FORMULATION AND EVALUATION OF HERBAL BASED MICRONEEDLE PATCH FOR AN ACNE INFECTION

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ABSTRACT:
The aim and objective of this study to formulate and evaluate the herbal based microneedle patch for an acne infection here, totally two herbal extracts were used (ie) hydroalcoholic extracts of Curcuma longa and Azadirachta indica, both the extracts can potentially inhibits the activity of many bacterial pathogens, these extracts already used in many cosmetic formulations for to control the acne infection. PVP and PVA were used as polymer for dissolving microneedle patch because of its better compatibility studies and physical nature. There are three different microneedle patches were formulated (F1, F2 and F3) and standardization procedure were done with this patches. By the results of standardization F2 formulation is better one, it was selected for further evaluation studies of SEM analysis and antimicrobial studies. Till now there are 11 types of bacterial stains prominently affects the human population, among these two stains (ie) Staphylococcus aureus, Micrococcus luteus were collected Antimicrobial study was performed using F2 microneedle patch, it’s potentially inhibits the growth of microorganism. In future in-vitro drug releasing study and in-vivo animal studies can be continued for F2 formulation.

Key words: Microneedle patch, Novel dosage forms, TDDS, Acne infection, SEM analysis.

INTRODUCTION:

Acne vulgaris (AV) is chronic inflammations of the pilosebaceous unit with clinical polymorphic lesion consist of non-inflammatory (open and closed comedones) and inflammatory lesions (papules, pustules, and nodules) with varying degree of inflammation. AV commonly is self-limiting disease and often found in the adolescence period [1].

The pathogenesis of acne vulgaris is multifactorial, including increased sebum production, comedogenesis, Propionibacterium acnes proliferation, and inflammation. P. acnes play an important role not only in the process of inflammation but also in the formation of comedones. P. acnes contribute to the inflammatory nature of acne by inducing monocytes to secrete pro-inflammatory cytokines including interleukin (IL)-1b, IL-8, and tumour necrosis factor (TNF)-α [2].

Acne vulgaris is one of the most common skin diseases, predominantly seen in adolescence and also a multifactorial disease in which Cutibacterium acnes is thought to play an essential role in the pathogenesis of inflamed lesions [3].

Lesions occur primarily on the face, neck, upper back and chest. When assessing the severity of the acne, one needs to consider the distribution (back, chest, and upper arms), type and number of lesions (comedones, papules, pustules, nodules) and the presence or absence of scarring [4].

It affects areas containing the largest oil glands, including the face, back, and trunk. Propionibacterium acnes (P. acnes), an anaerobic pathogen, plays an important role in the pathogenesis of acne. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils [5].
Propionibacterium acnes is a gram-positive and anaerobic bacteria that colonies in the human skin’s sebaceous glands and hair follicles [6].

PATHOPHYSIOLOGICAL FACTORS OF ACNE:

The pathophysiological factors that cause the development of acne are

➢ Excessive sebum production,
➢ Abnormal keratinisation and desquamation of pilosebaceous follicular epithelium (comedogenesis),
➢ Follicular proliferation of propionibacterium acnes and
➢ Inflammation [7].

CAUSES OF ACNE [8]

Table 1.2 – Causes of acne

<table>
<thead>
<tr>
<th>Causes</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>Microorganism like P. acne is one of the causative agents for acne. They have ability to adapt the abnormal production of oil, inflammation and inadequate sloughing of acne pores.</td>
</tr>
<tr>
<td>Diet</td>
<td>Acne vulgaris is seen to be associated with foods which have high glycaemic index like milk, salt, chocolates etc. studies have shown the relation of obesity with acne.</td>
</tr>
<tr>
<td>Genetics</td>
<td>In some peoples, the cause of acne might be genetic rate of acne is seen among first degree relatives and in twin studies as well. The genes, which attributed to acne, are polymorphisms in IL-1α, TNF-α and CYP1A1.</td>
</tr>
<tr>
<td>Hormonal changes</td>
<td>Puberty and menstrual cycles which cause hormonal changes and this contributes to acne vulgaris. Androgen is the sex hormone that increases during puberty and pregnancy; it may produce more sebum in follicular glands. Also anabolic steroids can lead to development of acne vulgaris in adult women.</td>
</tr>
<tr>
<td>Psychological causes</td>
<td>Several researchers have suggested the relationship between stress and acne severity. An increase in stress level can affect the acne flare.</td>
</tr>
</tbody>
</table>

