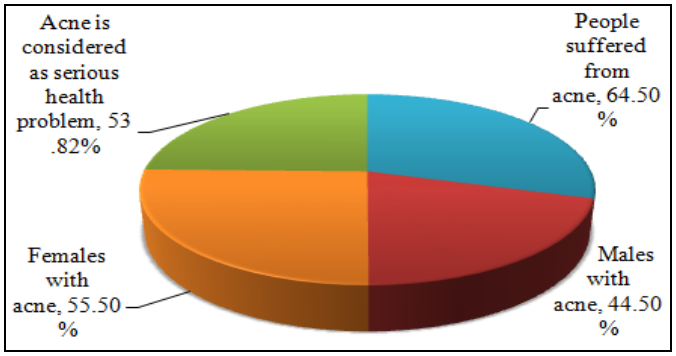


FIG. 7: PIE CHART REPRESENTING THE ACNE STATISTICS IN JEDDAH

From the data, it is clear that the percentage of people affected with acne in Jeddah is only 64.50% which is comparatively less as compared to the statistics in America.

French Studies on Acne with Adolescents Revealed the Following Data:

TABLE 2: THE ABOVE TABLE REPRESENTS THE LIST OF FACTORS THAT CAUSE AND DOESN'T CAUSE ACNE

Factors don't have any influence on acne	Factors that have an influence on acne
Gender	Eating chocolates
Overweight	Smoking
Consumption of dairy products	Sweating
Physical activity	Squeezing pimples, beauty products and pollution

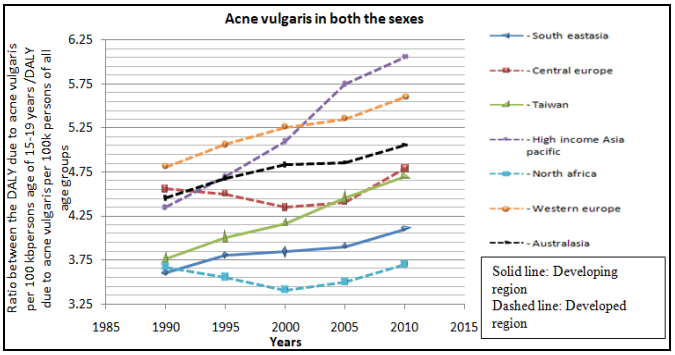


FIG. 8: GRAPH REPRESENTING THE RELATIONSHIP BETWEEN ACNE VULGARIS AND NATIONALITY [7]

Treatment: Most commonly used medication is topical creams and gels and they are used in case of mild and moderate acne. Whereas, the oral antibiotics are used in the case of severe acne vulgaris.

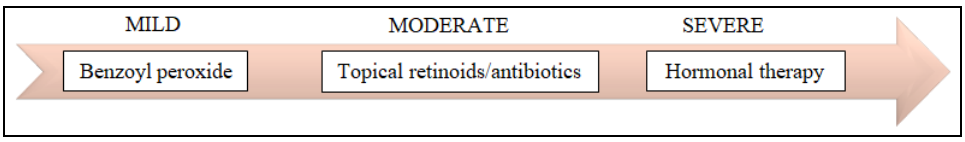
FIG. 9: THE TYPES OF TREATMENT AVAILABLE TO TREAT ACNE VULGARIS

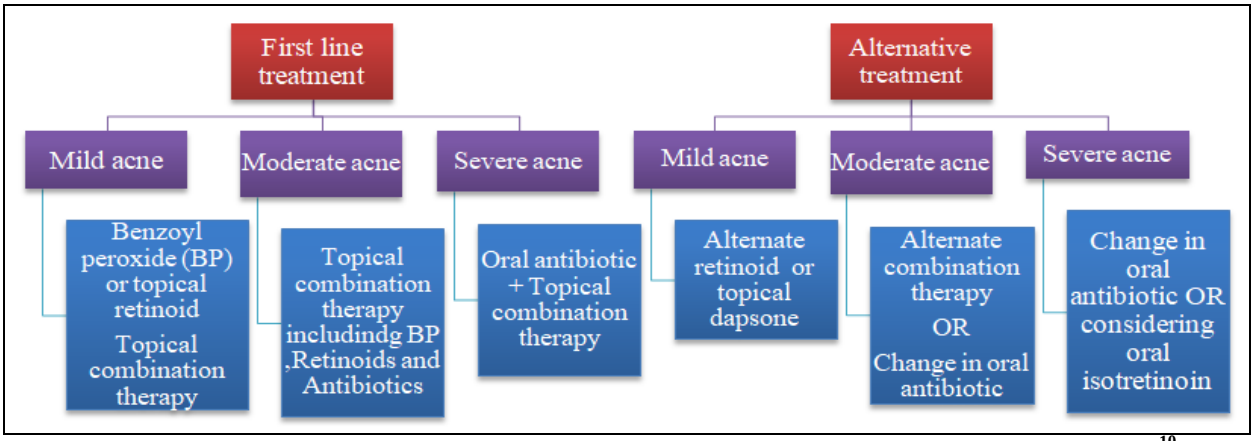
Before Treating acne there are Certain Factors to be considered: [9]

1. Type of acne and its severity.
2. Type of skin (*i.e.*: oily skin or dry skin).
3. Hyperandrogenism in women and their menstrual history.
4. Past medical history.
5. Acne stimulating medication.
6. Presence of scarring.
7. Patient's age.

Main Aim of Treating Acne Includes: While treating *Acne vulgaris* the medication should be capable of decreasing the inflammation, reducing the hyperproliferation of follicular epithelial cells and lower the production of sebum from the sebaceous gland. Along with these parameters, it should also be capable of preventing the acne scars.

Approaches to Treat Acne: [2]





FLOW CHART 3: MANAGEMENT OF ACNE IN YOUNG ADULTS AND ADOLESCENTS [10]

From the above flowchart, we can say that for both the treatment (First line treatment and Alternative treatment) topical medication is given first in case of mild acne while coming to severe acne oral antibiotics are administered.

Topical Therapies in Treatment of Acne Vulgaris: Topical therapy for treating acne includes both OTC (Over the counter) and prescription medicine.

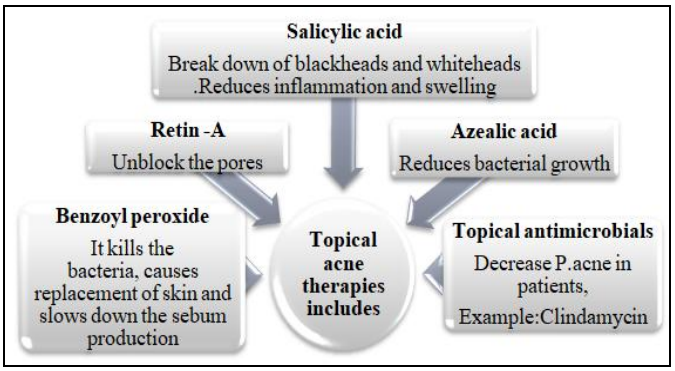


FIG. 10: PICTURE DEPICTS THE ACTIVE INGREDIENTS USED IN THE OTC MEDICATION

- Acne vulgaris can be treated using a single topical therapy or a combination therapy along with other topical agents or in combination with oral antibiotics.
- The main objective of topical treatment is that the drug should be capable of penetrating into the skin (lipophilic environment).
- Various topical treatments (formulation) are available like topical gels, creams, lotions, face washes, solutions and ointments.
- The above 6 entity is considered as a vehicle in which the drug is dispersed and administered. Vehicle plays a vital role in selecting the active ingredient or therapeutic agent. The activity of a therapeutic agent depends upon the vehicle because it has influence on the absorption of the drug through the skin. The vehicle selection depends on many parameters like type of skin (oily or dry in nature), the patient who is affected, affected area, and even depends on humidity and climatic conditions.
- Topical products can be directly administered to the affected area thereby increasing the exposure of drug to skin and reducing the systemic absorption of the drug [8].
- Despite being a good formulation in treating acne vulgaris, the topical medication may lead to some of the side effects like skin irritation and some other allergic reaction.
- Topical antibiotics are used in treating acne vulgaris because they have both anti-bacterial as well as anti-inflammatory property. The anti-bacterial property of antibiotics is essential in order to inhibit the bacterial protein synthesis and the anti-inflammatory property is essential in order to inhibit the chemotaxis of polymorphonuclear leukocytes. This is because the interleukin-8 (IL-8) is linked with the polymorphonuclear leukocytes (PMN) which in turn stimulates the keratinocyte proliferation [11].
- Topical treatment's absorption depends on many factors like the amount or the quantity of medication applied, the surface area of the application, application time and its duration, time interval involved in the application, site of application, the thickness of stratum corneum and the selection of the vehicle [12].

