RESEARCH ARTICLE

Preclinical Assessment of Aceclofenac Transdermal Patch by Freund's Complete Adjuvant-induced Arthritis in Rats

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ABSTRACT

The hydroxypropyl methylcellulose (HPMC) based transdermal patches of aceclofenac were prepared. On the basis of *in-vitro* potential, the formulation was additionally chosen and assessed for their *in-vivo* anti-arthritic activity and acute skin irritation tests using the primary skin irritation (draize) test and freund's complete adjuvant (FCA) caused arthritis technique. Furthermore, stability studies were conducted in compliance with ICH standards. The studied compositions had no irritant potential, and no edema production was ever noticed. All of the formulations had a zero irritation score (primary skin irritation index), indicating their safety and acceptability for transdermal administration. The stability investigations confirmed the produced patch's stability, and the *in-vivo* analysis showed a considerable decrease in the volume of irritated paws compared to the marketed formulation. The created transdermal patches may serve as a platform for the delivery of medication for the treatment of arthritis

Keywords: Aceclofenac, Arthritis, Freund's adjuvant, Transdermal.

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INTRODUCTION

Nowadays transdermal patches are in great demand as they are capable of avoiding highlighted issues associated with the oral drug delivery system, which include problems such as gastric irritation, drug hydrolysis, enzymatic degradation, first-pass hepatic metabolism, drug fluctuations, etc.[1] The transdermal drug delivery system (TDDS) also known as a patch, is a non-invasive method of delivering medications to the skin surface. TDDS is potentially used as an alternative to administering hypodermic injections as well as oral route drugs. In order to achieve the systemic or local effect, this drug delivery system can deliver an analgesic at a predetermined rate across the dermal layer of the skin.[2] Creams and ointments, which are semisolid formulations, do not guarantee sustained contact with the skin surface and are easily removed by the patient's clothing. Therefore, in the case of chronic conditions like arthritis, repeated application is necessary.[3]

Therefore, it is necessary to design a dosage form for arthritis that allows for less frequent administration while maintaining intimate contact with the skin for an extended period of time, improving patient compliance, especially in the case of older patients. The topic of this research is the stability, *in-vivo*, and skin irritation experiments of the manufactured transdermal patch.

MATERIALS AND METHODS

Materials

Aceclofenac was gifted from Shiva Biogenetic Pharmaceuticals Pvt. Ltd., Solan, India. The Freund adjuvant was bought from Sigma Aldrich in Mumbai, India. The study's other compounds were all of the analytical variety.

Development of Transdermal Patch

Due to merits like economic viability, ease of preparation, and reproducibility, the solvent evaporation technique was employed to formulate the aceclofenac-loaded patches. The patches were fabricated in a way to load around 11.25 mg of drug per 2.25 cm^2, covering all the possible ranges of the drug-to-polymer ratio.[4]

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As per the disclosed procedure, the desired patches loaded with aceclofenac were developed using the most widely employed solvent evaporation technique. The polymer was dissolved by mixing in solvent acetone. Aceclofenac was added to the polymer-solvent mixture and stirred well until a homogeneous solution was obtained. For the complete dissolution of the polymer, the system was left undisturbed overnight. Then, propylene glycol as a plasticizer and oleic acid as the permeation enhancer were added and dissolved with mixing. A petri dish was filled with the prepared solution. Additionally, the funnel was positioned above the petri dish to limit the rate of evaporation. The dried patches were removed, trimmed, packaged in aluminum foil, and kept in a desiccator for later usage after drying at room temperature for 24 hours.[4]

In-vivo Evaluation Studies

Skin Irritation Studies

The Institutional Animal Ethical Committee (CPCSEA/IAEC/SBS/2017-18/009) of the Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research in Dehradun, India, examined and approved the skin irritation study's techniques and protocols. This study was performed as per the OCED/OECD guidelines and previous studies.[5] For skin irritation studies, 03 albino rats (300–500 g) of either sex were used.[6] Backs are shaved, and care was taken to avoid abrading the skin, and only animal with healthy and intact skin was used. The test patch P13 was applied to the skin (approximately 6 cm^2) and covered with gauze patch. Untreated skin areas of the test animal serve as a control. The skin was observed at 0, 24, 48, and 72 hours. for any sign of irritability. The mean erythemal scores were recorded and compared with the control. The standards score of irritancy studies is depicted in Table 1.