TRANSDERMAL DRUG DELIVERY:

TDDS was introduced more than 200 years ago, it is only recently that the method appears to have reached a practical stage. Closely related term is percutaneous delivery, which is transportation of drugs into the tissues, with an attempt to avoid systemic effects. The concept of percutaneous absorption of drugs was given by Stoughton in 1989 [9].

MICRONEEDLE PATCHES:

Microneedle (MN) patches are topical Transdermal drug delivery systems consisting of arrays of micrometer-sized needles that create transport pathways through the skin to deliver a variety of therapeutic molecules, such as small molecules, (bio)macromolecules, and nanoparticles (vesicles) [10].

Microneedle (MN) is a micron scaled needle-like structure which measures maximum up to a length of 1mm with thickness in microns. MNs pierce the skin in a non-invasive and painless way to deliver drugs beneath the epidermis. MNs do not trigger the pain receptors and do not enter the blood vessels which enable painless delivery without damage to the blood vessels [11].

MATERIALS AND METHODS

Collection of plant material

Crude plant powdered material was purchased from Dhanvandhi Siddha Pharmacy and check basic quality control parameter then powdered material was subjected to extraction process by Soxhlet apparatus.

Procedure for extraction
The finely powder neem undergo the Soxhlet extraction method by using hydroalcoholic solvent (70% ethanol and 30% water) for a duration of three successive days (72hrs), the temperature should not exceeding more than 100°C. The dark green colour hydroalcoholic extract was collected, filtered and the filtrate was subjected to concentrate under reduced pressure using rotator vacuum evaporator. Finally the dried extract was stored in desiccator.

The same procedure was followed for the crude powdered drug turmeric \[^{[12]}\].

**PROCEDURE FOR FORMULATIONS**

**Fabrication of microneedle mould cavities**

Microneedle moulds are prepared by using mixture of resin and hydrate material (emseal). The resin and hydrate material taken in a 1:1 ratio and mixed well properly by hand mixing. The mixed preparation was making a flattened surface and micro sized derma roller was rolled in a surface. The surface was between 1.5mm diameter was prepared cut and coined shaped mould array was prepared. After preparation of mould arrays, they were dried for 24 hours at room temperature for formation of hard solid moulds \[^{[13]}\].

![Fig 1.1 - Microneedle mould](image)

**Formulation of microneedle patch**

The polymer solution was prepared using PVP: PVA ratio is given in the table 1.1. The required quantity of distilled water was taken and maintains the temperature between 80 to 90°C using hotplate magnetic stirrer. To this PVA was added, and stir it well, simultaneously take another beaker and add required quantity of PVP, little amount of ethanol and herbal extract mix it well until reaches the clear solution now add the PVP mixture into PVA solution and mix it \[^{[46]}\]. Prepared polymer gel solution was transferred into microneedle moulds and immediately centrifuged at 3000 rpm for 30 minutes for even and uniform distribution polymer solution to the microneedle cavities and to remove the air spaces. After completion of the process the mould cavities was removed from centrifuge tube, dried for 2 hours in hot air oven and then remove the patch from microneedle mould kept in desiccators \[^{[13]}\].

