RESEARCH ARTICLE

Efficacy of cling film dressing on palmoplantar psoriatic lesions among patients with psoriasis: A randomized controlled trial

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ABSTRACT

Introduction: Caring for patients with skin disorders is “more than skin deep”. Psoriasis is a systemic immune-mediated disease that generally does not affect survival, but certainly has major negative effects on patients. The previous research has reported that artificial restoration of a permeable barrier by occlusion results in regression of lesions in psoriasis. The aim of this research is to assess the efficacy of cling film dressing versus conventional treatment on palmoplantar psoriatic lesions among patients with psoriasis.

Methods: The research design used in the study was a true experimental time series design. Block randomization was used to assign the study participants into either study or control group in a Psoriatic Out-Patient Department of tertiary care center at Southern part of India. As usual, the intervention group participants received the application of topical corticosteroid Eczivate MF with cling film wrap while the control group participants followed the conventional treatment (topical corticosteroid Eczivate MF without occlusive dressing). The modified psoriasis severity index (MPSI) score was used to measure the outcomes.

Results: There was a high statistical significance difference on the severity of psoriatic disease level between the control and study groups (p < 0.05).

Conclusion: Steroidal cream application with occlusive dressing yielded better results than non-occlusive open treatment.

Keywords: Cling film; dressing; psoriatic lesions; psoriasis

INTRODUCTION

Skin conditions, though not life-threatening, do have enormous effects on an individual’s life, ranging from self-esteem to personal happiness and health. Psoriasis is an intrinsic immune-mediated disease, characterized by white scaly patches, that can involve scalp, elbow, or any part of the body (1). The condition was first described by Celsius (c. 25 BC–c. 50AD), a Roman scholar who referred to it as impeto. England’s Dr. Robert Willan, around 1809, first recognized psoriasis as a specific clinical entity and described it accurately (2).

The disease can range from a few scattered red scaly plaques to involvement of almost the entire body surface. It goes through cycles of flaring up, improving, and remission. While the intensity of psoriasis may wax and wane, it is generally a lifelong condition with no cure. Psoriasis affects 3% of the global population and the prevalence varies among different geographical areas and ethnic groups (3). It is estimated to affect 2–4% of the Western population. The reason for these variations is unclear, but both genetic and environmental factors likely play a role (4). The prevalence of psoriasis ranges from 0.4 to 3% and very common among men than female (5).

Due to its visibility and profound cosmetic effect on body image, palmoplantar psoriasis is an emotional burden. Patients with palmar psoriasis experience higher stigmatization and live in concealment due to their inability to conceal their lesions with clothing (6). The literature shows that ≥90% of patients with psoriasis have demonstrated symptoms related to depression and anxiety disorders (7). Evidence also suggests that patients with severe psoriasis exhibit a higher risk of self-harm and suicidal ideation (8). There is also evidence suggesting that unemployment (16.7%) in psoriatic patients is highly attributable to the severity of the disease condition (9).

Psoriasis is associated with a significant economic burden, which increases over time as the disease progresses.
A benchmark survey on the economic burden of psoriasis showed that the estimated annual expenses of psoriasis can be as high as $25,796 per person—or $135 billion for everyone with psoriasis in the United States (10).

The World Health Organization (WHO) estimates the prevalence of psoriasis by country is between 0.09% (2) and 11.4% (3), making psoriasis a serious global problem, with 100 million individuals affected worldwide (11). The National Psoriasis Foundation has similarly reported that 125 million people worldwide (2–3% of the total population) have psoriasis (12). Psoriasis is a chronic, non-communicable, painful, disfiguring, and disabling disease for which there is no cure, with a great negative impact on quality of life (11).

The etiology of psoriasis is not fully understood, though some literature has cited risk factors including family history and environmental factors, such as smoking, stress, obesity, and alcohol consumption (4). The Global Burden of Disease Study estimated a high global burden of psoriasis, with average disability-adjusted life years as high as 1,050,660 — twice as much as for acute hepatitis C (13).

One of several clinical classifications of psoriasis, palmoplantar psoriasis, affects <5% of the total body surface area and is confined to the palms and the soles, but has a large impact on quality of life. The painful symptoms can have a disabling effect on daily living (14). Janoska found that patients with psoriasis experienced greater stigmatization and lower self-esteem and may live in concealment, especially if the lesions are on body parts such as hands and feet that are exposed during the course of daily activities. They emphasized in their case study analyses that patients often face rejection and misunderstanding, leading to social isolation (14).