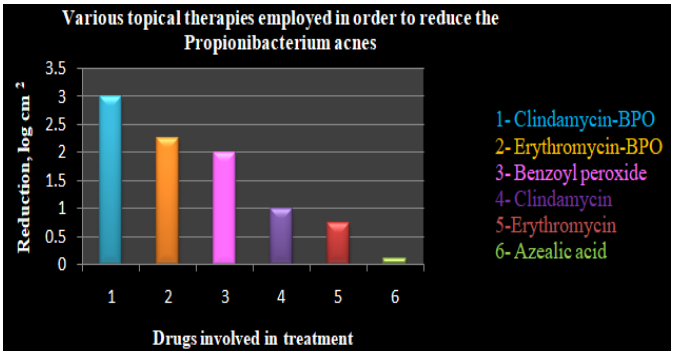


FIG. 11: A GRAPH REPRESENTING THE USE OF TOPICAL THERAPIES TO DECREASE THE *P. ACNES*

Topical Agents Involved in Treating Acne:

1. Topical Retinoids:

- Retinoids are otherwise called as comedolytics and they are obtained from vitamin A. It is used as monotherapy or in combination therapy along with other topical or oral antimicrobial agents. They also act as an anti-inflammatory agent as well as comedogenic activity.
- The first line of treatment for patients having mild-moderate acne is topical retinoids.
- Topical retinoids create a harsh and difficult environment for *P. acne* instead of inhibiting them.

- In addition to these features, retinoid increases the penetration of other topical agents into the skin (pilosebaceous unit) [9].
  - ✓ The main aim of topical retinoids include [13]:
  - ✓ Inhibition of the keratinocyte proliferation.
  - ✓ Increase in the turnover of follicular epithelial cells.
  - ✓ Reducing or suppressing the formation of microcomedones.
- Topical retinoids used in acne treatment can be classified in the following manner: [12]
  - **a)** Tretinoin (Cream, gel or microsphere gel vehicle).
  - **b)** Isotretinoin
  - **c)** Adapalene (Cream, gel, lotions).
  - **d)** Tazarotene (Cream, gel or foam).
- Multiple pathogenic features including the inflammatory and non-inflammatory lesions can be targeted by administering topical retinoids along with other anti-microbial agents[18].

a) Tretinoin:

- It has been used for the past three decades in the treatment of acne. Tretinoin is a derivative of vitamin A. It is a comedolytic agent which is helpful in treating acne. It has an anti-inflammatory property and prevents the blockage of the pilosebaceous unit [8].
- Tretinoin is present in the form of cream, gel, and liquid.
- Tretinoin binds to different sets of a retinoic acid receptor-like alpha, beta, and gamma which modify its activity, efficacy, and tolerability. Tretinoin is inactivated by benzoyl peroxide when they are administered together [10]. In order to avoid the oxidation and inactivation of tretinoin by benzoyl peroxide, they are given in the alternative timing. Example tretinoin is given at night time and benzoyl peroxide is given in the morning time.
- The side effect of using tretinoin includes:
  - ✓ Dryness in the skin
  - ✓ Peeling of the skin
  - ✓ Irritation in the skin
  - ✓ Leads to redness of the skin
  - ✓ Photosensitivity
  - ✓ Burning sense
  - ✓ Contraindicated during pregnancy
- However, these side effects can be reduced by application of a suitable quantity of medication, the frequency of application, by avoiding exposure to sunlight, using protective clothing and hats and also by applying suitable sunscreen with a sun protection factor (SPF) greater than 15. [14]

b) Isotretinoin: Isotretinoin undergoes an isomerization to tretinoin. Isotretinoin has a similar efficacy as that of tretinoin. When we compare adapalene and isotretinoin, it is said that the isotretinoin is having an increased effect compared to adapalene. Isotretinoin lowers inflammatory as well as non-inflammatory lesions. When isotretinoin is combined with erythromycin, it shows better activity compared to isotretinoin alone [13].

c) Adapalene:

- Adapalene is used as the first line treatment in the acne vulgaris. It is known as a synthetic retinoid compound. It has an anti-inflammatory property which acts on acne lesions. The differentiation in the follicular epithelial cells is normalized [8].
- They are less effective as compared to other retinoids. But, adapalene is having less irritation compared to other topical retinoids and in addition to this, there is no photosensitivity [9].
- It is more lipophilic in nature, which allows it to penetrate more rapidly into the skin. Studies revealed that a concentration of 0.1% of adapalene and 0.025% of tretinoin are having similar efficacy. When adapalene is combined with benzoyl peroxide, it is having more activity. Even a combination of 0.1% adapalene and 1% clindamycin is having a better activity than using both the drugs individually [13].
- In order to attain a follicular targeting, the adapalene is integrated with the microcrystals (with a size range of 3-10 μm) [14].

d) Tazarotene:

- Tazarotene which is marketed as TAZORAC is a synthetic retinoid with a comedolytic property (causes lysis of comedones in acne). It

is used in the treatment of mild to moderate acne. Commercially, it is available as a gel (0.05 %, 0.1%) which is applied daily in the evening only once [14].

- They are having more activity as compared to tretinoin (0.025% and 0.05%). Increased efficacy is found by combining tazarotene and clindamycin together. Better efficacy is observed when using a triple preparation (tazarotene 0.1% gel, clindamycin 1%/Benzoyl peroxide 5% gel and erythromycin/Benzoyl peroxide) [13].
- Tazarotene is a pro-drug which gets converted into tazarotenic acid in the keratinocytes. Tazarotene acts as an anti-inflammatory agent. It is considered a second-line treatment. Since, this drug causes skin irritation and dryness, they are administered only when there is no response to adapalene and tretinoin [8].

2. Benzoyl Peroxide (BP):

- BP is available as cream, gel, and lotion and it possesses anti-microbial, anti-inflammatory and comedolytic property. It is employed as a peeling agent in the treatment of acne.
- Used in treating the inflammatory acne like papule, pustules, and cysts. The presence of oil on the skin is reduced as well as the size of the sebaceous gland is decreased.
- BP is lipophilic in nature which allows it to enter into the stratum corneum and easily penetrate into the pilosebaceous unit.
- In order to obtain a better efficacy, BP is combined with clindamycin[9]. Benzoyl peroxide rapidly degraded into benzoic acid and hydrogen peroxide and degrades the bacterial protein by releasing the free radical oxygen against the *P. acnes*.
- It is used as a monotherapy in mild to moderate acne. In order to decrease the resistance of the *P. acnes* species and to improve the efficacy, BP is combined with topical antibiotics.
- BP is available in various concentrations like 2.5%, 5% and 10% comprising of lotion, creams, and gels [14]. It is also available as topical washes, foams, and also used as a leave on or wash-off agents [10].
- The side effects of BP comprise of dryness, burning sensation, erythema, peeling, itchiness and sometimes bleaching of the clothing and skin.

3. Azelaic Acid:

- It is a natural dicarboxylic acid which leads to inhibition of *P. acne* protein synthesis. It has several properties like anti-inflammatory, antioxidant, anti-keratinizing properties and bacteriostatic [8]. The concentration *Propionibacterium acne* is reduced on the surface of the skin. Azelaic acid inhibits the pro-inflammatory cytokines thereby reducing the inflammation.
- It also causes the induction of peroxisome proliferator - activated receptor - gamma (PPARG). PPARG is also called as glitazone receptor which decreases the inflammatory responses.
- Azelaic acid is marketed as gels and creams for topical application. Since, these formulations contain a high quantity of azelaic acid; it leads to many side effects like burning, irritation, stinging and redness of the skin. In order to overcome these side effects, lamellar liquid crystal (LLC) system containing azelaic acid is prepared with a smaller quantity of azelaic acid for topical application [16].

