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Research Article

# FABRICATION AND SCREENING OF SOLID LIPID NANOPARTICLES-LOADED MICRONEEDLE PATCH FOR POLYCYSTIC OVARY SYNDROME TREATMENT

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**ABSTRACT**

**Objectives:** The study aimed to develop a novel microneedle (MN) patch for the transdermal delivery of clomiphene citrate. This approach seeks to facilitate sustained drug release through the skin, improving therapeutic efficacy and minimizing side effects associated with oral administration.

**Methods:** The patch was constructed using biocompatible polymers – polyvinyl alcohol, polyvinylpyrrolidone, and polyethylene glycol 400 – commonly utilized in medical applications. Each MN measured approximately 915 µm in height, optimized to penetrate the outer skin layer without causing pain. Durability was assessed by folding the patch repeatedly; it withstood nearly 300 folds before exhibiting signs of wear. Analytical tests confirmed that clomiphene citrate remained stable within the patch matrix, with no adverse interactions between the drug and the polymer components. In vitro experiments using simulated human skin models demonstrated a biphasic release pattern: an initial rapid release followed by a sustained release over 5 days. Human skin samples were employed to evaluate the patch's drug release profile, corroborating the in vitro findings. The patch's efficacy was tested on rats with letrozole-induced fertility issues, simulating human infertility conditions.

**Results:** Microscopic examination revealed uniformly shaped MNs of appropriate dimensions for effective skin penetration without discomfort. The patch demonstrated high durability, maintaining structural integrity after extensive mechanical stress. The drug and polymer components exhibited compatibility, ensuring the stability of clomiphene citrate within the patch. Approximately 89% of the drug was released over 5 days in vitro, while ex vivo tests showed a 78% release, indicating effective sustained delivery. In vivo studies indicated that the transdermal delivery of clomiphene citrate through the MN patch (MNP) resulted in prolonged drug activity and reduced side effects compared to oral administration.

**Conclusion:** The MNP developed in this study offers a promising alternative for the transdermal delivery of clomiphene citrate. Its design ensures painless application, sustained drug release, and improved therapeutic outcomes, potentially reducing the side effects associated with traditional oral administration.

**Keywords:** Nanoparticles, Microneedle, Transdermal drug delivery, Animal study, Clomiphene citrate.

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## INTRODUCTION

Transdermal drug delivery (putting medicine on the skin to absorb into the body) already works well for some treatments like hormone therapy, quitting smoking, and pain relief. However, it doesn't work for all types of medicine. Large and water-loving (hydrophilic) drugs such as proteins, vaccines, and peptides can't easily pass through the skin's outer layer, called the stratum corneum, which acts like a strong barrier [1,2].

Some methods such as using chemicals or scraping the skin can help drugs pass through, but they may also irritate or damage the skin. The big challenge is to get these important medicines into the body through the skin without hurting it [3,4,5].

Microneedles (MN) – tiny needles that barely poke into the skin – offer a promising solution. They make tiny, temporary holes that allow medicine to get in. Because they are so small, they cause much less pain than regular needles, and the amount of pain depends on how deep they go and how many are used [6-8].

Recently, scientists have started using MNs made from special materials that dissolve or break down in the skin. These include materials such as polyvinyl alcohol (PVA), carboxymethyl cellulose, and maltose. They work well for delivering protein-based drugs, but they dissolve very fast, which can cause the medicine to be released too quickly [9,10].

Another type of MN is made from biodegradable materials such as polylactid acid, polyglycolic acid, or polylactic acid-co-glycolic acid (PLGA). These break down more slowly, allowing the medicine to be released over time. However, making these can involve high heat or strong chemicals, which can damage sensitive medicines like proteins or vaccines [11,12].

To solve these problems, a new kind of MN patch (MNP) made from solid lipid nanoparticles (SLNs) has been developed. This type of patch is designed to deliver larger, delicate drugs like human growth hormone or vaccines that need to stay in the body longer. SLNs are very safe for the body, break down naturally, and don't cause harm. As they break down, they slowly release the drug, offering a steady effect over time. Depending on their size, SLNs can be removed from the body by the kidneys or broken down by enzymes [13,14].

There are a few different ways MNs are used:

- Solid MNs can make tiny holes in the skin. After removing them, a gel or patch with the drug is placed over the area[15]
- Coated MNs are covered in the drug and applied directly to the skin
- Biodegradable MNs are made to stay under the skin layer and slowly let the medicine out over time [16]
- Dissolving MNs melt under the skin surface and push the drug in through a mix of pressure and spreading.