Note: 10 ml of polymer solution contains 2 mg of turmeric extract and 2 mg of neem extract.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>INGREDIENT</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Azadirachta indica extract</td>
<td>2 mg</td>
<td>2 mg</td>
<td>2 mg</td>
</tr>
<tr>
<td>2.</td>
<td>Curcuma longa extract</td>
<td>2 mg</td>
<td>2 mg</td>
<td>2 mg</td>
</tr>
</tbody>
</table>

Table 1.1 – Composition of Microneedle patch
STANDARDIZATION OF THE MICRONEEDLE PATCHES

Physical Examination of patches:

All the formulated patches were evaluated visually for appearance in terms of brittleness, transparency, stickiness, flexibility, and homogeneous appearance \[^{[14]}\].

Surface pH:

The patches were allowed to swell by keeping them in contact with 1 ml of distilled water for 2 h at room temperature and pH was noted down by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1 min \[^{[14]}\].

Weight Uniformity:

Weight uniformity was tested by selecting ten patches of area 1.5 cm\(^2\) randomly out of each formulation and the average weight was determined. The individual patches were weighed and compared with the average weight \[^{[14]}\].

Folding Endurance:

The folding endurance of patches was evaluated by repeatedly folding the 1 × 1 cm film at the same point until it broke. The 1 × 1 cm of the film was taken from the centre as well as from the edge of the patch. The test was conducted on three randomly selected patches from each formulation \[^{[14]}\].

Percentage Moisture Content:

The percentage moisture content was determined for each formulation. A film of 1 × 1 cm was taken from each patch. These films were weighed individually using a digital weighing balance. These polymeric films were then placed in labelled Petri dishes and stored in desiccators containing silica beads at 25 °C. The films were weighed until a constant weight was achieved. The percentage moisture content was calculated using the following formula \[^{[14]}\],

\[
\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]

Percentage Moisture Uptake:

The percentage moisture uptake was determined for each formulation. Transdermal film of 1 × 1 cm was cut from each patch. Films were weighed individually by using a digital weighing balance. These films were then placed in labelled Petri dishes and stored in a humidity chamber at 25 °C with 84% relative humidity (RH). The transdermal films were continuously weighed until a constant weight was achieved. The percentage moisture uptake was calculated using the following formula: \[^{[14]}\],

\[
\text{Percentage moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

Drug Content:

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>PVA</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>PVP</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>Qs</td>
<td>Qs</td>
<td>Qs</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol</td>
<td>Qs</td>
<td>Qs</td>
<td>Qs</td>
</tr>
</tbody>
</table>
A specified area of one microneedle patch was dissolved in phosphate buffer pH 7.4 and ethanol (1:1). The clear solution was obtained by sonicated the solution on bath sonicator. After filtration the drug was estimated spectrophotometrically at wavelength of 200 to 800nm using UV 1800 double-beam spectrophotometer and drug content was determined \[14\].

**Scanning Electron Microscopy (SEM) Analysis of microneedle patch:**

The scanning electron microscope was used to understand the surface morphology and size and shape of the MNs. The magnification, tilt degree, spots, width and other imaging characteristics were reported on the SEM images. Device = VEGA3 LMU, Magnification = 51.572 \[14\].

**ANTIMICROBIAL ACTIVITY OF MICRONEEDLE PATCH ANTIBACTERIAL ASSAY**

**Test bacterial organisms:**

The test microorganisms used in the study were *Staphylococcus aureus*, *Micrococcus luteus*. The morphologically identified microorganisms were then subjected to a biochemical test for identification.

**Antibacterial assay using agar well diffusion method:**

The well diffusion method was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates could solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly, and the inoculums could dry for 5 minutes. Wells were cut and 20 μl of the different concentration of test drug (25, 50, 75 and 100μl) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Chloramphenicol disc was used as a positive control.