4. Topical Antibiotics:

- Topical antibiotics mainly focus on the *Propionibacterium acne*. Clindamycin and erythromycin are usually used as a topical application for treating acne. Clindamycin and tetracyclines are possessing bacteriostatic activity. Quinolone scaffold exhibits its bactericidal activity by interacting with the DNA supercoiling [17].
- Dapsone is considered as an anti-inflammatory agent as well as an antibiotic agent. It inhibits the bacterial DNA synthesis. It has been stated that the dapsone is safe and effective for treating long-term acne.
- Side effects of dapsone include mild irritation and dryness of skin. The orange-brown coloration of the skin occurs when dapsone and

Benzoyl peroxide is administered together due to oxidation of dapsone [13].

- Studies show that after a certain period of time bacterial resistance develops while using the topical antibiotic as an only treatment. So, in order to avoid the bacterial resistance, the topical antibiotics can be administered along with the benzoyl peroxide or any other suitable topical agents like retinoids.
- Studies address that the frequency of topical antibiotic monotherapy for treating acne has decreased when comparing the combined treatment of topical antibiotic with Benzoyl peroxide [19].

Oral Antibiotics and Isotretinoin: [20]

1. Oral Antibiotics:

- The oral antibiotics are used in the treatment of moderate to severe acne and also the acne which is resistant to topical therapies. Oral antibiotics are for long-term use.
- These compounds express their activity by inhibiting the *Propionibacterium acnes* and the formation of the inflammatory mediators.
- Tetracycline, doxycycline, minocycline, erythromycin, and azithromycin are used as a choice of systemic antibiotics in the treatment of acne.
- However, there are certain side effects of erythromycin and tetracycline like photo-sensitivity, vulvovaginal candidiasis, and gastrointestinal irritation. In addition, to this tetracycline has got another side effect like permanent bone and tooth pigmentation.
- List of oral antibiotics used to treat acne:

a. Tetracyclines:

1. Doxycycline – Duration of 4-6 month. Side effects including gastro-intestinal upset and photosensitivity.

2. Minocycline – Duration of 4-6 months. Side effects including vertigo and hyper-pigmentation of the skin.

b. Macrolides: 1. Erythromycin- Duration of 4-6 months. Side effects including gastro-intestinal upset and vaginal candidiasis.

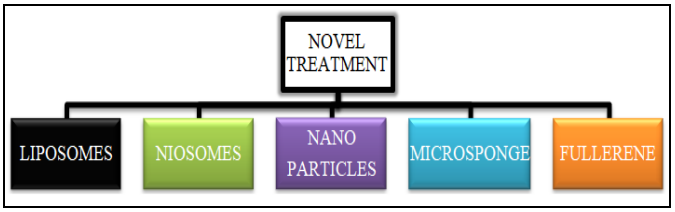
c. New antibiotics:

1. Tetracyclines (Lymecycline)
2. Macrolides (Azithromycin)

2. Oral Isotretinoin:

- It has been used in treating acne by exhibiting its action against multiple factors like decreasing sebum secretion, the formation of comedeons, colonization of skin with *Propionibacterium acnes*. They also exhibit anti-inflammatory activity.
- Initially, the treatment is started with a low dose and subsequently increased based on the tolerability. When the drug is administered along with the food, its bioavailability is increased.
- There are also a number of side effects associated with systemic isotretinoin therapy like teratogenicity, dryness of skin, lips, nasal passages and eye. But, most of the side effects can be tolerated and treated.
- In case, if any problem occurs during oral isotretinoin therapy, then immediately it should be reported to physician and laboratory monitoring is required during the treatment.
- Suggested tests comprise of blood count, low-density lipoprotein, high- density lipoprotein, liver enzymes, triglycerides, total cholesterol level during fasting condition.

Novel Treatment for Acne:



FLOW CHART 4: NOVEL TREATMENT FOR ACNE

1. Liposomes:

- They are spherical vesicles composed of lipid bilayer which is used as a carrier or a vehicle for the delivery of drugs into a particular site and thus, improving the therapeutic effect of liposomal drugs. In liposomes, both hydrophilic and hydro-phobic drugs can be incorporated [21].

- Recently reported anti-acne drug-loaded liposomes include clindamycin, tretinoin, salicylic acid, and tea tree oil loaded liposomes [22].
- Lauric acid was found to be a typical free fatty acid in the human sebum with a strong antimicrobial activity.
- According to research, they found that the lauric acid is having a strong anti-microbial activity against *P. acne* than OA (Oleic acid) and PA (palmitic acid).
- **Fig. 12:** A graph representing the anti-microbial action of free fatty acids on *Propionibacterium acnes*. Colony forming unit (CFU) of *P. acnes* was identified by incubating *P. acne* with lauric acid (LA), palmitic acid (PA) and oleic acid (OA) [22].
- From the graph, it can be concluded that lauric acid is having a strong bactericidal activity. Additionally, in order to overcome the poor water solubility of lauric acid, it has been incorporated into liposomes to form Lipo LA.
- Studies reveal that the clindamycin hydrochloride liposome has a potential effect in the treatment of acne vulgaris.

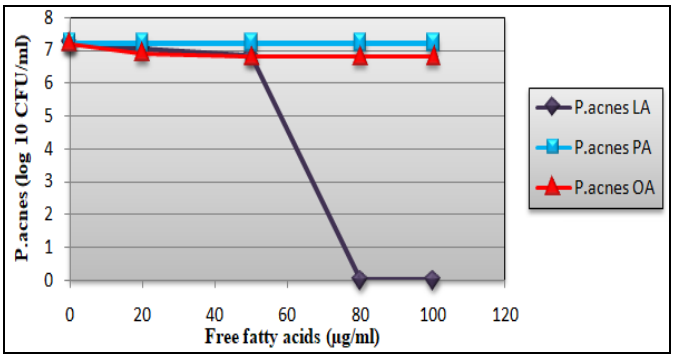


FIG. 12: ANTIMICROBIAL ACTION OF LA, PA, OA ON *P. ACNES*

2. Niosomes:

- Niosomes are obtained from synthetic non-ionic surfactants. They are either unilamellar or multilamellar vesicles used as a carrier for transport of drugs to the target site. Non-ionic surfactants are used as solubilizer and stabilizer of the insoluble drug in the pharmaceutical area. In order to overcome the disadvantages caused by liposomes, niosomes are used. They have several advantages including improved therapeutic effect, drug stability, increased chemical stability, lower cost, improved penetration capacity and prolonged circulation time in the biological system. Both hydrophilic and lipophilic drugs can be incorporated and delivered to a target site [23].
- Various mechanisms are available in order to provide a support for the penetration capacity of niosomes. It acts by decreasing the trans-epidermal water loss thereby enhancing the hydration of stratum corneum and loosening of the organized structure. The efficiency of the drug is increased when niosomes are incorporated with surfactants thereby promoting the drug uptake by the target cells.
- Researchers have formulated dapsone niosomes (dapsone is used as an antibiotic) for the topical application in order to control and prolong the drug release for healing mild to moderate acne vulgaris with enhanced skin penetration. They found that the dapsone niosomes for topical application were safe, tolerable and effective with minimum side effects [24].

3. Nanoparticles:

- Nanotechnology is intended to improve the transdermal drug delivery system. Before designing a transdermal/dermal drug delivery system, it is important that we should have known about the interaction and long-term as well as short-term toxicity studies between the skin and the nanocarriers. To begin with, the interaction of skin with nanocarriers depends upon the following components:
  - ✓ Physicochemical properties like size and surface charge.
  - ✓ Drug loading capacity.
  - ✓ Nanomaterials property.
  - ✓ The flexibility of the structure.
  - ✓ Means of application.
- Some nanocarriers may adhere to the skin surface and release the drug into the skin layer while some nanocarriers may not release the drug. In the case of acne, the hair follicle itself is considered as a target site. So, recently they

have said that the nanocarriers can be used for follicular and trans-follicular drug delivery system [25].

