

Research Article

Formulation And Development Of Sustained Release Anti-Inflammatory Transdermal Pad Using Herbal Extracts

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ABSTRACT

The present investigation was aimed to formulate anti-inflammatory transdermal pad by incorporating herbal extracts. The incorporation of herbal drugs such as boswellic acid (*Boswellia serrata* Rox.), shivlingi extract (*Bryonia laciniosa* Linn.), guggul extract (*Commiphora mukul* Hook.) and isolated compounds from raladhupa namely CS1 and CS2 (*Canarium strictum* Rox.) were envisaged. The drugs were selected on the basis of their synergistic action in suppressing inflammation. The anti-inflammatory transdermal pads were evaluated for their physical properties like thickness of film, moisture absorption and diffusion studies across shaved rat skin. The qualitative drug release of each constituents of formulation on TLC plate indicates drug release occurred at a constant rate. The skin irritation study on albino rabbit skin showed that the formulation does not produce any irritation.

Key words: Transdermal drug delivery, Boswellic acid, Rosin, Transdermal pad

INTRODUCTION

Rheumatic diseases have affected mankind since ages and is a major prototype disease causing disability [1, 2]. RA is a both extra vascular immune complex disease and disorder of cell-mediated immunity leading to chronic inflammation, granuloma formation and joint destruction. The etiopathogenesis of RA involves diverse and complex factors such as genetic background, rheumatic factor (circulating antibodies), immune complexes, compliment activation, lymphocytes, arachidonic acid metabolites, free oxygen radicals etc. [3].

Currently, synthetic drugs form a major line of treatment in the management of arthritis. The conventional drug treatments of RA consist of analgesic, non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs) and cortico-steroids. These agents act at various sites in schema of pathogenic mechanism. Transdermal delivery offers a convenient route of drug delivery and reported to have better patient compliance [4]. Transdermal drug administration generally refers to topical application of agents to healthy intact skin either for localized treatment of tissues underlying the skin or for systemic therapy. For transdermal products the goal of drug design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin [5].

TDDS can minimize first pass metabolism associated with gastrointestinal administration of drugs and maintain constant drug level in blood. It is possible to enhance the transdermal permeation of drug using penetration enhancers [6]. The present investigation was aimed to formulate transdermal pad incorporating herbal extracts such as boswellic acid (Boswellia serrata Rox.), shivlingi extract (Bryonia laciniosa Linn.), guggul extract (Commiphora mukul Hook.) and isolated compounds from raladhupa (Canarium strictum Rox.). Boswellic acid, a constituent of *Boswellia serrata* (Family Burseraceae) reported to have anti-inflammatory and antirheumatic activities along with anti-pyretic effect with no ulcerogenic effect and is well tolerated in as high as dose of 2 gm/kg in mice. It improves blood supply to joints and restores integrity of vessels obliterates by spasm of internal damage. Boswellia serrata (Salai guggul) shows its superiority over conventional drugs being used since ages and is absolutely free from toxic and side effects [7]. Capsicum plaster reported to have used in the treatment of chronic low back pain [8]. The compounds isolated from Canariun strictum (Family Burseraceae), commonly known as black dammer resin has been known to be an effective anti-inflammatory and analgesic activity and is well tolerateted at 100 mg/kg in mice [9]. It undergoes extensive first pass metabolism and hence is a suitable candidate for transdermal patch formulation. The capsicum oleoresin as an anti-inflammatory agent, good penetration enhancer and a pharmaceutically effective carrier [10]. Bryonia laciniosa (Linn.), commonly known as shivlingi has been reported for anti-inflammatory activity [11]. *Commiphora mukul* (Hook.) commonly known as guggul used as effective anti-rheumatic agent. Rosin and rosin esters has been reported to have good film forming property [12]. The herbal drugs (boswellic acid, guggul extract, menthol and capsicum oleoresin) have been reported for producing synergistic action in suppressing inflammation and are time tested and proven safe [13].

MATERIALS AND METHOD

Materials

Saiba Industries Private Limited, Mumbai, gifted capsicum oleoresin. Indrajit Herbal Private Limited Nagpur gifted Boswellic acid. Rosin N grade (ISI) and all the chemicals used in the study were of Analytical Reagent grade. The leaves of *Bryonia laciniosa* Linn. was procured from the campus of Department of Pharmaceutical Sciences Nagpur. The above material was botanically identified and confirmed from the Department of Botany, Rashtrasant Tukdoji Maharaj, Nagpur University, Nagpur. The plant specimen was dried and its herbarium sheet was prepared and it is available in Department of Botany, R.T.M., Nagpur University, Nagpur. (Specimen voucher No. is 6511/A)

Animals

Albino rats of either sex weighing between 120-150 gm were used for the present investigation. Animal Ethical Committee approved experimental protocol under guidelines of CPCSEA, New Delhi. The rats were housed at controlled temperature $(25\pm2^{\circ}C)$ with food, water *ad libitum*.