**RESULTS AND DISCUSSION**

Formulation F1, F2 and F3 was successfully formulated with respective procedure and then the formulations F1, F2 and F3 was evaluated for its stability, so the basic standardization parameters were analyzed and the report was plotted in the table 1.2 and 1.3

<table>
<thead>
<tr>
<th></th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Table 1.2 - Formulation image**

**Table 1.3 – Standardization test result of microneedle patch**
<table>
<thead>
<tr>
<th>S.N O</th>
<th>PARAMETERS</th>
<th>F1 (1:1)</th>
<th>F2 (1:2)</th>
<th>F3 (1:3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Microneedle formation</td>
<td>Minimum microneedle formed</td>
<td>Maximum microneedle formed</td>
<td>Minimum microneedle formed</td>
</tr>
<tr>
<td>3.</td>
<td>Surface pH</td>
<td>7.7 ± 0.03</td>
<td>7.5 ± 0.03</td>
<td>7.2 ± 0.02</td>
</tr>
<tr>
<td>4.</td>
<td>Weight variation (mg)</td>
<td>0.0226 ± 0.00207</td>
<td>0.0236 ± 0.0024</td>
<td>0.0216 ± 0.002074</td>
</tr>
<tr>
<td>5.</td>
<td>Folding Endurance</td>
<td>&lt;300</td>
<td>&gt;300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>6.</td>
<td>Moisture Content (%)</td>
<td>3.02 ± 0.004</td>
<td>3.26 ± 0.002</td>
<td>3.68 ± 0.003</td>
</tr>
<tr>
<td>7.</td>
<td>Moisture Uptake (%)</td>
<td>6.43 ± 0.02</td>
<td>5.85 ± 0.02</td>
<td>5.10 ± 0.03</td>
</tr>
<tr>
<td>8.</td>
<td>Drug Content (%)</td>
<td>0.7 ± 0.003 (Curcumin)</td>
<td>0.8 ± 0.002 (Curcumin)</td>
<td>0.8 ± 0.004 (Curcumin)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7 ± 0.004 (Azadirachtin)</td>
<td>0.9 ± 0.003 (Azadirachtin)</td>
<td>0.7 ± 0.003 (Azadirachtin)</td>
</tr>
</tbody>
</table>
The totally three formulations were prepared (F1, F2 and F3) and evaluated by the standardization studies; among all the formulations F2 formulation was better than F1 and F3 formulation. Because of the maximum microneedle was formed in F2 formulation. So far, the better formulation F2 was choosed for further studies (i.e.) SEM analysis and in-vitro antimicrobial study.

**SEM ANALYSIS OF MICRONEEDLE PATCH (F2):**

The surface morphology of microneedle patch was examined by scanning electron microscope and the figures given the table 1.4

<table>
<thead>
<tr>
<th>S.NO</th>
<th>PARAMETERS</th>
<th>F2</th>
<th>IMAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Length of the microneedle</td>
<td>161.11µm</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>2.</td>
<td>Width of the microneedle base</td>
<td>162.05µm</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>3.</td>
<td>Distance between two microneedle</td>
<td>315.62 – 388.05µm</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
</tbody>
</table>

The scanning electron microscopy (SEM) had been used to study the morphology and dimension of microneedles. The result shows the formation of array, sharpness, length, width and diameter (base) of the microneedle. The needle has mechanical strength to breach the stratum corneum and deliver drug directly into the systemic circulation.

**ANTIMICROBIAL ACTIVITY OF MICRONEEDLE PATCH (F2):**

The herbal based microneedle patch (F2) was evaluated for in-vitro antimicrobial activity, here totally two bacterial strains were evaluated for this study, i.e Staphylococcus aureus and Micrococcus luteus. This study revealed that, the herbal based microneedle patch (F2) exhibits significant antimicrobial activity. The observed results were given in the table 1.5,1.6 and fig 1.2
Table 1.5 – Zone of Inhibition

<table>
<thead>
<tr>
<th>S. NO</th>
<th>MICROORGANISMS</th>
<th>ZONE OF INHIBITION (MM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100µl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td><em>Micrococcus luteus</em></td>
<td>17</td>
</tr>
</tbody>
</table>

GRAM POSITIVE BACTERIA

Fig 1.2 – Graphical representation of antimicrobial activity of microneedle patch

Table 1.6 - Pictorial representation of anti microbial activity of microneedle patch

<table>
<thead>
<tr>
<th>S.NO</th>
<th>BACTERIAL STRAIN</th>
<th>MICROBIAL CULTURE IMAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td></td>
</tr>
</tbody>
</table>
2. **Micrococcus luteus**

*Staphylococcus aureus*: The zone of inhibition of standard drug (Chloramphenicol) and test sample (microneedle patch) was found to be 24mm and 21mm respectively.