- It is said that pharmaceutical ingredient can be delivered by various routes like intravenous, intramuscular, oral, topical using SLN (Solid Lipid Nanoparticles) as a novel drug delivery system. SLN has several advantages like [26].
  - ✓ Protecting the drug from chemical degradation.
  - ✓ Releasing of the drug in a controlled manner.
  - ✓ Retards the water loss from the skin (occlusive property).
  - ✓ SLN blocks the UV (Ultra violet) rays and acts as a physical sunscreen by scattering the sunlight.
  - ✓ They do not have any biotoxicity.
- Large-scale production can be done readily.
- Solid lipid nanoparticles are found to be the viable alternative to liposomes because of the important features of SLN like the ability to target a particular site, penetrating capacity, enhanced physical stability and low cost as compared to that of phospholipids.
- SLN has also got advantages compared to that over fat emulsions and polymeric nano-particles [27].

4. Microsponge:

- Microsponges are used as a carrier for topical delivery of drugs. They are porous spherical microparticles with a size range of 5-300 µm. They are capable of entrapping a broad range of active pharmaceutical ingredients.
- A microsphere act as microscopic sponge and stores the active ingredient were its release is triggered when they are applied on the skin.
- Rubbing, concentration gradient, application of pressure and elevated skin temperature, all these factors act as a triggering agent in the release of the drug into the skin. A microsponge appreciably decreases the skin irritation without affecting the efficacy of the drug by preventing the excessive buildup of drugs in epidermis and dermis layer.
- Microspheres along with the drug or active ingredient are incorporated into various formulations like creams, gels, powders, and lotion. The porous surface present on the microsponge helps the drug to release in a controlled manner which is useful in decreasing the irritation as well as improving the efficacy of drugs like benzoyl peroxide [28].
- A study demonstrated that the production of ethylcellulose microparticles containing Benzoyl peroxide has controlled the release of benzoyl peroxide into the skin. Emulsion solvent diffusion method was carried out for the preparation of microparticles. The diffusion of solvent from the microparticles surface leads to the formation of pores thereby forming the microsponges.
- In order to construct a microparticle with a desired characteristic like an *in-vivo* release, certain parameters should exist like:
  - ✓ The selected method should produce a high yield of microparticles.
  - ✓ Should not undergo an agglomeration.
  - ✓ High encapsulation of the core substance.
  - ✓ The release of drug from batch to batch should be reproducible.
  - ✓ Should be capable of altering the *in-vitro* release when the process parameter is altered.
- Researchers said that the size of the microparticles is very large and they cannot pass through stratum corneum thereby remaining on the surface of the skin and releasing the drug in a gradual manner. The excessive accumulation of the drug on the epidermis is avoided due to the above release pattern and thereby increases the safety of the drug [29].
- The release of the active ingredient from the microsponge depends on pore diameter as well as the number of pores. The diameter of the pore may affect the drug release by either slowing down or improving the migration of drug from microsponge into the vehicle into which the compound is dispersed.

- It has been stated that the release rate of the drug will be lower when the microsponge is having low porosity, low surface area, and low volume [29].

5. Fullerene: Fullerene is having a cage structure, which is a unique structure. It is having a spherical carbon molecule. Compared to other antioxidant compounds, fullerene is having a hundred times greater activity. It is said that, the fullerene can be used in treating neurodegenerative disorders and arthritis. And it is also said that they can act effectively against various oxidative disorders.

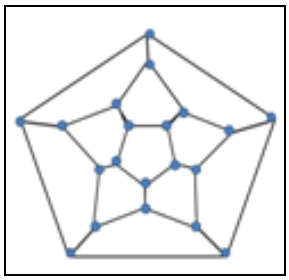


FIG. 13: 20-FULLERENE (C_{20}). DEPICTS THE SMALLEST FULLERENE

- Fullerene was observed to be effective against Acne vulgaris because of its penetration capacity (they can penetrate deep into the epidermis layer of skin) and it doesn't cause any skin irritation.
- Based on a study, it was observed that the fullerene was having a notable action against acne vulgaris. But, after 8 weeks of use it is said that, only 37.8% reduction in the inflammatory lesions had been observed which was lower as compared to adapalene gel. They have also declared that there was a significant decrease in the accumulation of neutrophils (responsible for the formation of pustules). And an *in-vitro* study revealed that 75µM of polyvinylpyrrolidone-fullerene was having an inhibitory action against sebum production. They have stated that fullerene exhibits its action by decreasing neutrophils accumulation as well as reducing sebum production [30].

Miscellaneous Therapies Available for the Treatment of Acne:

Hormone Based Therapy for Treatment of Acne:

- Acne can be effectively treated with hormonal treatments. In this treatment, the androgen level has been lowered and their effect on the sebaceous gland has also been opposed.
- Anti-androgens or androgen receptor blocker, agents in the ovary or adrenal gland which is responsible for decreasing the production (endogenous) of androgens such as estrogens, a combination of oral contraceptives, gonadotropin-releasing hormone agonists are used in hormonal therapies for the treatment of acne [31].

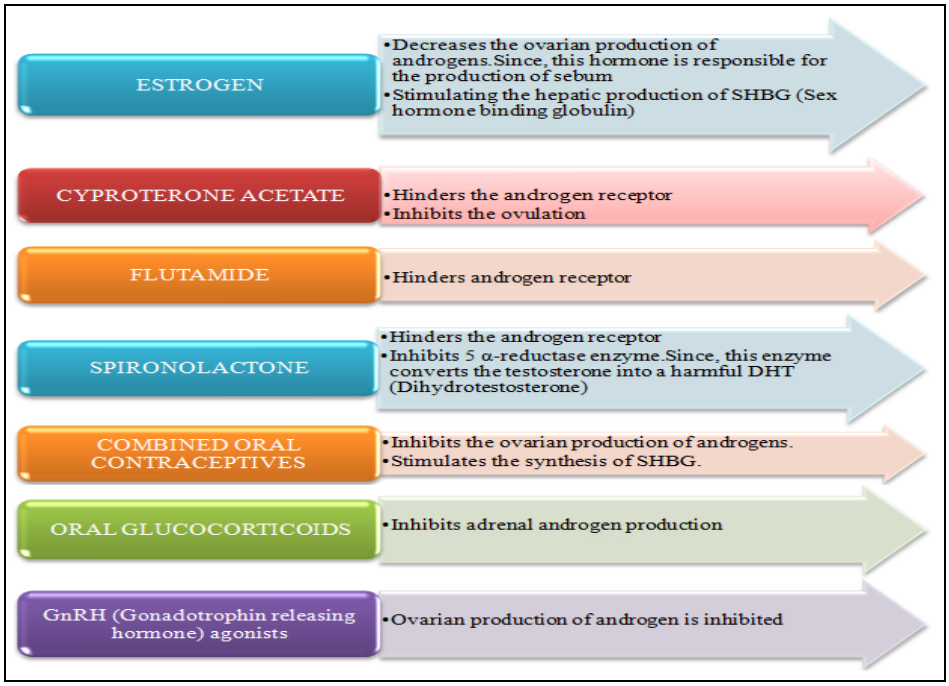
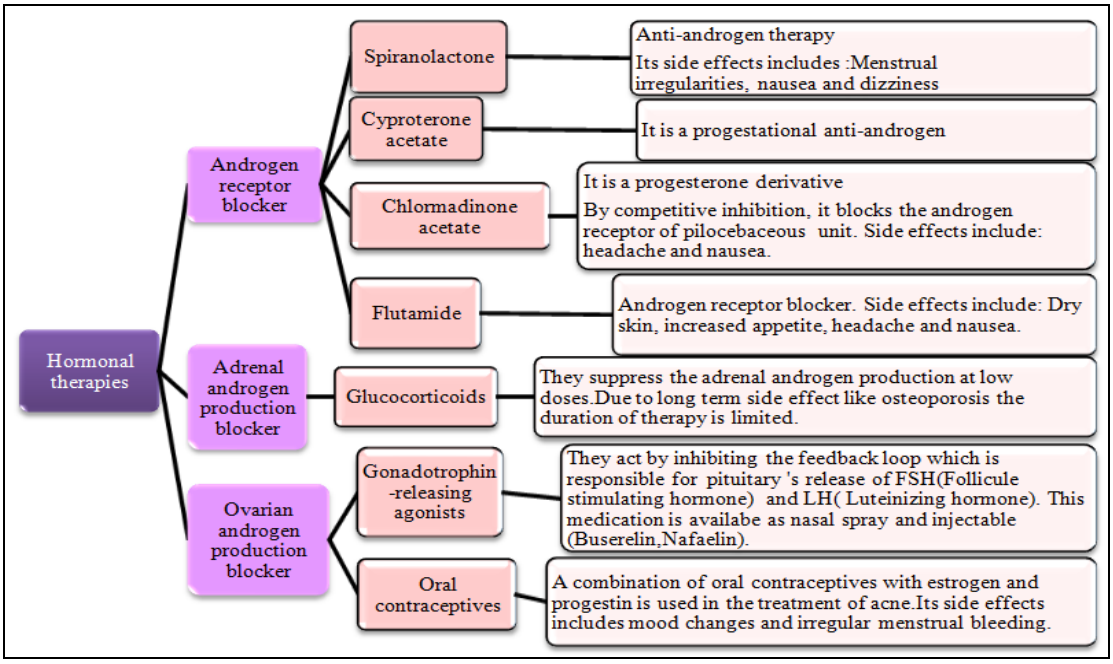