In -vivo Anti-arthritic Activity

The FCA-induced arthritis model was used to test the anti-arthritic activity of the ACF-loaded transdermal patch in albino rat model.[7]

Animals

For the investigation, 200–250 g adult inbred Wistar albino rats of either sex were employed. The animals were kept in common polypropylene cages with a 12:12 hours light/dark cycle and at a controlled ambient temperature of 22°C and relative humidity of 55°C.[8] All of the animals received free access to water and commercially available rat regular pellet food. The Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Dehradun, India, followed the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India guidelines and received prior approval from the Institutional Animals Ethical Committee (CPCSEA/IAEC/SBS/2017-18/009) for conducting the experimental animal studies.

Experimental Protocol

The anti-arthritic activity of the ACF-loaded patch P13 was evaluated in Wistar albino rats by using Freund's complete adjuvant (FCA) induced arthritis model.[9] Each rat in the groups was placed in an observation chamber for 10–15 minutes to minimize the stress-related behaviors. Arthritis was induced in all groups of animals excluding naive control, with 0.1 mL of complete Freund's adjuvant in the right hind paw.[10] As per the approved protocol, after administration of FCA, active control and test patch (P13) was applied onto the plantar surface of the right hind paw. The inflammatory reactions were rapid and can be observed as soon as the FCA was administered.[11]

The animals were divided into five groups, each having six animals. The animals received the following treatments:

- **Naive Control**: Animals without interventions were kept under experimental conditions and provided food and water *ad libitum.*
- **Arthritis Control:** Animals were treated with 0.1 mL of complete Freund's adjuvant to induce arthritis.
- **Active Control**: Animals were treated with a marketed gel of aceclofenac.
- **Negative Control**: Animals were treated with a patch (HPMCK100M, Propylene glycol, oleic acid, acetone) without aceclofenac.
- **Test group**: Animals were treated with test formulation P13 transdermal patch.

According to the methodology, the inflamed albino rats' paw volume and the percentage of edema inhibition in each group of animals were measured on days 0, 7, 14, 21, and 28. Data on analgesic and anti-inflammatory effects were expressed as mean and standard deviation and subjected to two-way analysis of variance analysis.[12] Using the following formula, the percentage inhibition in hind paw edema was determined,[13]

$$\% \text{ Inhibition in oedema} = (Vc - Vt/Vc) \times 100$$

Where Vt is the is-mean edema volume of the test and Vc-mean edema volume of control.

Stability Studies

The stability study of P13 was conducted as per the method described in ICH Guideline Q1A.[14] The prepared patch was

Table 1: Standard Score for skin irritation

S.No.	Grading of Skin reaction	Score
(a)	Erythema and Eschar formation	
	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well defined erythema	2
	Moderate to severe erythema	3
	Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
(b)	Edema formation	
	No edema	0
	Very slight edema (barely perceptible)	1
	Slight edema (edges of the area well defined by definite raising)	2
	Moderate edema (raised approximately 1mm)	3
	Severe edema (raised more than 1mm and extending beyond the area of exposure)	4

Table 2: Stability studies of P13 transdermal patch, after being stored at 30 ± 2°C and relative humidity 65 ± 5%.

Formulation code	*Thickness (µm)*	*Mass (mg)*	*Drug content (mg per 2.25 cm^2/patch)*	*Folding endurance*
0 month				
P13	0.220 ± 0.002	427.283 ± 1.884	11.194 ± 0.347	211 ± 5.00
1 month				
P13	0.220 ± 0.001	427.832 ± 1.215	11.185 ± 0.233	210 ± 4.00
3 months				
P13	0.220 ± 0.002	427.990 ± 1.352	11.189 ± 0.211	215 ± 7.00
6 months				
P13	0.220 ± 0.004	428.701 ± 1.421	11.170 ± 0.347	211 ±5.00

Note: All data are presented as mean value ± SD and n=3

Table 3: Stability studies of P13 transdermal patch, after being stored at 40 ± 2°C and relative humidity 75 ± 5%.