Preparation of medicated transdermal pad

Rosin (3.25 gm) and bees wax (3.25 gm) in equal proportion (1:1) was melted in china dish and extract of guggul (0.4 gm), boswellic acid (1 gm), isolated compounds from raldhupa namely CS1 (0.5 gm) and CS2 (0.5 gm) were added in them followed by menthol (1 gm), capsicum oleoresin (0.5 gm) and 0.1 gm of liquid paraffin were added and stirred to get uniform paste. The 10 gm above paste was speared on special type of crape bandage cloth and dried at room temperature for 10 min to obtain anti-inflammatory transdermal pad. These pads were then subjected to further evaluation.

Evaluation of transdermal pad

The transdermal pads were evaluated for followings physiochemical parameters.

Thickness

The thickness of the transdermal pad was determined using micrometer screw gauge (Mitoyoto, Japan), recording mean of six were determinations [14]. The observations are shown in Table 1.

Moisture content

The prepared transdermal pad was cut into 20 x 50 mm strips. The strips were then weighed individually and kept in a dessicator containing activated silica at room temperature for 12 hours.

The films were reweighed individually until a constant weight was obtained. Percentage of moisture content was then calculated based on the change in the weight with respect to the initial weight of the film [15]. The observations are shown in Table 1.

Water absorption studies

The water absorption capacities of various pads were determined at 75 % and 93 % relative humidity (RH). Transdermal pads were cut into 25 x 60 mm strips. A strip was weighed and kept in a dessicator at 40° for 24 hours, removed and exposed to relative humidity conditions of 75 % (containing saturated solution of sodium chloride) and 93 % (containing saturated solution of ammonium hydrogen phosphate) in different desiccators at room temperature. Weight was taken periodically until a constant weight was obtained. The water absorption capacity of the pads (in weight %) was calculated in terms of percentage increase in the weight of pad over the initial weight of the specimen [16]. The observations are shown in Table 1.

Qualitative evaluation of drug release by thin layer chromatography

In vitro diffusion study

Herbal transdermal pads measuring 3.14 cm^2 were subjected to *in-vitro* diffusion testing using Franz diffusion cell [17]. Suitably prepared shaved rat skin was clamped between the donar and receptor compartments and the pad was placed over the skin. The receptor compartment contained phosphate buffer having pH 7.2 and ethanol (8:2) at 37° C \pm 1° C. The medium was magnetically stirred and the drug diffusing into the receptor compartment across the skin were determined by withdrawing 2 ml samples after 1 h interval up to 12 h and an equivalent amount of diffusion medium was added to the receptor compartment to maintain a constant volume [18, 19].

The samples for qualitative evaluation of drug release were concentrated and these samples were analyzed by using Thin Layer Chromatography (TLC). The samples were applied on precoated Silica gel plate as bands. The plate was developed in Ethyl acetate: Acetone (4:6) as mobile phase and R_f values was determined. The R_f values of released drugs were compared with R_f values of pure drugs [20]. In this way drugs permeation through the skin were determined [21]. The observations are shown in Fig. 1 and Table 2.

Skin irritation studies

The results of skin irritation studies showed negligible erythema with prepared films. The absence of edema indicates that the transdermal pads are compatible with the skin and hence can be used for the transdermal application.

Anti-inflammatory studies

The transdermal pads of formulation and diclofenac diethyl ammonium salt as standard were prepared. Rosin (3.5 gm) and Bees wax (3.5 gm) in equal proportion (1:1) melted in china dish and salt of diclofenac diethyl ammonium (1 gm) were added in them followed by menthol (1 gm), capsicum oleoresin (0.1 gm) and 0.1 gm of liquid paraffin were added and stirred to get uniform paste. The 10 gm above paste was speared on special type of crape bandage cloth and dried at room temperature for 10 min to obtain transdermal pad of



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Fig 1: Qualitative evaluation of drug release by thin layer chromatography



Where,

(A = Boswellic acid, B = Sample withdrawing after 2 hrs, C = Guggul extract, D = Sample withdrawing after 4 hrs, E = Capsicum oleoresin, F = Sample withdrawing after 6 hrs, G = Shivlingi extract, H = Sample withdrawing after 8 hrs, I = CS1, J = Sample withdrawing after 10 hrs, K = CS2, L = Sample withdrawing after 12 hrs.)