FIG. 14: THE MECHANISM OF ACTION EXHIBITED BY VARIOUS HORMONAL THERAPIES [31]



FLOW CHART 5: HORMONAL THERAPIES FOR TREATING ACNE CONTAIN SYSTEMIC MEDICATIONS LIKE [32]

2. Light-based Therapy:

- The treatment of acne with light-based therapy has gained popularity among the people. It plays an important role in treating the acne as well as the acne scars. Many therapies like; Chemical peeling, surgical excision and tissue augmentation with various fillers have been used to treat acne. But, all these treatments have some adverse effects because of which their use is limited. These treatments also lead to worsening of acne scars, incomplete removal of scars thereby showing an unsatisfactory result. However, these undesirable effects can be reduced by using the latest laser technology [33].

- Following are some of the techniques available: Visible light, specific narrowband light, intense pulsed light (IPL) and pulsed dye laser (PDL) [34].

- The Federal Drug Administration (FDA) in the US and the regulatory bodies in the European Union approved certain devices like [35]:

✓ Blue light	-	Approved in 2002
✓ Red light	-	In combination with blue light in the year 2005
✓ 450nm diode	-	Approved in the year 2002
✓ Long-Pulsed Dye Laser	-	Approved in 2004
✓ Photopneumatic 400-1200 nm broad band light therapy	-	Approved in 2007

- The main objective of light based therapy was to decrease the *Propionibacterium acne* count and also to suppress the sebum secretion from the sebaceous gland. In addition, to this, they also possess anti-inflammatory action.

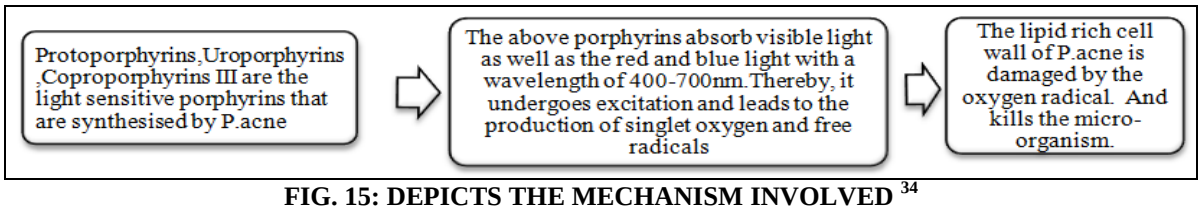


FIG. 15: DEPICTS THE MECHANISM INVOLVED [34]

- ALA (Aminolevulinic acid) is used for Photodynamic therapy. The ALA is topically administered to the affected area, and the protoporphyrin IX synthesized by *P. acne* get stimulated by the light source and leads to the formation of singlet oxygen and free radicals which is responsible for decreasing the *P. acne* count. The mechanisms involved in treating

acne by ALA-PDT are as follows: Decreasing the *P. acne*, directly damaging the sebaceous gland and altering the follicular keratinocyte shedding [36].

3. Natural Remedies:

- In order to overcome the side effects caused by synthetic drugs and bacterial resistance caused by antibiotic drugs, herbal extracts are used for the preparation of anti-acne products.
- Various formulations are prepared using natural plant extracts for the acne treatment like [37] *Rosmarinus officinalis, Pelargonium asperum, Ocimum tenuiflorum, Azadirachta indica* Temulawak (*Curcuma xanthorrhiza*).

Acacia nilotica

Calendula officinalis

Purified honey bee venom

***Mangifera indica* L. Kernel extract**

Matricaria chamomilla

***C. sativum* (Coriander oil is used)**

FIG. 16: DEPICTS THE NATURAL PLANTS EXTRACTS AVAILABLE FOR THE TREATMENT OF ACNE

- *Acacia nilotica* shows multiple medicinal properties because of which it has been used for decades. Research suggests that, *A. nilotica* act effectively against *P. acne* but, it is not effective against *Staphylococcus aureus* and *Escherichia coli*.
- *Matricaria chamomilla* possesses various properties like cytotoxic effect, anti-proliferative and anti-inflammatory effects. They have been used in the preparation of cosmetic products.
- Research suggests that *Rosmarinus officinalis* are having better activity compared to other plant extracts. It is obtained from rosemary; it is a culinary spice and possesses various properties like: anti-oxidant, anti-inflammatory, anti-carcinogenic, and anti-microbial activity.
- Research states that the ERE (Ethanolic rosemary extract) containing three main constituents like: Carnosol, carnosic acid and rosmarinic acid.
- The above constituents possess anti-inflammatory activity. And they act by inhibiting pro-inflammatory cytokines since; the *P. acne* causes the inflammation by the production of pro-inflammatory cytokines.
- Following are the examples of pro-inflammatory cytokines (*i.e.*) interleukin-8 (IL-8) and tumor necrosis factor-α which are produced by human monocytic THP-1 cells. *P. acne* induces the inflammation by stimulating the NF-κB (Nuclear factor kappa light chain enhancer of activated B cells) transcriptional activity in human monocytic cells which is responsible for the production of the above - mentioned cytokines [38]. Additionally, in another research, they suggest that rosmarinic acid has inhibitory action against IL-8 production [39].

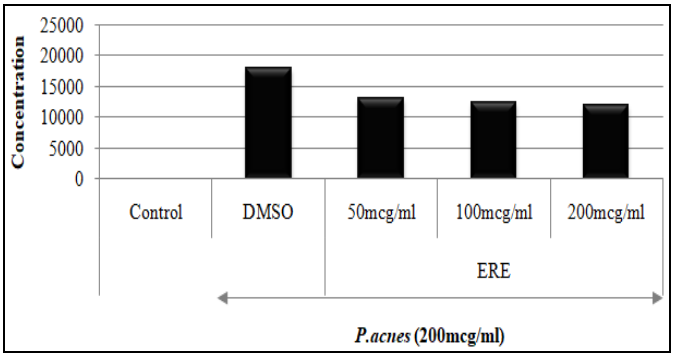


FIG. 17: GRAPH REPRESENTING THE INHIBITORY ACTION OF ETHANOLIC ROSEMARY EXTRACT ON THE PRODUCTION OF IL-8

- Purified honeybee venom (*Aspis mellifera* L.): Bee venom is obtained from the honey bee. It has been used in cosmetics product for its anti-aging, anti-inflammatory and anti-bacterial property. The bee venom in the pure form is collected from honey bee with the help of bee venom collector.
- Research reveals that bee venom exhibits anti-microbial activity by decreasing the number of colony forming unit by *P. acne*.