MNs are made from a variety of materials. But because they are small, they can only carry a limited amount of drug, and it can take time for the

full dose to enter the skin. So, drug formulas must be specially designed for this kind of delivery [17-19].

**Aim of work**

Combining nanoparticles with a MNP offers a synergistic effect and enhances the delivery of clomiphene citrate. The nanoparticles can encapsulate the drug, and the MNs can facilitate its transdermal delivery, potentially improving absorption rates [20]. The combination aims to provide a more targeted and localized delivery of clomiphene citrate to the ovaries, potentially minimizing systemic side effects. Combining these technologies offer a more convenient and patient-compliance method of delivering clomiphene citrate compared to other traditional routes of administration such as:

Overcome swallowing difficulties exhibited by tablets and capsules

The skin heals more quickly where this is used than it does with a regular needle.

a. Localized delivery
b. Reduced systemic side effects
c. Steady and controlled release
d. Convenience and compliance
e. Avoidance of first-pass metabolism
f. Flexible and painless administration [21,22].

## MATERIALS AND METHODS

**Material**

MNPs were made using a mixture of PVA, polyvinylpyrrolidone (PVP), and polyethylene glycol 400 (PEG 400), along with SLNs loaded with clomiphene citrate. To create the molds for the MNs, a soft material called polydimethylsiloxane was used. Physical paramaters were observed in given below Table 1 [23].

The patches were made using a slightly modified two-step method:

First, PLGA and PVA (500 mg each) were mixed into 10 mL of dimethyl sulfoxide or methyl pyrrolidone, and stirred continuously for 3 h at 500 revolutions per minute (RPM). This mixture was poured into a round dish (Petri plate) and pressed using a mold punch. It was left to harden for 24 h to form the base structure [24,25].

In the next step, PVP, PEG 400, and PVA were mixed together in a specific ratio in two separate containers. Span 80 and Tween 80, which are surfactants that help mix oil and water, were added to the solutions. The mixture was stirred with a homogenizer at 1200 RPM for 15 min. After that, the SLN-loaded drug solution was poured into each mold [26].

The molds were then placed in a sonicator (a device that uses sound waves to mix and remove air bubbles) and treated in three 15-min cycles.

Once this was done, a drug-free layer of 10% PVA solution was poured on top of the MN molds to create a backing layer without the drug. The patches were then left out to dry on their own overnight at normal room temperature [27].

Finally, once dry, the patches were carefully taken out of the molds MNs were examined under a microscope to ensure the tips were sharp and the needles were evenly spaced and well-formed as shown in Fig. 1.

*Animal*

Female, non-pregnant, 4–5 days regular estrous cycle Albino Wistar rats [28,29].

*Chemicals*

Letrozole suspension, clomiphene citrate loaded MNP, saline.

**Animal study of MNP**

All the tests and procedures in this study were done with the right approvals. The research was checked and approved by the Institutional Animal Ethics Committee. Official permission was also taken from a government body called Committee for Control and Supervision of Experiments on Animals (CPCSEA), which oversees the care and use of animals in experiments in India. The study was conducted under Proposal Number: 921/PO/ReRcBi/S/05/CPCSEA.

**Experimental design**

Female albino Wistar rats, weighing between 200 and 220 g and showing normal reproductive cycles of 4–5 days, were selected for this study. All animals used were non-pregnant and kept under controlled laboratory conditions. The animals were kept in a place where the lights were on for 12 h and off for 12 h, with the air kept slightly moist, and they had unlimited access to regular food and clean water. The rats were randomly split into two groups, with three rats in each. To create a condition similar to polycystic ovary syndrome (PCOS), the animals were given a daily oral dose for 21 days. One group received normal saline (used as the control group), whereas the other group was given a suspension of letrozole, a medication known to induce PCOS-like symptoms in rats [30].

At the end of 21 days:

- Group 1: Animals continued on normal saline administration. Blood samples were collected to confirm the induction of PCOS through hormonal analysis.
- Group 2: Animals were treated with a single SLN-loaded MNP application, administered once every 5 days up to day 28. This treatment regimen was continued for 3 months. Blood samples were amassed periodically and analyzed for hormonal profiling.

**Estimation of physical parameters of microneedled patch**

*Physical estimation*

Physically MN was observed by structural equation modeling (SEM) study sample was given I-Con lab Navi Mumbai, Maharashtra. In the study height, length, width, and tip were estimated along with the surface of MNP [31]. As shown in Fig. 7.