*Micrococcus luteus*: The zone of inhibition of standard drug (Chloramphenicol) and test sample (microneedle patch) was found to be 23mm and 17mm respectively.

The *in-vitro* antimicrobial study was so important for reduce the usage of animals in research work. Generally, four methods used for *in-vitro* antimicrobial assay. Here we choose well diffusion method, this study performed by using different concentration of the sample (25, 50, 75 and 100 µl) was used for this study and finally the *in-vitro* antimicrobial study shows significant action against *Staphylococcus aureus* and *Micrococcus luteus*. We can observe the maximum zone of inhibition at maximum concentration of the test sample. So, it gives dose dependent activity. The antibacterial activity of microneedle patch shows good action against *Staphylococcus aureus* and *Micrococcus luteus* but lesser than the chloramphenicol. This notable antimicrobial activity of microneedle patch may be exhibited by due to the presence of turmeric extract (curcumin) and neem extract (Azadirachtin).

**SUMMARY**

The aim and objective of present work is to formulate and evaluate herbal based microneedle patch for acne infection. It should kill acne bacteria. So, our research study focused on selections of pharmacologically potent herbal drugs (Neem and Turmeric), and it was subjected to suitable extraction.

Then the microneedle mould array was fabricated, by the help of mould array the microneedle patch was successfully prepared using three different ratio of polymer solution (F1, F2 and F3). This microneedle patch type is coming under the category of dissolving microneedle. This type of microneedles are generally facilitates rapid release of macromolecules and ease of administration for patients with one step application. Microneedles are tiny micron sized structures that upon application can breach the stratum corneum (SC) and penetrate to the upper dermal layers. Microneedles do not trigger the pain receptors and do not enter into the blood vessels, which enable painless delivery without damage to the blood vessels.

The basic standardization parameters like physical examination, microneedle formation, surface pH, weight variation, folding endurance, percentage moisture content, percentage moisture uptake and drug content of microneedle were done for all the formulation. The maximum microneedle was formed in F2 formulation so, it was subjected to further evaluation studies (i.e.) SEM analysis and antimicrobial studies. From the SEM analysis, we ensured that length, width and distance between two microneedle and the results claimed that the micro size of the needle. Then *in-vitro* antimicrobial study was performed using two different pathological strain (acne) *Staphylococcus aureus* and *Micrococcus luteus* the zone of inhibition was found to be 21mm and 17 mm respectively. We can observe the maximum zone of inhibition at maximum concentration of the test sample. So, it gives dose dependent activity. The antibacterial activity of microneedle patch shows good action against acne producing strains (i.e.) *Staphylococcus aureus* and *Micrococcus luteus*.

**CONCLUSION AND FUTURE PROSPECTS**

This is an attempt to develop herbal based microneedle patch with minimal combination of herbal extract, from the last 15 years, the deportment toward transdermal delivery of larger peptides, protein, vaccine compound as well as traditional molecule has led to the microneedle based product development. Globally, the arrival of various commercial microneedle products is highly anticipated. Microneedle may extend a remarkable impact on clinical medicine and cosmetic application over the upcoming future. From this research study we concluded that the herbal based microneedle patch having potential pharmacological action against an acne producing microorganism. Based on the results, F2 formulation was better than F1 and F3 formulation it was confirmed by evaluation studies. In future *in-vitro* drug releasing study and *in-vivo* animal studies can be continued for F2 formulation.

**REFERENCE**