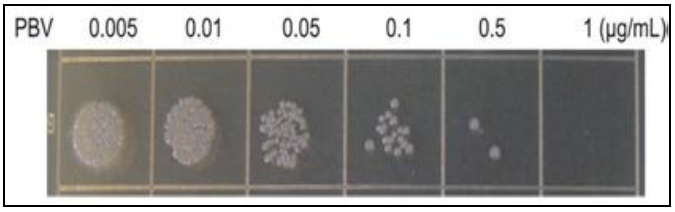


FIG. 18: THE IMAGE SHOWS THE INHIBITORY ACTION OF PBV AGAINST THE BACTERIAL COLONIES

- It shows that as the concentration of PBV (Purified bee venom) increases, the no of colonies formed by bacteria decreases. (Picture was reproduced from "Effects of cosmetics containing purified honeybee (*Apis mellifera* L.) venom on Acne vulgaris").
- Based on electron microscopy the anti-microbial activity of PBV was determined in a research. It uses both SEM (Scanning electron microscope) and TEM (Transmission electron microscope) in order to determine the structure of bacteria before and after the treatment with PBV. In this, the structural integrity of bacteria was lost after the treatment with PBV [40].
- *Mangifera indica* L. Kernel extract: *Mangifera indica* L. is also known as mango. In the study, they state that *Mangifera indica* possesses different properties like anti-microbial, anti-inflammatory, and anti-oxidant property. In addition to this, the ethanolic extract of *M. indica* also has inhibitory action against *P. acnes*. Mango contains polyphenolic compounds like gallic acid. Studies state that *Mangifera indica* L. possesses toxic action against various micro-organisms due to the presence of hydroxyl group in the gallic acid. And they also reveal that the microorganism which causes acne is inhibited by this compound (*Mangifera indica* L.) through inhibiting the IL-8 secretion [41].
- *Coriandrum sativum*: They possess several activities like anti-inflammatory, anti-oxidant, and anti-scarring properties due to the existence of salicylic acid. In a research, the antibacterial activity of coriander oil was determined by using the disc diffusion method. They have also revealed that the zone of inhibition for *P. acne* was more as compared to that of *S. epidermidis (Staphylococcus epidermidis)* [42].
- Wild bitter melon (WBM) is consumed as a vegetable and it contains various components like phenolics, alkaloids, and steroidal glucosides. An article published in the "Journal of Food chemistry" showed that the *in-vitro* studies using ethyl acetate extract of WBM decreased the level of both pro-inflammatory cytokines as well as matrix metalloproteinase (MMP)-9 levels in case of *P. acne* [43]. MMP is considered as a group of zinc-dependent endopeptidases, which causes the degradation of ECM (extracellular matrix) components and its activity is controlled by TIMPs (Tissue inhibitors of matrix metalloproteinases) [44].
- Temulawak is a plant species which belongs to a ginger family. It shows anti-oxidant, anti-fungal and anti-bacterial activity. A study revealed that the Temulawak essential oil has more activity in inhibiting the *P. acne* growth. And they say that the lipase formed from *P. acne* also plays a role in causing inflammation and this lipase activity is inhibited by ethyl acetate extract. The essential oil in the flower bract which inhibits the lipase activity is α-curcumene and the growth of *P. acne* is inhibited by xanthorizol [45].

Problems Faced by Acne Patients: Our face will be the most obvious part of our outward appearance that will be noted by others during our speech or when communicating with others. When there is any discomfort in their appearance then they'll be facing various problems like:

Psychological Problems: It may spoil the self image of the patient, lowers the self- esteem, patient become frustrated, get anger, embarrassment and depression

Psychosocial Problems: The self consciousness that arise during a interpersonal relationship, feeling what others may think about their appearance, lacking self confidence, lack of involvement in academic performances, use of camouflage makeup, decreased dating and increased unemployment.

- Adolescence and early adulthood undergo depression when they are having a high risk of suicide. Whereas, when we consider other age groups they have got the following clinical features like loss of interest in work, unhappiness, having negative thoughts, not having a proper sleep, suicidal ideation [46].
- In a study, 4 instruments have been used in order to evaluate the patients before and after the treatment. The four instruments are as follows:
  1. Hopkins' Symptoms checklist.
  2. The National Institute of Mental Health Mood Scale.
  3. The Profile of Mood State Scale.
  4. Rosenberg's Self-Esteem Questionnaire.
- They observed a significant cutaneous improvement by using isotretinoin and also the psychologic improvement was observed in the anxiety score of Hopkins' Symptoms checklist and the National Institute of Mental Health Mood Scale.
- The following conditions are observed:
  - ✓ Decrease in their self-esteem/ self-confidence: When people have acne they feel like they are looking ugly and they lose their self-confidence. Especially, college students think that they are very insecure about going and talking in front of their classmates.
  - ✓ Problems that arise with the body image: Patient says that they feel shy to look at the mirror and it turns into a painful feeling for them.
  - ✓ Social withdrawal due to embarrassing situations: People feel embarrassed due to the negative impact on acne in the society.
  - ✓ Confused and frustrated: Sometimes people get confused because of the medication prescribed by practitioners saying that medication will cure their problem which actually doesn't have much impact on the acne treatment.
  - ✓ Patient's lifestyle gets affected: Patients are having limited lifestyle because of acne. People say that they feel embarrassed in the following situations: (eg): Sportsperson feels embarrassed to get into the gym, people feel insecure when they enter into a store to purchase eatables [47].
- A review was done by "Richard G. Fried & Amy Wechsler" on "Psychological problems in the acne patient" has got the following data [48]:
  - ✓ **Acne affecting the emotional behavior of patient:** Emotional impact of the patient depends on many factors including age, psychosocial developmental period, coping abilities, the clinical seriousness of the disease and, family as well as supporters. Due to the availability of a variety of Over the Counter medications and miracle-cures available online, people become confused and overwhelmed.
  - ✓ **Effect of Stress and Emotional Parameters on the Evolution and Development of Acne:** Elevated levels of glucocorticoids and androgens are observed due to stress and this elevated level of hormones can interfere with acne.
  - ✓ **Influence of Acne on Psychosocial and Functional Status:** Acne patients having a higher unemployment rate as compared to that of the control group (16.2% *vs.* 9.2%).

- ✓ **Acne Leads to the Manifestation of Psychiatric Disorders:** Acne leads to the development of 2 Types of psychiatric disorders like:

1. Primary psychiatric disorder: They are independent of acne. Examples: Obsessive-compulsive disorder, disorders related to anxiety and depression and, body dysmorphic disorder.
2. Secondary psychiatric disorder: Related to stress caused by acne. Examples: anger, anxiety and, depression.

- ✓ **High- Risk Patient: People who are High Risk Show the Following Behaviors (*i.e.*):** Having poor interpersonal skills, not involving in family functions and, changes in academic performances.
- ✓ **Identifying Patient who are at High Risk:** Identified by some of the characteristics like restricted verbal productivity, poor eye contact while communicating with others, anger and negative voicing and excessive picking of pimples.

Induction of Acne Vulgaris in Animal Model: Acne can be induced by 2 methods. The first method is by injecting the *P. acne* in animal and the second method is by using chemicals. Various animal models are available for mimicking the inflammatory response of human acne starting from mice, rabbit and even Mexican hairless dog.