Solubility and drug content of SLN loaded MNP.

MNP solubility is determined by dissolving a known mass in a fixed solvent volume (e.g., water or phosphate-buffered saline [PBS]) under stirring at 37°C. After equilibrium, undissolved particles were removed through centrifugation, followed by filtration. The dissolved fraction is quantified by drying the filtrate and weighing the residue. Solubility (mg/mL) is calculated as given in the below formula providing essential data for drug release studies. Solubility were observed in given below Table 2.

$$\text{Solubility} = \frac{\text{Dissolved mass (mg)}}{\text{Solvent volume (mL)}}$$

Drug content the drug content of the MNP is determined using the formula: As shown in Fig. 3.

$$\%\text{Drug content} = \frac{\text{Dissolved drug}}{\text{Total patch weight}} \times 100$$

*In vitro study*

Scientists tested how the drug comes out of the MNP in a lab using a special tool called a Franz diffusion cell. To act like skin, they used a thin piece of cellophane that had been soaked in water for 12 h before starting the test [28]. The MNP was placed in the top part of the device, and the bottom part was filled with a salty liquid (called PBS) that's similar to fluids in the human body. This liquid was kept at a warm temperature – around 35°C – to copy body heat, and it was stirred constantly at a steady speed (300 spins/min) [31]. To see how much

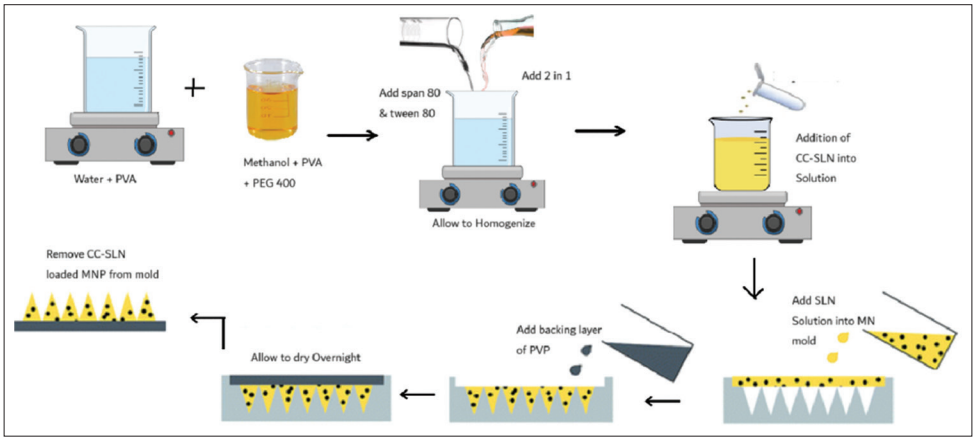


**Fig. 1: Preparation of microneedle patch**

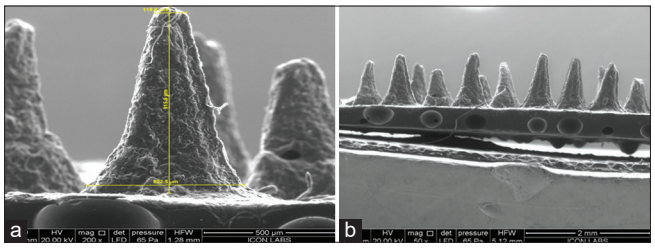


**Fig. 2: Microneedle height, width of needle (a), shows the structural equation modeling study of microneedle patch (b)**