Treatment for psoriasis is often focused on controlling symptoms. These include topical therapy, phototherapy, and systemic therapy. Approximately 80% of patients with psoriasis receive topical medication, and more notably, topical corticosteroids are the first-line treatment option (15). Thus, the challenge and need exist to develop specific, safe, and effective long-term therapies. The treatment for psoriasis, like that for other immune-mediated conditions, is complex, since it has many different clinical presentations. The extent of skin involvement can vary from only a few small localized plaques to total body involvement. Anatomical differences in skin thickness in different areas affect penetration of medication and, hence, the efficacy of topical therapies in reducing disease severity (16).

The effects of occlusive dressing on the stratum corneum water-holding capacity have been studied previously. Oclusive therapy, in which the topical medication is applied and the skin is covered with a plastic membrane, enhances the penetration of topical agents such as corticosteroids. Occlusive dressings retain heat and moisture, thereby promoting wound healing and increasing the transcutaneous penetration of topical drugs (17).

Covering the affected area with impermeable dressings, tapes, gloves, or transdermal devices decreases the water gradient across the stratum corneum. This increases the hydration of the outermost layers of the skin, ultimately leading to increased permeation of the applied drug (18).

Alberti et al. compared the effectiveness of occlusive and open-application dressing containing mapracorat 0.1% ointment on 24 patients with plaque-type psoriasis over a period of 2–4 weeks. A comparison of pre-treatment and post-treatment observations revealed that the total anti-psoriatic effect size appeared to be higher in the occluded group than the open group (19).

Hydrocolloid occlusive dressings have been found to be effective in enhancing the efficacy of topical corticosteroids and reducing the amount of medication required to control plaques (20). Glade CP et al. analyzed the epidermal proliferation, differentiation, and inflammation in epidermal single cell biopsies taken from 15 patients with psoriasis vulgaris, treated with clobetasol solution under hydrocolloid occlusion once weekly versus clobetasol ointment twice daily unoccluded. Substantial changes in flow cytometric parameters were observed, but clearance was induced earlier in the group treated with corticosteroid under hydrocolloid occlusion (20).

The changes in psoriasis severity scores for erythema, plaque thickness, and scaliness in 39 patients diagnosed with the diagnosis of palmoplantar psoriasis were investigated (21). The patients were observed for 6 weeks and they were randomized into two different group. One group had calcipotriol ointment under occlusion for overnight for twice weekly and the other group had same ointment twice daily under topical non-occlusive. After 6 weeks of treatment, the investigators observed that there was a significant reduction from the baseline score among the occlusive group. The mean score of occlusive group was 6, whereas the mean score of the non-occlusive group was 20.1. (21).

The aim of this research is to assess the efficacy of cling film dressing versus conventional treatment on palmoplantar lesions of psoriatic patients.

METHODS

The research design chosen for this study was a true experimental times series design as mentioned in Table 1. The study was conducted in the Psoriatic OPD of tertiary care center at Southern part of India. An average of 40-50 patients attends the dermatology OPD in a day. The target population was adult palmoplantar psoriatic patients who fulfilled the inclusion criteria of the study: All adult male and female patients attending the dermatology OPD with palmoplantar psoriasis with symmetrical and homogenous lesions, patients with MPSI score ≥13 (moderate to severe cases), patients who could be contacted on their own telephone numbers, and patients who were receiving the

| TABLE 1. Research design of the study |
|--------------------------------------|
| Group | Pre-test | Intervention | Post-test I | Reinforcement | Post-test II |
|-------|----------|--------------|-------------|---------------|-------------|
| Study | O1       | O2 ‡ X (IR)  | O1          | X (IR)        | O1          |
| Control| O1       | *            | O2 ‡        | *             | O2 ‡        |

O1: Pre-assessment using modified psoriasis severity index (0 week). O2: Post-assessment using modified psoriasis severity index after 4th week for study and control group. O2 ‡: Post-assessment using modified psoriasis severity index after 8th week for study and control group. IR: Intensive reinforcement (Telephonic reinforcement every night). *Routine care at the Dermatology OPD
same topical corticosteroid (Eczivate MF). Patients who were known to have any associated complications such as diabetes, hypertension, or psoriatic arthritis, as well as patients receiving other therapy such as PUVA or oral systemic medication, were excluded from the study.