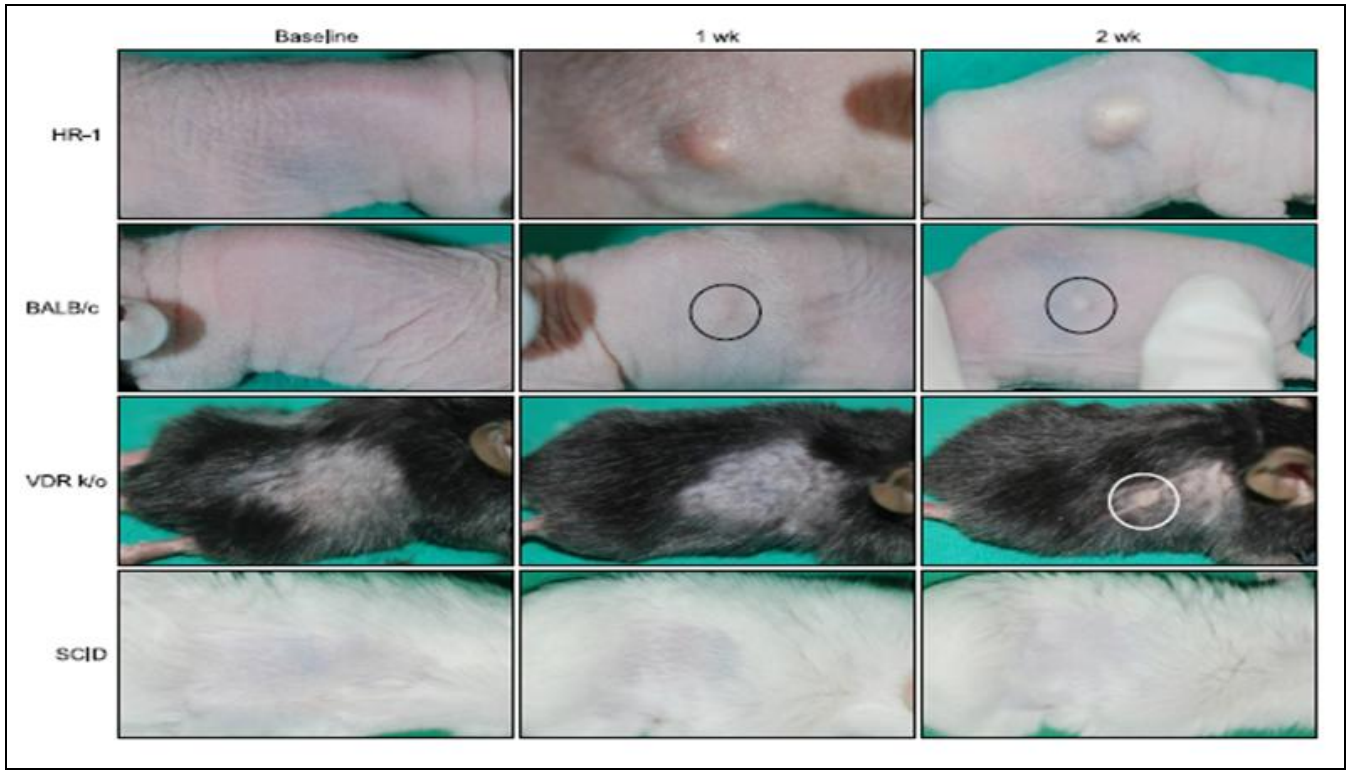


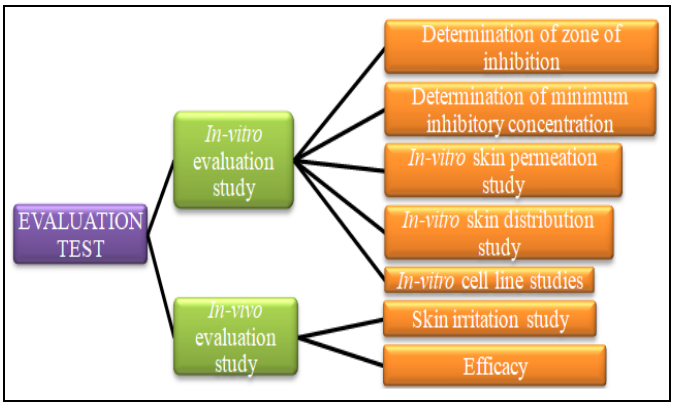
FIG. 19: PICTURE REPRESENTS THE INFLAMMATORY RESPONSE AFTER INJECTING *P. ACNE* IN DIFFERENT STRAINS. (PICTURE WAS REPRODUCED FROM "HR-1 MICE: A NEW INFLAMMATORY ACNE MOUSE MODEL")

- A study carried out using the HR-1 Mice states that the HR-1 mice are having more inflammatory response as compared to that of others like: BALB/c, VDR k/o- vitamin D receptor-knockout mice and SCID-Severe combined immune-deficiency mice.
- All these mouse strains were injected with 10^8 colony forming unit/ µl *Propionibacterium acnes* suspension. And they observed that the inflammation was more in HR-1 mice. The reason behind this is that the expression of inflammatory mediators such as interleukin-1ß, Matrix metalloproteinase 9 (MMP 9), and toll like-receptor-2 were more in HR-1 mice as compared to that of other 3 strains [49].
- All most in every study, acne has been induced by injecting *Propionibacterium acne* bacteria intradermally.

- In another study they have injected the Formalin killed *P. acne* in female Sprague-Dawley rats and they have determined the ear thickness in order to find out the inflammation caused by the bacteria. And they found out that one day after injection of bacterial strain CN6134, the ears of the rat was 2-3 times thicker as compared to that of the saline-injected controls. Lesion counting was also determined with the help of microscope [50].
- Acne can also be induced without injecting *Propionibacterium acne* in animal. An alternative method for this is inducing acne with the help of chemicals.
- A study has been carried out to induce pustules (moderate form of acne) in rabbit. In this study they have stated that the pustules can be induced by application of 8 chemicals including sodium lauryl sulphate, mercuric chloride, ammonium fluoride, croton oil, sodium arsentate, nickel sulphate, potassium iodide and, benzalkonium chloride.
- These chemicals were applied individually on the rabbit skin at different concentrations. In this study, they have taken rabbit model because they are applying chemical on different areas of the animal including posterior right, posterior left, anterior left, anterior right and also mid-right as well as mid-left. They have observed a reproducible result (pustulation) only in the case of sodium lauryl sulphate and mercuric chloride. Whereas, other chemicals showed the following characteristics because of which it was not considered as a best chemical to induce pustules.

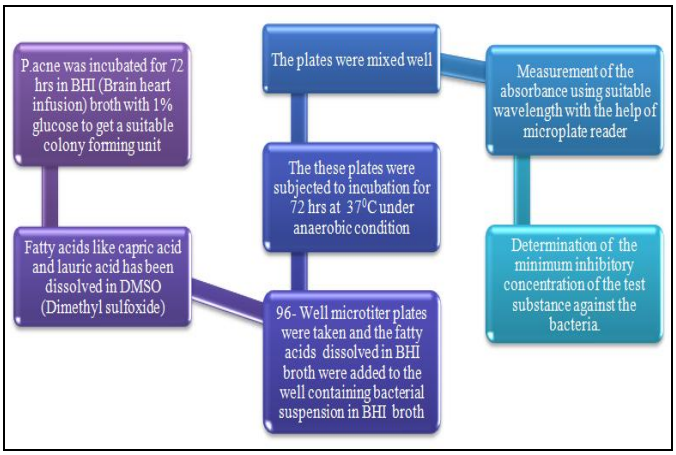
  The characteristics of other chemicals are as follows: Ammonium fluoride didn't give reproducible results, pustules produced by croton oil were more difficult to evaluate because along with pustules it has also produced edema as well as erythema, prior skin damage in the animal was required for chemicals like sodium arsentate, nickel sulphate and, potassium iodide to induce pustules, benzalkonium chloride produced only yellow staining and edema but it didn't produce any pustules. After considering all these issues they have concluded that only mercuric chloride and sodium lauryl sulphate readily produces pustules [51].

Evaluation Studies:



FLOW CHART 6: EVALUATION STUDIES

- Flow chart-6 describes both *in-vitro* and *in-vivo* evaluation test for acne.
- Since, the main cause for acne is bacterial infection the traditional method for antibacterial study *viz*., zone of inhibition and minimum inhibitory concentration can be used. In addition, *in-vitro* skin permeation and *in-vitro* skin distribution study can also be used for evaluation of the test substance.
- In the *in-vivo* evaluation, the animal models can be used to determine the skin irritation and efficacy of the test substance. A study was conducted by "Wen-Cheng Huang" with the use of *propionibacterium acne* for the determination of the above said evaluation which describes the method of incubation and inhibition [52].

