ANTI-INFLAMMATORY ACTIVITY OF INDOMETHACIN TRANSDERMAL PATCHES CONTAINING NATURAL PENETRATION ENHANCER

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Abstract

The greatest hindrance for transdermal drug delivery (TDD) is the barrier property of skin, especially the stratum corneum (SC). Various methodologies have been investigated and developed to enhance the penetration of drugs through the skin. Among them, the most popular approach is the application of penetration enhancers (PEs), including natural terpenes, a very safe and effective class of PEs. In the present study, the transdermal patch of indomethacin with camphor and menthol as permeation enhancer formulated and evaluated for anti-inflammatory potency. The study revealed that indomethacin transdermal patch with natural enhancer (camphor and menthol) have batter antiinlammatory action as compare without containing natural enhancer. Percentage inhibition of edema by indoethacin transdermal patch without containing permeation enhancer (F1 formulation) in rat's left hind paw was observed to be- 32.19% (at 1 hr.) and 38.60% (at 2 hr.), where as in case of indomethacin patch containing permeation enhancer (F5 formulation) was observed to be- 42.02% (at 1 hr.) and 48.50% (at 2 hr.).

Keyword : Transdermal drug delivery, patch, penetration enhancers, antiinlammatory.

Introduction

Transdermal drug delivery offers a very advantageous route for drug delivery compared with the other routes of drug administration having advantages such as bypassing the hepatic first pass metabolism, and longer duration of action [1,2]. However, the barrier function of the skin outermost layer, the stratum corneum (SC) is one of the main limitations to it, and for this reason, skin penetration enhancers are gaining the greatest interest in pharmaceutical research

Penetration enhancers help in the permeation of the desired drug (penetrant) through the skin by lowering the impermeability of the skin. Some properties which are desired in permeation enhancers are the must be pharmacologically inert, nonirritating, nontoxic, nonallergic, compatible with drugs and excipients, odorless, tasteless, colorless, and inexpensive and also have good solvent properties . [3-4]

Due to their high enhancement effect and low skin irritation, terpenes of natural origin are now receiving much attention in pharmaceutical and cosmetic formulations as PEs. Terpenes, primarily

extracted from medicinal plants, are volatile compounds with molecular components that are composed of only carbon, hydrogen and oxygen atoms. [5-6]

Menthol

Menthol, which is one of the potent penetration enhancers, is obtained from the flowering tops of *Mentha piperita*. Menthol and limonene together can be used as a prototype of terpenes that can be used as permeation enhancer [7]

Camphor has a wide variety of topical uses due to its antibacterial, antifungal, and anti-inflammatory properties. It can be used to treat skin conditions, improve respiratory function, and relieve pain. Continue reading to learn more about the different uses for **camphor** and its supporting scientific evidence. Camphor oil is extracted from the wood of camphor trees, known scientifically as *Cinnamomum camphora*, and it has a strong aroma. It can also be synthesized from turpentine. [5,7]

Methodology

Preparations of transdermal patches

The transdermal patches of composition listed in Table no.2 were prepared by solution casting technique employing a glass substrate (Bangles wrapped with aluminium foil). Membrane type transdermal systems with containing 75 mg Indomethacin prepared by employing various proportions of $HPMCK_{15}M$, $PVPK_{30,}$ and Ethyl Cellulose. The polymers was accurately weight and dissolved in a suitable solvent mixed until clear solution formed with magnetic stirrer then added drugs and placed for 30 mint in ultra sonicator bath machine (Elmasonic S150**)** for complete dissolution after that this sonicated solution mixed with PEG400 as a plasticizer .The polymer solution was poured into bangles placed in a suitable level, hard rigid surface and patches were dried at a room temperature in a dust free environment for 24 hrs. An inverted funnel was covered over the bangles to avoid fast evaporation of the solvent. Patches of 3.14 cm^2 were prepared by cutting and packed in an aluminum foil and kept in a desiccator. [8]

Evaluation of Transdermal Patches

Thickness of patches [9]

The thickness of Patches was measured by digital vernier calipers with least count 0.001mm at three different sites average of three reading was taken with standard deviation.