The sample size for the present trial was estimated according to the previous study (22). The sample size was estimated using power analysis. The relationship between the sample size, statistical power, level of significance, and the effect size was considered. In this study, the two tailed level of significance was set at 0.05 and the minimum expected statistical power will be 0.80. After adding a 10% for non-response rate total sample size with be 30 participants (n1 = 15 participants and n2 = 15 participants). Block randomization was used to assign the patients in to the study and the control group. Mondays, Wednesdays, and Fridays were assigned to select study group and Tuesdays, Thursdays, and Saturdays were assigned to select control group participants. The OPD in-charge nurse assigned every fifth patient with inclusion to be included in the research group. As the main objective of the study was to determine the efficacy of cling film dressing in reducing the severity of psoriatic disease compared to conventional non-occlusive topical therapy. To obtain pronounced results within 2 weeks, moderate-to-severe intensity patients were chosen, with patients with mild psoriasis excluded from the study.

The efficacy of the treatment was assessed using the modified psoriasis severity index (MPSI). The severity index consists of grading the four elements of erythema, plaque elevation, scaling, and pruritus. Psoriatic plaques in the bilateral palmar and plantar regions were graded based on symmetrical distribution of erythema, plaque elevation, scaling, and pruritus, with severity rated for each element on a 0-4 scale.

The MPSI score is used to evaluate the severity of psoriasis at a given time and to monitor the response to therapy. The score was calculated as the sum of erythema, plaque elevation, scaling, and pruritus in the bilateral palmar and plantar regions as follows:

\[ \text{MPSI} = (\text{Epa} + \text{Ppa} + \text{Spa} + \text{PRpa}) + (\text{Epl} + \text{Ppl} + \text{Spl} + \text{PRpl}) \]

\[ \text{Epa} \text{ Epl} = \text{Erythema score of psoriatic lesions of palmar and plantar regions} \]

\[ \text{Ppa} \text{ Ppl} = \text{Plaque elevation score of psoriatic lesions of palmar and plantar regions} \]

\[ \text{Spa} \text{ Spl} = \text{Scaliness score of psoriatic lesions of palmar and plantar regions} \]

\[ \text{PRpa} \text{ PRpl} = \text{pruritus score of psoriatic lesions of palmar and plantar regions.} \]

The patients were assessed for psoriatic disease severity levels using the MPSI scale. The severity was graded as very mild (1-4), mild (5-11,23), moderate (12-19), severe (20-22,24-28), and very severe (29-32). The score was evaluated at baseline and during first and second follow-up visits.

The patient's sociodemographic data (age, sex, level of education, occupation, personal habits of the patient, place of residence, marital status, and family income) were collected. Data on clinical parameters such as family history of psoriasis, duration of disease in months, and body mass index (BMI) were also obtained.

The study obtained the ethical approval from the Institutional Research Ethics Committee. Authorization to conduct the research in the skin Out Patient Department was gotten from the concerned authorities. The purpose of the research study and the patient's right to participate in or to withdraw from the study were described in a written informed consent. The eligible participants were identified after baseline (week 0) assessment was conducted, using the MPSI score for both study and control groups. Patients who had a score of ≥13 were included for the study and randomly allocated to either the study or control group. The investigator ensured that privacy was maintained throughout the assessments and procedures.

The control group was directed to continue the routine conventional treatment, application of topical corticosteroid (Eczivate MF) to the psoriatic lesions in the palmar and plantar regions. Cling film treatment (application of topical corticosteroid to the psoriatic lesions in the palmar and plantar regions, with the entire region wrapped in cling film and secured in place with adhesive tape) was demonstrated to study group patients by the investigator, who also took a return demonstration by each patient in the group. The patients in the study group were instructed to apply the cling film dressing overnight for a minimum period of 6 h. The investigator also followed up the patients by intensive telephonic reinforcement every night for 8 weeks. The participants in the study group were instructed to stop applying cling film and inform the investigator if they felt discomfort, itching, and any other unpleasant feelings.