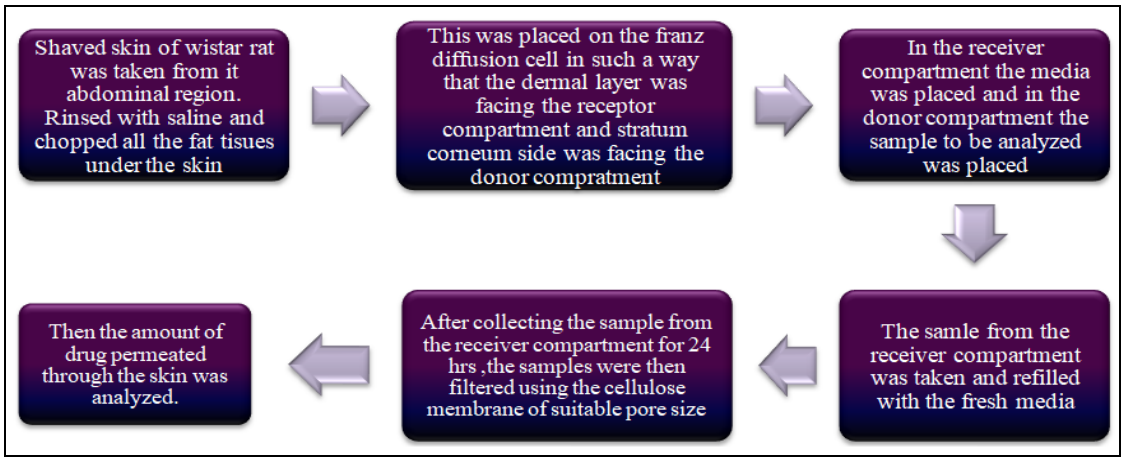


FLOW CHART 7: FLOW CHART REPRESENTS THE DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

Minimum Bacterial Concentration [52]:

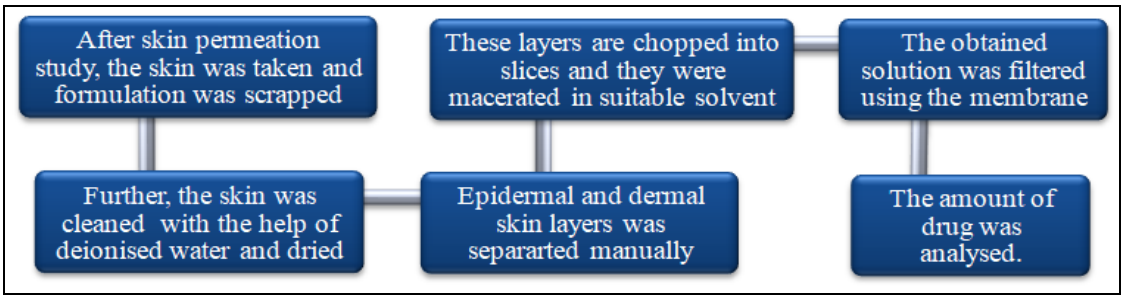
- In the previous study along with the minimum inhibitory concentration, they have also determined minimum bacterial concentration (MBC).
- In which they have incubated the *P. acne* along with fatty acid at various concentration in PBS (Phosphate buffer saline solution) on a 96 well plate for 5 h. After incubation the mixture was diluted with the help of PBS and further spotted on BHI agar plates.
- Further, the colony forming units were counted. The same was used for minimum bacterial concentration required for the study. The study involved the use of fatty acids for the inhibitory effect.

***In-vitro* Skin Permeation Study:** [27] The following flow chart describes the method used for the skin permeation study by "Amit Kumar Jain Ashay Jain Neeraj Kumar Garg Atul Jain Som Akshay Jain Rajeev K. Tyagi Govind P. Agrawal".



FLOW CHART 8: *IN-VITRO* SKIN PERMEATION STUDY

Skin Distribution Study: [27] The following flow chart describes the method used for the skin distribution study by "Amit Kumar Jain Ashay Jain Neeraj Kumar Garg Atul Jain Som Akshay Jain Rajeev K. Tyagi Govind P. Agrawal".



FLOW CHART 9: SKIN DISTRIBUTION STUDY

***In-vitro* Cell Line Studies:** Mainly to understand the pathophysiology of acne, 3 cell lines are available like SZ95, SEB-1 and SEB-E6E7. These three are considered as an immortalized human sebaceous gland cell line. In addition, to pathophysiology, they are also helpful in finding out the activity and regulation of sebaceous gland and also in the treatment of disease like acne [56].

SZ95 has been widely utilized for the acne investigation. In 1999 "Christos C. Zouboulis" cultured human sebocytes from an 87 year old women and cultivated and further transfected with simian virus-40 large T antigen for the immortalization. These cells resembles to that of the human sebocytes [58].

In 2017 "Yasaman Mirdamadi" carried out a study and revealed that the isotretinoin suppresses SZ95 sebocytes cell proliferation [59]. In the year 2003 the second immortalized human sebaceous cell line was discovered by "Diane Thiboutot". The second

cell line is SEB-1 which was obtained from the sebaceous gland of normal skin of 55 year old men [57]. Finally, the third immortalized sebaceous cell line named SEB-E6E7 was obtained from adult human facial skin [57]. They were transfected with HPV16 E6 and E7 genes.

Apart from the above three cell lines, there is another cell line named HaCat cell line which is called as a human keratinocyte cell line. "H. Kum" has carried out a research to determine the anti-acne properties of phloretin both *in-vitro* as well as *in-vivo*. In this work they have considered HaCat cells for their *in-vitro* cell line studies. The cells were cultured in Dulbecco's modified Eagle's medium. Inflammation in the HaCat cells have been produced by p.acne- induced inflammatory mediators like prostaglandin and COX-2 (cyclooxygenase- 2). Further, they have examined the effect by using ELISA (Enzyme-linked immunosorbent assay) [15].

***In-vivo* Evaluation Study:**

1. *In-vivo* Skin Irritation Study: [53] *In-vivo* skin irritation studies has been carried out by "SHREYA NIKAM". The study has been carried out in the following manner. In order to check the primary skin irritation study, Albino rats were taken and divided into equal groups as test and control. Hairs at the back of the rats were clipped 1 day prior the experiment. The test substance at different concentration was applied on the animal and they were observed for 7 days.

TABLE 3: BASED ON THE SCORING PATTERN THE SEVERITY OF IRRITATION HAS BEEN DETERMINED

S. no.	Score	Severity of skin irritation
1.	A	No reaction
2.	B	Slightly patchy erythema
3.	C	Moderate but patchy erythema
4.	D	Moderate erythema
5.	E	Severe erythema with or without edema

2. Efficacy: [26] A study has been carried out by "Pouran Layegh 1, Navid Mosallaei 2, Danial Bagheri 2, Mahmoud Reza Jaafari 3, Shiva Golmohammadzadeh" in order to compare the efficacy of the prepared (Isotretinoin solid lipid nanoparticle) formulation with that of marketed formulation in a human population. IT-SLN was compared with Isotrex (*i.e.*: marketed formulation) to find out the efficacy in curing the acne patients. The treatment regimen was accompanied with clindamycin 2% solution. IT-SLN was more effective as compared to marketed formulation. In addition, the global assessment scores were also determined and the scoring was given in the following manner: 0- Completely cleared, 1- Almost cleared, 2- Marked response, 3- Moderate response, 4- Slight response, 5- Condition unchanged, 6- Condition worsened.

The recovery percent was calculated using the below formula:

Lesion count at the base line - Lesion count after the medication/ Lesion count at the baseline × 100

The IT-SLN was having higher recovery rate as compared to that of isotrex. And it was concluded that after 8 weeks IT-SLN had better treatment profile in both inflammatory as well as non-inflammatory lesions. Additionally, the global score for 70% population was 0 and 1. In a wider point of view 54 and 46 % of patient who were treated with IT-SLN had a global score of 1 and 2 respectively. Whereas, 26 and 66% of patient who were treated with isotrex was having a global score of 2 and 3 respectively.

CONCLUSION: Though, Acne is a common inflammatory disorder among the adolescent age group, it affects people both psychologically and psychosocially. It may also lead to depression and suicidal attempts. We have included the possible consequences and methods to overcome the same.

Combination therapies are available in the market in order to achieve improved efficacy and to decrease the treatment time. Instead of going for a monotherapy with the conventional delivery system we can choose either a combination therapy or product which is obtained by implementing nanotechnology for improved efficacy.

ACKNOWLEDGEMENT: The authors would like to thank Department of Science and Technology- Fund for Improvement of Science and Technology Infrastructure in Universities and Higher Educational Institutions (DST-FIST), New Delhi for their infrastructure support to our department.

CONFLICT OF INTEREST: Authors declare that there no conflict of Interest.

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How to cite this article:
Janani SK and Sureshkumar R: A comprehensive review on acne, its pathogenesis, treatment, *in-vitro* and *in-vivo* models for induction and evaluation methods. Int J Pharm Sci & Res 2019; 10(7): 3155-77. doi: 10.13040/IJPSR.0975-8232.10(7).3155-77.