Fig 2: Effect of formulation on percent inhibition of carrageenan induced rat paw oedema

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Formulation	Thickness in (cm)	% Moisture Content	Water Absorption (%)		
			RH 75 %	RH 93 %	
1	0.06	0.35	1.77	2.01	
2	0.05	0.41	1.75	2.03	
3	0.06	0.35	1.69	1.99	
4	0.07	0.36	1.73	1.98	
5	0.08	0.37	1.79	2.37	
6	0.07	0.35	1.72	2.49	
$Mean \pm SD$	0.065 ± 0.0104	0.37 ± 0.020	1.74 ± 0.036	2.14 ± 0.224	

Table 1: Thickness in (cm), moisture content and water absorption capacity in (wt %) of formulation

Data represents Mean \pm SD of 6 determinations, RH indicates relative humidity

Table 2: Thin Layer Chromatographic study of various extracts

Test Extracts	Mobile Phase	R _f values
Shivlingi Extract	Ethyl acetate: Aceton	ie (4: 6) 0.9
CS1	Ethyl acetate: Aceton	e (4: 6) 0.8
CS2	Ethyl acetate: Aceton	e (4: 6) 0.7
Guggul Extract	Ethyl acetate: Aceton	e (4: 6) 0.9
Capsicum oleoresin	Ethyl acetate: Aceton	ie (4: 6) 0.5
Boswellic acid	Ethyl acetate: Aceton	e (4: 6) 0.9

Table 3: Effect of formulation on percent inhibition of inflammation of carrageenan induced rat paw oedema

Test Group (N=6)	0 hr	1 hr	2 hr	3 hr	Percent Inhibition		
					1 hr	2 hr	3 hr
Control	1.038 ± 0.014	1.501 ± 0.020	2.388 ± 0.011	2.888 ± 0.010	-	-	-
Test	0.961 ± 0.014	1.26 ± 0.011	1.65 ± 0.005	1.815 ± 0.022	40	50	60
Standard(Diclofenac diethyl ammonium salt)	1.011 ± 0.011	1.398 ± 0.020	1.713 ± 0.013	1.856 ± 0.0076	20	50	50



diclofenac diethyl ammonium salt. Albino rats were used in a group of six animals. All animals were marked with the ink so as to dip in the cell to the same height. The test preparation or transdermal pad of our formulation were applied at abdominal region after depilating the abdominal region. The standard group were applied the pads prepared by using diclofenac diethyl ammonium salt.

After an hour 0.1 ml of 1 % carrageenan was injected into the plantar tissue of right hind paw and immediately paw volume was measured, by dipping the paw up to the mark in the cell. The paw volume was again recorded after one, two and three hours. The same experiment was repeated using diclofenac diethyl ammonium salt containing pads [22]. The observations are shown in Table 3 and Fig. 2.

The activity of the drug is expressed as percent inhibition of oedema.

% Inhibition = 100 x (1 - Vt/Vc)

Where, Vt and Vc are the average increase in paw volume of

drug treated and control group respectively. **RESULTS AND DISCUSSIONS**

Bees wax and rosin in (1:1) proportion was selected for formulation of anti-inflammatory transdermal pads because these bases have good consistency and stick up properly without staining the skin. By using all these extracts and isolated compounds, formulation of anti-inflammatory transdermal pad was prepared followed by Thin Layer Chromatographic (TLC) studies were done for each isolated compounds and extracts. The R_r values are shown in Table 2. TLC study shows that all the extracts and isolated compounds are in pure form. By using Franz diffusion cell and shaved rat skin, drug diffusion study of the formulation was done. The drug diffusion study of 12h was observed. In order to evaluate the physicochemical characters of transdermal pad such as thickness, moisture content and moisture absorption studies were determined. The skin irritation study showed the anti-inflammatory transdermal pad does not produce irritation to the skin.



To evaluate anti-inflammatory activity of formulation, carrageenan induced rat paw oedema test is carried out. The prepared anti-inflammatory transdermal pad (test) showed 59% inhibition while the diclofenac diethyl ammonium pad (standard) showed 48% inhibition (Fig. 2). This indicates that the activity of the test pad is comparable to the standard pad and with further optimization of the formulation the activity can be further enhanced.

CONCLUSION

Through the present experimentation, it has found that the drugs of Ayurvedic origin can be utilized in a better form with enhanced efficacy by incorporating in modern dosage forms.

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