The measurement of the disease severity in the palmar and plantar regions was done the same way as in the pre-assessment, using the MPSI scale. The first post-test was taken after one week of cling film or conventional treatment. The second post-test for both groups was taken a week after the first by the OPD nurses (Research Assistants (RAs)) those who volunteered to do the data collection. The RAs were blinded to patient group, they were not informed whether the patient belonged to control or study group. After analyzing the data, the study revealed the benefits of cling film compared to control group. Hence, the investigator recommended and demonstrated the control group also the same intervention to satisfy the ethical consideration.

The data collected from the study and control groups were analyzed and interpreted as follows: Distribution of demographic and clinical variables and comparison of efficacy of cling film dressing versus conventional treatment on palmoplantar lesions of psoriatic patients. Descriptive statistics were used to describe data variables including psoriatic disease severity. ANOVA was used to test the association of demographic and clinical variable with the efficacy of cling film dressing. The Paired t test between pre-test and post-test II and II of study and control Group was used to identify the efficacy of cling film dressing.

**RESULTS**

The distribution of demographic and clinical variables of study participants is shown in Table 2. Six patients (40%) in the study group and 7 (46.7%) in the control group were in the age group of 40–49 years. Four patients (26.7%) in the study group and 11 (73.3%) in the control group...
were males. With regards to educational status, 7 patients (46.7%) in the study group and 3 (20%) in the control group had a primary level of education, while 2 patients (13.3%) in the study group and 4 (26.7%) in the control group had higher secondary education. Considering occupational status, most were employees of private concerns: 5 patients (33.3%) in the study group and 4 (26.7%) in the control group. Few were manual laborers: 3 patients (20%) in the study group and 2 patients (13.3%) in the control group. Assessing family income, most patients (33.3%) in the study group and six patients in control group were receiving a monthly income in the range of 44–63 USD, while very few received more than 63 USD: 1 patient (6.7%) in the study and control group.

With regard to family history of psoriasis, 7 patients (46.7%) from the study group and 10 (66.7%) from the control group had a family history of psoriasis.
control group had no family history of psoriasis, while an equal number of five patients (33.3%) in the control group had a family history. Considering duration of the disease, 8 patients (53.3%) in the study group and 7 (46.7%) in the control group had the illness for >24 months. Assessing personal habits, 1 patient (6.6%) in the study group and 5 (33.3%) in the control group were smokers. One patient (6.6%) in each of the control and study groups reported alcohol use. A majority of 13 patients (86.8%) in the study group and nine (60%) in the control group were neither smokers nor alcohol users. Regarding BMI, 8 patients (53.8%) in the study group and 7 (46.6%) in the control group were in the average category. Equal numbers of 7 patients (46.6%) in each of the study and control groups were in the category of overweight.

The distribution of psoriatic disease severity among the study and control groups based on MPSI scoring is showed in Table 3. In pre-tests among the study group, 2 patients (13.3%) had a moderate level of disease severity, 12 (80%) had a severe level, and 1 (6.7%) had a very severe level of disease. In the control group, 7 patients (46.7%) had a moderate level of severity and 8 (53.3%) had a severe level of disease. In the study group, post-test I showed that 3 patients (20%) had a mild level of disease severity, 10 (66.7%) had a moderate level, and 2 (13.3%) had a severe level of disease. In the second post-test among the study group, 11 patients (73.3%) had a very mild level of disease severity, 3 (6.7%) had mild level, and 1 (6.7%) had a moderate level. In the control group, post-test I showed that 2 patients (13.3%) had a mild level of disease severity, 9 (60.0%) had a moderate level, and 4 (26.7%) had a severe level of disease. In the second post-test among the study group, 1 patient (6.7%) had a very mild level of disease severity; 2 (13.3%) had a mild level, and 12 (80.0%) had a moderate level.

The comparison of psoriatic disease severity between pre-test and post-test I and II among the study and control groups is showed in Table 4. The paired t-test revealed a statistically significant decrease in disease severity between the pre-test and post-test I and II among the study group: 2.88 (p < 0.05) and 4.02 (<0.001), respectively. There was no significant association between demographic and clinical variables and the effect of cling film dressing among the study group between pre-test and post-test II except for occupation, family income, and BMI (p < 0.01), with F values of 3.00, 3.74, and 3.05, respectively (Table 5).