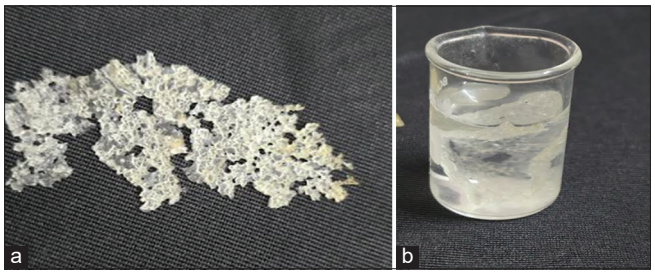


**Fig. 3: Result of drug solubility in patch (a), soluble patch in buffer (b)**

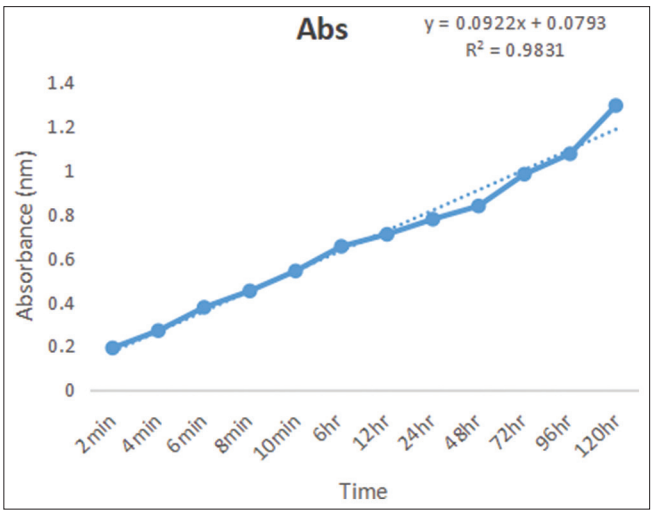


**Fig. 4: Graph shows *in vitro* study up to 120 h. Values represent mean absorbance standard deviation, calculated from triplicate measurements. A linear regression was applied, showing a strong correlation ($R^2$=0.9831)**

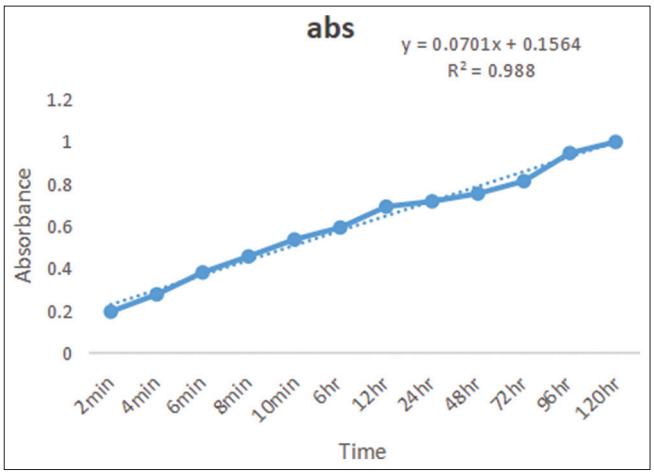


**Fig. 5: Graph shows *ex vivo* drug release up to 120 h. Absorbance values represent the mean±standard deviation from triplicate samples. Linear regression shows a strong correlation across time ($R^2$=0.988)**

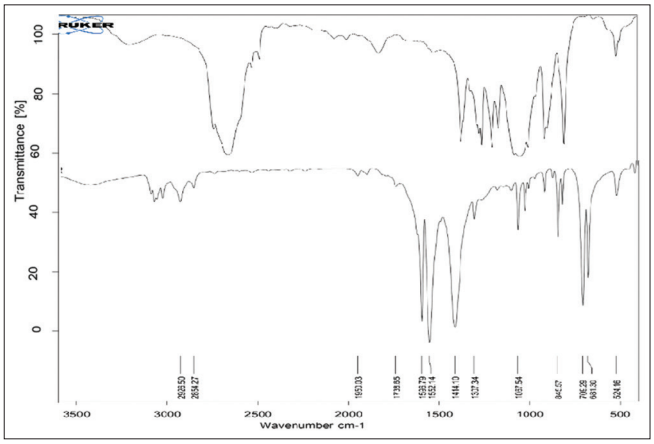


**Fig. 6: Graph shows Fourier transform infrared spectroscopy of pure drug and microneedle patch loaded solid lipid nanoparticles**

drug was released over time, small samples (1 mL each) were taken at set times – after 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 h. Each time a sample was taken, they added the same amount of fresh liquid to keep the conditions the same. The amount of drug released in each sample was measured using a ultraviolet (UV)-visible spectrophotometer [32]. The results were used to calculate the cumulative percentage of drug