Weight variation [9]

The three disks of 3.14 cm^2 was cut and weighed on electronic balance for weight variation test. The test was done to check the uniformity of weight and thus check the batch- to- batch variation.

Table 1: Formulation of Transdermal Patch of Indomethacin

Formulation Code	Drug (mg)	$HPMCK_{15}M$ (mg)	$PVPK_{30}$ (mg)	EC (mg)	PEG-400* (ml)	Solvent (M:DCM) (1:1) (ml)	Natural Penetration Enhancer
F1	75	50	250	100	0.2	4	-
F5	75	50	250	100	0.2	4	2:2:2 (oleic acid, camphor& menthol)

Drug content

Accurately weighed patches were individually dissolved in minimum quantity of ethanol and made volume up to 100 ml with PBS pH 7.4 solutions; 10 ml was transferred to flask and made volume 100 ml. The absorbance was recorded. The blank solution was made in the same manner except the patches without drug were used.[9]

Percentage Moisture content [10]

The films were weighed & placed in desiccators containing calcium chloride at 40^0c in a dryer for at least 24 hrs or more until it gives a constant weight. The % of moisture content was the difference between constant weight taken and the initial weight and as reported with percentage by weight moisture content.

$$\text{Percentage moisture lost} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Percentage Moisture absorption/uptake [10]

The films of which the size $3.14cm^2$ were put in a desiccators with silica gel for 24 hrs and weighed the patches were transferred to another desiccators containing saturated solution of KCL(84% RH) after equilibrium was attained. Patches were taken out and weighed. Moisture uptake was calculated with following formula

$$\text{Percentage moisture absorption} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Swelling index[11]

The patches of 3.14 cm² were weighed and added into Petri dish which contains 10 ml double distilled water and were permeated to absorb moisture at a fix time interval check the increase weight of the patches. Continue this process till same weight observed until weight remaining the same over a period of time. Swelling index (% S) was determined by applying the formula.

S (percentage) = Wt – Wo / Wo x 100

Where, S percent swelling, Wt patch weight at time t.

Wo patch weight at time zero.

Folding endurance[12]

This was obtained by constantly folding one patch at the same place without breaking gave the value of folding endurance. This test performed to check folding ability of transdermal patches also indicate brittleness of patches, more brittle patch when folding endurance value1.

Percentage Elongation [12]

A film strip (4 x 1cm) was cut on a glass plate with a sharp blade. The % elongation break is to be determined by observing the length just before the breaking point with formula by pointer on the graph paper.

% Elongation = [Final length – Initial length] * 100/Initial length

Tensile Strength [13-14]

The tensile strength of the patches was found by the apparatus and the design of instrument such that, it had one wooden frame that horizontally placed having fixed scale. On the top of frame two clips were attached to hold patches that under study. From two clips one clips fixed & other moved. Instrument also has pulley to hold weight a patch, weight applied to one end of pulley and other end attached to the fixed clip. During the test wooden platform not dislocate from the original place so platform was fixed carefully to avoid dislocation. Three patches were cut for study having 3.14 cm^2 sizes. Thickness and breadth of patches were noted at three sizes and calculated average value. Rate of stress changes was maintained constant with the addition of 0.5g per 2 minutes. The elongation was observed and the total weights taken were used for calculation. Formula for tensile strength :

$$\text{Tensile strength} = F/a.b\ (1+L/l)$$

Where,

F is the force required to break; 'a' is width of film; 'b' thickness of film; L is length of the film; l is an elongation of film at break point

Evaluation Methods of Anti-Inflammatory Action

Inflammation is one of the most important natural defense mechanisms. Its main purpose is to destroy the injurious agent and/or to minimize its ill effects by limiting its spread. Though inflammation is protective in some situations if untreated can lead to serious complications. Inflammation is the dynamic pathological process consisting of a series of interdependent changes.