### DISCUSSION

According to the results of this study, the effect of conventional treatment on palmoplantar lesions of psoriatic patients shows no significant change in disease severity between the pre-test and post-test I and II in the control group who followed conventional treatment. The control group of patients was instructed to continue the routine conventional treatment, application of topical corticosteroid (Eczivate MF) on psoriatic lesions in the palmar and plantar regions. Post-test I and II were taken after the 4th and 8th week, respectively. Other studies have shown that long-term use of conventional systemic treatments causes cumulative toxicity, including liver toxicity from methotrexate, renal toxicity from cyclosporine, and skin carcinogenesis from phototherapy, due to poor tolerability among psoriatic patients (23,24).

All the study participants in the study group had cling film treatment (application of topical corticosteroid to the psoriatic lesions in the palmar and plantar regions, with the entire region wrapped in cling film and secured in place with adhesive tape) for 8 weeks. All the patients were instructed to keep the cling film on for 6 h/night. Very few studies have been conducted on the same topic. Duweb et al. studied the same effect of occlusive against non-occlusive application of ointment among psoriatic patients for 6 weeks. The aim of the study was to assess the efficacy of occlusive calcipotriol 50 mcg/mg ointment versus non-occlusive therapy. The study found that twice-weekly occlusive calcipotriol ointment was as effective as the twice-daily application and also revealed a significant reduction in the severity of the lesions among occlusive patients compared to the non-occlusive patients (22).

Another study by Wigger et al., concluded with the same recommendations. That study tested psoriatic plaques after

### TABLE 3. Frequency and percentage distribution of psoriatic disease severity gradings based on modified psoriatic severity index (MPSI) score among study and control group (n=30)

| MPSI GRADING | Study group (n=15) | Control group (n=15) |
|--------------|-------------------|---------------------|
|              | Pre-test | Post-test I | Post-test II | Pre-test | Post-test I | Post-test II |
| Very mild    | F | % | F | % | F | % | F | % | F | % |
| Mild         | - | - | - | - | 11 | 73.3 | - | - | - | - |
| Moderate     | 2 | 13.3 | 3 | 20.0 | 3 | 20.0 | - | - | 2 | 13.3 |
| Severe       | 12 | 80.0 | 2 | 13.3 | - | - | - | - | - | - |
| Very severe  | 1 | 6.7 | - | - | - | - | - | - | - | - |

### TABLE 4. Paired t test between Pretest and Post-test I and II of Study and Control Group (n=30)

| Groups        | Effect of Pre-test and Post-test I | Effect of Pre-test and Post-test II |
|---------------|----------------------------------|-----------------------------------|
|               | Mean | SD  | t     | p value | Mean | SD  | t     | p value |
| Study group   | 8.8  | 36.46 | 9.88 | 0.042* | 20.1 | 4.02 | 19.5 | 0.000*** |
| Control group | 2.2  | 3.18  | 2.68 | 0.370  | 6.1  | 4.90 | 4.84 | 0.943   |

**Significant at p<0.001, *Significant at p<0.05**
TABLE 5. ANOVA of demographic and clinical variable with the efficacy of cling film dressing in the post-test I and II among study group