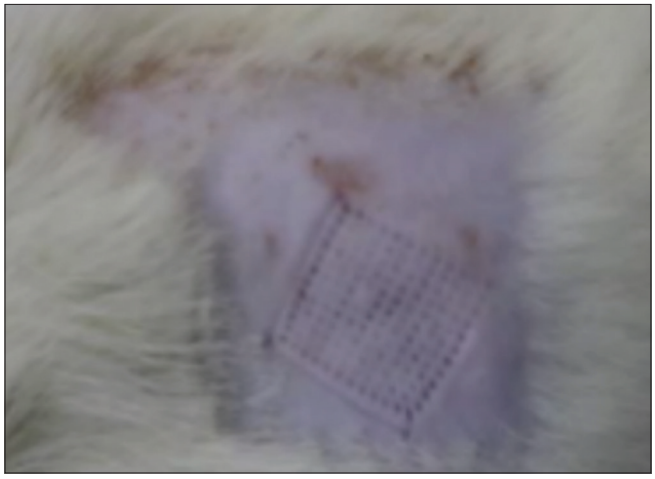

**Fig. 7: Applied microneedle patch on rat skin**

**Table 1: Physical parameters of clomiphene citrate**

| S. No. | Parameters | Purpose |
|---|---|---|
| 1. | Physical characteristic | Characteristics parameters of clomephene citrate such as state, odor, taste, melting point, and solubility study were done |
| 2. | FT-IR study | To study drug-excipient interaction and compatibility |
| 3. | Analytical method development | Analytical method was performed to identify drug |
| 4. | Differential scanning calorimetry | To study the thermal compatibility of drug [33,34] |

FTIR: Fourier transform infrared

**Table 2: Solubility**

| Patch weight (mg) | Volume of solvent (ml) | Dissolved weight (mg) | Solubility (mg/mL) |
|---|---|---|---|
| 210 | 10 | 170 | 17 |
| 210 | 10 | 160 | 16 |
| 210 | 10 | 185 | 18.5 |

Dissolved weight: The mean dissolved weight was 171.67 mg (standard deviation±12.58). Solubility: The mean solubility was 17.17 mg/mL (standard deviation±1.25)

**Table 3: Blood report of rat sample**

| Hormonal report | Control | PCOS | Treatment (MNP) |
|---|---|---|---|
| Testosteron (ng/mL) | 0.17±0.05 | 0.18±0.73 | 0.21±0.41 |
| Estrogen (pg/mL) | 12.45±1.23 | 10.81±0.29 | 11.52±0.82 |
| Progesteron (pg/mL) | 51.87±9.97 | 50.26±6.52 | 57.01±2.23 |
| FSH (ng/mL) | 3.16±0.35 | 3.27±0.37 | 3.72±0.46 |
| LH (ng/mL) | 0.15±0.003 | 0.39±0.05 | 0.72±0.037 |

Values are expressed as mean±standard deviation (n=3). Hormone levels in the treatment group showed improvement compared to the polycystic ovary syndrome group

released, which helped to evaluate how steadily the drug was released over the 120-h period. Kinetic models were also used to understand the pattern and rate of drug release [35,36].

*Ex vivo study*

To see how well the drug from the MNP could pass through real skin, scientists did a lab test using goat skin and a device called a Franz diffusion cell. First, the goat skin was cleaned and soaked in a special saltwater solution (PBS) for 12 h to keep it soft and moist. Then, the skin was placed in the device with the outer layer (the part you'd see on the surface) facing up, where the patch was placed. The lower part of the device was filled with the same saltwater solution, warmed to 35°C (close to body temperature), and stirred constantly to copy what happens inside the body. To find out how much drug went through the skin, small amounts of the liquid (1 mL) were taken from the bottom part at different times – after 2, 4, 6, 8, and 10 min, and then after 6, 12, 24, 48, 72, 96, and 120 h. Every time they took out a sample, they added fresh liquid to keep the amount the same. The drug levels in the liquid were checked using a special light-based machine called a UV-visible spectrophotometer. This helped the scientists calculate how much drug passed through the skin over time and understand the pattern of its movement [37].

*Analytical data*

Fourier transform infrared spectroscopy (FTIR) was used to check how the drug, lipid, and other ingredients in the SLN-loaded MNPs interact with each other. This technique helps identify the different chemical groups present and ensures that all the components are chemically compatible and stable when combined [38].

*Moisture content*

First, each MNP was weighed (W1), and then it was dried at 24 h for 50°C before being weighed again (W2). The amount of moisture was determined using the formula:

$$([W1-W2]/W1) \times 100$$

This test helps determine how much moisture is present in the patch, which is important for assessing its stability during storage.

*Folding endurance*

The patch was tested by folding it over and over at the same place until it tore. The number of times it could be folded before breaking was counted. Withstand before tearing indicates the mechanical strength and flexibility of the patch, which are important for ensuring it remains durable during handling and use [40].

## RESULTS AND DISCUSSION

### Physical parameters

The SEM image of a MN (×200, 20.00 kv) shows a 915.0 μm height, 602.5 μm base width, and 118.8 μm tip diameter, confirming a well-defined structure for transdermal drug delivery. Its rough, porous surface enhances drug loading and dissolution. Imaging under low vacuum (65 pa) prevents artifacts, ensuring accurate analysis of its penetration and release potential. As shown in Fig. 2.