Formulation code	*Thickness (µm)*	*Mass (mg)*	*Drug content (mg per 2.25 cm^2/patch)*	*Folding endurance*
0 month				
P13	0.220 ± 0.002	434.283 ± 1.884	12.114 ± 0.471	209 ± 4.00
1 month				
P13	0.220 ± 0.004	434.453 ± 1.115	10.000 ± 0.313	212 ± 4.00
3 months				
P13	0.219 ± 0.003	435.510 ± 1.152	09.850 ± 0.311	209 ± 5.00
6 months				
P13	0.219 ± 0.002	435.751 ± 1.141	07.670 ± 0.114	213 ±4.00

Note: All data are presented as mean value ± SD and n = 3

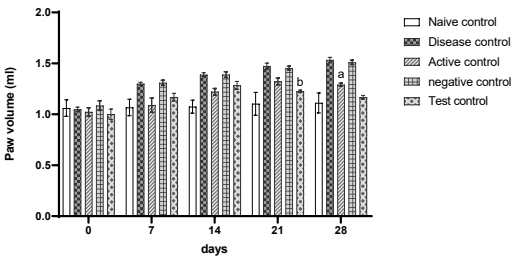

Figure1: *In- vivo* anti-arthritic activity of transdermal patch of aceclofenac using Paw volume.

kept at a temperature 30 ± 2°C/65% ± 5 %RH for intermediate-term testing conditions and at a temperature 40 ± 2°C with relative humidity 75 ± 5% for accelerated testing conditions for 6 months, respectively. The stability of the formulation was analyzed for thickness, mass, drug content, and folding endurance.[15]

RESULTS AND DISCUSSION

Skin Irritation Studies

For skin irritation studies, formulation (P13) was applied on the hairless skin of the rat. The rat skin was observed at predetermined time intervals of 0, 24, 48, and 72 hours for any possible sign of irritation and compared with the controlled.[7] Film-forming gels had a zero-irritation score after a 72 hours observation, indicating their safety and suitability for transdermal administration.

Skin Irritation Measured by Erythema Score

Erythema was assessed visually after each exposure of rat skin to the control and the test patch.[16] Erythema which is caused by the increase of the blood flow in dermis enables us to monitor the response of that layer in the preparation. The score for each rat at the end of each day was zero for test formulation. The control patch did not cause any feasible change daily on any rat. However, there is no discernible change in the erythema score prior to and following daily patch use. Additionally, on any given day, there was no discernible difference between the control and test patches. From these observations, we conclude that the transdermal patch containing aceclofenac was significantly non-irritant and had the same safety profile as compared to control patch.

In-vivo Anti-arthritic Sudy

The study aimed to determine how well the P13-optimized transdermal patch worked to prevent paw edema. Using Freund's complete adjuvant (FCA) induced arthritis model 13, the activity of ACF-loaded transdermal Patch (P13 anti-inflammatory) was assessed in albino rats. Albino rats will be given 0.1 mL of the complete Freund's adjuvant mixed in the right hind paw to cause arthritis. The paw volume was obtained using a plethysmometer to determine the inhibition (%) of edema in each treated group.[17,18] Days 0, 7, 14, 21, and 28 were the different time intervals used to measure the paw volume in each group (Figure 1). The mean% inhibition rate of edema[19,20] was used to express the anti-inflammatory

activity of the applied materials. According to the findings, the improved transdermal Patch (P13) demonstrated a significantly lower level of inflammation than the commercial formulation when evaluated using two-way ANOVA and Tukey's multiple comparison test (p 0.0001).

Each group (n=6) represents mean ± standard deviation. Two-way ANOVA followed by Tukey's multiple comparison test, F (4, 125) = 166.6, $^{a}P<0.0001$ anti-arthritic effects of active control. F (4, 125) = 169.7, $^{b}P<0.0001$, antiarthritic effect of test control.

Stability Studies

In compliance to the International Conference on Harmonisation (ICH), the selected patch, *viz*. P13 was evaluated for stability at 30 ± 2°C/65 ± 5% RH and at 40 ± 2 °C/75 ± 5% RH for 6 months and was observed for the physicochemical attributes as well as drug assay.[20] The results of the stability have been represented in Tables 2 and 3.

As shown in Tables 2 and 3, there was no significant decrease in the selected formulation's thickness, mass, drug content, and folding endurance for a period of 6 months. The drug content remained consistent during the storage period along with the other physicochemical attributes, assuring the stability of the selected transdermal patch.

CONCLUSION

It is evident that the inflammation was significantly reduced as compared to the commercial formulation. Following a 72 hours observation period, the transdermal patch's irritation score was discovered to be zero, indicating both its safety and acceptability for transdermal delivery. According to a six-month stability assessment, the improved formulation P13 was relatively stable and suitable for transdermal administration. This formulation might be a better option than the currently marketed twice-daily conventional oral pills. In the case of stubborn patients, it may promote patient compliance.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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