***In-vivo* Anti-inflammatory study of optimized formulation**

Formalin-induced Paw Edema

This model based upon the ability of test drug to inhibit the edema produced in the hind paw of the mice after injection of formalin. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue-mediated response. In the first phase there is release of histamine, 5-HT and kinin, while the second phase is related to the release of prostaglandins

Animals

Healthy young adult albino (100-120 gm) of either sex and of approximate same age were used throughout the study were housed under standard laboratory conditions in polyacrylic cages, and were provided with pelleted food and water *ad libitum*. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. Animal studies were approved by Institutional Animal Ethics Committee (IAEC) of R.K.D.F college of Pharmacy, Bhopal, M.P. and carried out in accordance with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

Formalin induced paw edema model

In-vivo Anti-inflammatory study of optimized Transdermal patch formulation F5 containing permeation enhancer was conducted by formalin induced paw edema model using 12 albino rats and divided into three groups of four animals on each. In all groups, acute inflammation was induced by sub-planter injection of 0.1 ml of freshly prepared 1 % suspension of sterilized formalin in normal saline in left hind paw of the rats. The medicated formulations (0.3g) or base or standard were applied topically to the planter surface of hind paw with gentle rubbing with index finger to each rat of respective group one hour before and one hour after the formalin challenge. The paw edema volume was measured using plethysmometer at every 30 mint intervals for 4 hour after injection of formalin. The average paw edema volume of all the groups were calculated and compared with that of control. The percent inhibition of edema was calculated by using following formula. [15-19]

% Edema inhibition = (1-Vt/Vc) 100

Where, Vt = Mean edema volume of test, Vc = Mean edema volume of control

Eight groups of animals four each:

- Group I -Received transdermal patch base
- Group II –Received Indomethacin transdermal patch
- Group III- Received Indomethacin transdermal patch with permeation enhancer (F5 formulation)
- Group III- Received indomethacin marketed prepration

Statistical Analysis

The statistical analysis of various studies were carried out using analysis of variance (ANOVA) followed by Dunnett's 't' test and standard deviation, $p<0.05$ was accounted significant.

5.7 Skin Irritation Study

In-vivo skin irritation study was conducted by 15 albino rats of either sex weighing between (100-120 g) was used. Animals were divided in to 3 groups of 5 animals on each. Hairs were depleted from the back of rats with the help of depilatories and area 2 cm^2 was marked on both the sides. One side served as control while the other as test and animals were used after 24 hrs. After hair depletion herbal gel was applied (500mg / rat) on test side and gel base was applied on control side once a day for 7 days and site was covered with cotton bandage and observed for any sensitivity and the reaction if any was graded as under :- [16]

A – No reaction, B – Slight patchy erythema, C –Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

RESULT AND DISCUSSION:

Evaluation of Transdermal patches

Table 2 and 3 shows the physicochemical evaluation like the Thickness, Folding endurance, Percentage moisture absorbed, Percentage moisture lost, Drug content uniformity.

Table 2: Physicochemical Evaluation data of Transdermal Patches

Formulation Code	Thickness (mm)	Weight variation (mg)	% Drug Content	Folding endurance	Tensile strength Kg/mm^2
			Indomethacin		
F1	0.30±0.09	0.159±0. 01	98.12±2. 02	58±02.04	3.21±0.81
F5	0.31±0.29	0.153±0. 017	98.12±2.02	57±22.03	3.35 ±1.84

Table 3 .Physicochemical Evaluation data of Transdermal Patches of Indomethacin

Formulation Code	% Elongation	% Moisture Content	% Moisture uptake	Swelling index
F1	33.23±2.51	2.68±0.35	4.37±4.03	25.31±1.28
F5	36.94±4.71	3.35±2.78	5.25±1.25	24.12±0.15

In-Vivo Anti-Inflammatory Study of Optimized Formulation

Percentage inhibition of edema by indoethacin transdermal patch without containing permeation enhancer (F1 formulation) in rat's left hind paw was observed to be- 32.19% (at 1 hr.) and 38.60% (at 2 hr.), where as in case of indomethacin patch containing permeation enhancer (F5 formulation) was observed to be- 42.02% (at 1 hr.) and 48.50% (at 2 hr.). All the results were compared with standard indomethacin marked prepration.