### Solubility and drug content

The solubility of the MNP was determined by dissolving a known mass in 10 mL of solvent, measuring the dissolved drug content, and calculating solubility in mg/mL. Based on the table, the solubility values were 17 mg/mL, 16 mg/mL, and 18.5 mg/mL across different trials. The drug content was assessed by completely dissolving the patch and quantifying the drug using UV-Vis, ensuring accurate evaluation of drug loading efficiency and formulation consistency [37].

### *In vitro* study

The graph shows that the drug was released in two phases. At first, a large amount came out quickly during the first few hours. After that, the drug continued to come out slowly and steadily for up to 120 h. The fast release at the beginning probably happened because some of the drug was on the surface of the MNs and dissolved quickly. After that, the drug continues to be released slowly and steadily as it gradually diffuses from the SLN matrix embedded within the MNs. The cumulative drug release reached 88.69%, indicating a consistent and prolonged release, which makes the system suitable for transdermal drug delivery. Drug release is as shown in Fig. 4.

***Ex vivo* study**

The *ex vivo* release graph shows an initial rapid release phase, where a large portion of the drug quickly passes through the goat skin barrier within the first 10 min, indicating effective skin penetration. After the quick release at the beginning, the rest of the drug was released slowly and steadily over the next 120 h. Suggesting that a drug can continuously diffuse slowly and consistently from the SLN-loaded MNP into the receptor fluid [41]. The release profile indicates a two-phase release mechanism: The quick release at the start happens because some of the drug on the surface dissolves right away. Sustained release is driven by the drug's gradual diffusion through the skin layers. The total drug release after 120 h was found to be 77.9%. Drug release is as shown in Fig. 5.

**FTIR**

It is a comparison of both FTIR spectra helps evaluate drug-excipient interactions. If the MNP FTIR retains key functional groups of both SLN and clomiphene citrate, without significant chemical changes, it indicates good compatibility between drug, lipids, and polymers used in the MNP. This ensures that the formulation is physically stable, non-reactive, and effective for drug delivery. Drug intraction is as shown in Fig. 6.

**Moisture content and folding endurance**

Moisture content patch was kept with silica gel bead for 24 h at room temperature. Final and initial weight was measured by given formula [40,41]:

$$M_1 = \frac{(iw - fw)}{iw} * 100$$

Where,

*iw* =Weight of the MNP before drying.

*fw*=Weight of the MNP after drying.

Moisture loss was found to be 14.11%.

**Folding endurance**

Take a MNP of standard size (e.g., 5 cm × 5 cm), hold the patch at both ends, and fold it in the same location repeatedly. Folding the patch at the same spot until you see visible cracks or any of the tiny needles start to come off. Count how many times this happens before that point [42].

Patch was folded 294±3 before breaking.

**Animal study**

Blood sample was collected, and hormonal report was done, in which it was observed that PCOS was cleared with continue treatment of 3 months as shown in Table 3, also no skin irritation was observed on the lower abdomen part of the rat as shown in Figure [43].

## CONCLUSION

The study successfully developed a biodegradable MNP loaded with SLN, demonstrating its potential for controlled transdermal delivery of clomiphene citrate. The SEM analysis confirmed well-defined MN structures with appropriate dimensions for skin penetration and drug delivery. Solubility and drug content analysis ensured consistent drug loading, while FTIR studies confirmed compatibility between clomiphene citrate, SLN, and polymeric excipients, indicating a stable formulation without significant chemical interactions. Lab tests showed that the drug is released in two stages: A fast release at the beginning, then a slow and steady release that continued for up to 120 h, achieving 88.69% cumulative release, which suggests effective controlled drug delivery. Similarly, the Ex vivo release study confirmed successful skin penetration, with 77.9% release, demonstrating efficient drug permeation. The moisture content (14.11%) and folding endurance (294±3 folds) indicate good stability and mechanical strength, making the patch durable and suitable for application. Overall, the SLN -loaded MNP presents a promising, non-invasive, and patient-compliant alternative for transdermal delivery of clomiphene citrate, offering benefits such as enhanced absorption, localized delivery, it also causing fewer side effects throughout the body compared to regular pills or injections.

## AUTHOR'S CONTRIBUTION

I, Foram Bhatt have collected the data, conducted test and experiments, and have drafted the article. Dr. Dipti Patel is my guide and has reviewed the article.

## CONFLICT OF INTEREST

There are no conflicts of interest.

## AUTHORS FUNDING

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