| Demographic variable                  | Effect of cling film dressing (O−O₂) | ANOVA |
|---------------------------------------|--------------------------------------|-------|
|                                       | n  | Mean  | SD  | F value |
| Age in years                          |    |       |     |         |
| 20–29                                 | 1  | 17.0  | 0.00| 0.85    |
| 30–39                                 | 3  | 21.0  | 2.00|         |
| 40–49                                 | 6  | 21.8  | 2.48|         |
| 50–59                                 | 5  | 19.6  | 4.87|         |
| 60 and above                          | 0  | 0.00  | 0.00|         |
| Gender                                |    |       |     |         |
| Male                                  | 4  | 22.0  | 4.16| 0.22    |
| Female                                | 11 | 20.0  | 3.14|         |
| Education                             |    |       |     |         |
| Primary                               | 7  | 21.1  | 2.73| 0.75    |
| Secondary                             | 6  | 19.5  | 4.50|         |
| Higher secondary                      | 2  | 22.0  | 1.41|         |
| Graduate                              | -  | -     | -   |         |
| Occupation                            |    |       |     |         |
| Daily wages                           | -  | -     | -   | 3.00**  |
| Manual laborer                        | 3  | 5.00  | 1.73|         |
| Government                            | 1  | 11.0  | 0.00|         |
| Private                               | 5  | 11.6  | 3.58|         |
| Any other (Unskilled job)             | 6  | 8.80  | 2.19|         |
| Family income (Per month in USD)      |    |       |     |         |
| <25                                   | 5  | 7.20  | 3.40| 3.74**  |
| 26–43                                 | 5  | 11.2  | 2.86|         |
| 44–63                                 | 4  | 8.50  | 3.78|         |
| >63                                   | 1  | 6.0   | 0.00|         |
| Marital status                        |    |       |     |         |
| Married                               | 13 | 20.6  | 3.44| 0.001   |
| Unmarried                             | 2  | 20.0  | 4.24|         |
| Clinical variable                     |    |       |     |         |
| Family history of psoriasis           |    |       |     |         |
| No one                                | 7  | 20.1  | 3.07| 0.57    |
| Parents                               | 5  | 21.8  | 3.34|         |
| Siblings                              | 2  | 18.0  | 5.65|         |
| Grand parents                         | 1  | 23.0  | 0.00|         |
| Duration of disease in months         |    |       |     |         |
| 0–6                                   | 4  | 21.2  | 2.87| 0.76    |
| 7–12                                  | 3  | 19.3  | 1.52|         |
| 13–18                                 | -  | -     | -   |         |
| 19–24                                 | -  | -     | -   |         |
| >24                                   | 8  | 20.7  | 4.23|         |
| Personal habit                         |    |       |     |         |
| Smoking                               | 1  | 17.0  | 0.00| 0.25    |
| Alcohol                               | 1  | 27.0  | 0.00|         |
| None                                  | 13 | 20.3  | 2.98|         |
| Body mass index                       |    |       |     |         |
| Underweight = <18.5                   | -  | -     | -   | 3.05**  |
| Normal weight = 18.5–24.9             | 8  | 20.5  | 2.97|         |
| Overweight = 25–29.9                  | 7  | 20.7  | 4.07|         |
| Obesity = BMI of 30 or greater        | -  | -     | -   |         |

**Significant at p<0.01

using only ointment with occlusives and four different ointments without occlusives. Patients with more drugs reported side effects of prednicarbate 0.25% ointment, clobetasol 0.05% ointment, calcipotriene 0.005% ointment, and calcipotriene 0.005%/betamethasone dipropionate 0.05% ointment, and longer periods of application. The occluded group showed less severity of lesions and shorter duration of application of ointment (19).

The present study revealed no significant association between demographic and clinical variables and the effect of cling film dressing in the study group between pre-test and post-tests, except for occupation, family income, and BMI (p < 0.01), with F values of 3.00, 3.74, and 3.05, respectively. There are no related studies to corroborate these findings. However, few studies were conducted addressing comorbidities in psoriatic disease, assessing severity of the disease during treatment period. The study concluded that education status, obesity, blood sugar, cardiovascular disease, and blood pressure influenced the severity of psoriasis (25,26,27). The present study did not include these comorbidities.

Limitation

There was no much limitations notified in this intervention. Psoriasis symptoms worsen as the skin dries out, some people find it helpful to wrap the skin using specialist tape or cling film after applying moisturiser at night to help seal in moisture. However, the study group was instructed to stay away from heat after wrapping up with cling film. Since it was a very small sample, the generalizability would be a question mark.

CONCLUSION

This study concluded that topical corticosteroids are the most commonly prescribed drugs by dermatologists in an outpatient setting for patients with psoriatic lesions. Cling film is a kind of occlusive that helps in retaining the topical ointment over the skin and reduces the severity of the disease. In contrast, topical ointment with non-occlusive treatment does not retain the medication and needs long-term application of corticosteroids that can lead to complications and slower reduction in the severity of lesions. Thus, this study recommends that the use of occlusives along with conventional treatment improves the reduction in severity of the psoriatic lesions. A careful literature review on factors associated with severity of psoriatic lesions and inclusion of those clinical parameters will be helpful in addressing the influence of those factors.

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STATEMENT OF ETHICS

The Medical Ethics Committee of Sri Ramachandra Medical College and Research Institute (SRMCR/NU/IEC36), India, approved the study. The study was conducted in compliance with the ethical principles laid down in the Declaration of drug licensing authority in India, that is, the Drugs Controller General (India) (DCGI). All volunteers gave their written informed consent to participate in the study.
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CONFLICT OF INTEREST STATEMENT
The authors have declared no conflicting interests.

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