The result revealed that formulations containing enhancer camphor, oleic acid and menthol has better anti-inflammatory action as compared to formulation without it because camphor and menthol both are natural anti-inflammatory remedies as well as permeation enhancer. The Formulation F 5 show highest percentage inhibition of edema as compared to others formulation (Table.4 & fig.1).

Table 4: Mean Percentage inhibition of edema

% Inhibition (Mean±SEM)

Group	Percentage inhibition of edema			
	1 hr	2hr	3hr	4hr
Control	-	-	-	-
F1	32.19±0.16	38.60±0.70	43.74±0.24	44.03±0.93
F5	42.02±0.16	48.50±0.23	52.31±0.02	56.25±0.80
Standard Drug	43.02±0.16	49.00±0.13	51.11±0.05	55.85±0.90

Skin Irritation Study

The skin irritation test was conducted for a period of seven days and the results are tabulated in Table 5. The results indicated that the control preparation, F5 formulation containing both drug and permeation enhancer and marketed products did not cause any skin reaction. It can be assured that both drug

,permeation enhancer and other excipients did not cause any skin irritation and can be used in the gel formulation.

Figure1 : Percentage inhibition of edema

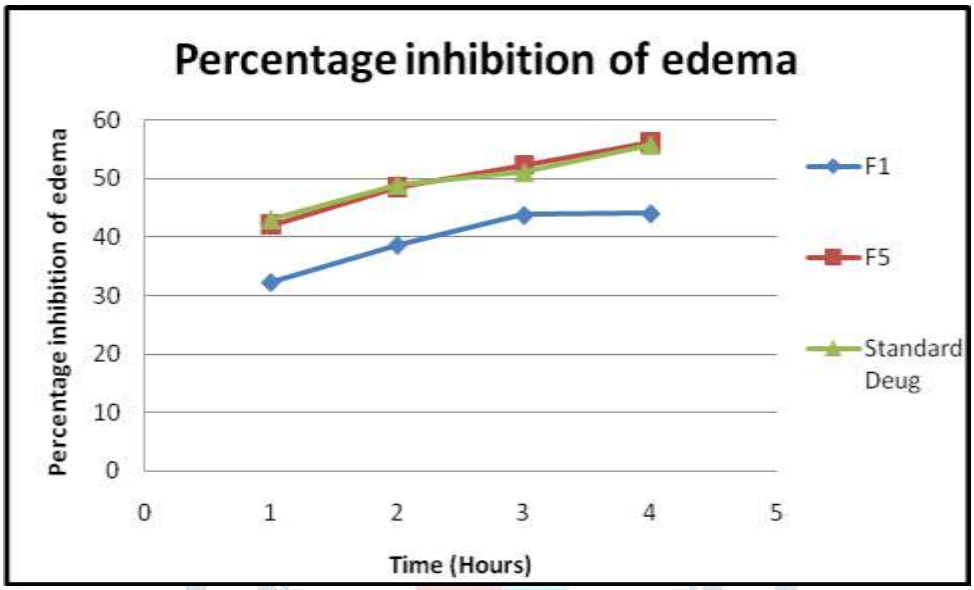

Table 5: Skin irritation study

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	A	A	A	A	A	A
F5	B	B	A	A	A	A	A
Standard Drug	A	A	A	A	A	A	A

A – No reaction, B – Slight patchy erythema, C –Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

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