Research Article

Formulation and Evaluation of Solasodine Transdermal Patches For Anti-Inflammatory Activity

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ABSTRACT

The objective of the current study is to increase the efficacy and to improve the patience compliance of the herbal medicine which can be achieved by developing alternative drug delivery system. Transdermal drug delivery system has become proven technology which delivers the drug in controlled and non-invasive manner. Presently available synthetic medicines for inflammation cause gastrointestinal irritation and reappearance of symptoms. There is a need for the development of better anti-inflammatory medicine. *Solanum surettense* grows as a wild plant in many parts of India, which is a main plant source for therapeutically active compound Solasodine. Solasodine is isolated from *S.surattense* and characterization of the compound is done using TLC, IR, NMR and MASS spectroscopy. Transdermal patches are formulated using isolated solasodine by solvent evaporation method with two different polymers (HPMC and Ethyl cellulose). The formulations were subjected to various evaluation parameters like weight variation, folding endurance, percentage moisture absorbance, percentage moisture loss and drug content. *In vitro* drug release studies were carried out using Franz diffusion cell. Formulation containing HPMC as skeletal material shows maximum drug release of 99.8 % at the end of 5 hours and it is selected for further investigations. *In vivo* studies for anti-inflammatory activity is performed by carrageen induced rat paw edema technique. Solasodine patches treated group shows 57.1 % inhibition compared with standard Indomethacine treated group which shows 52.3 % inhibition at the end of 4th hour.

Keywords: Anti-inflammatory activity, characterization, evaluation, formulation, isolation

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INTRODUCTION

Herbal medicine has been a backbone for revitalizing human body systems from early stages of human history. Herbal medicines have many advantages like cost effective and they are used in the treatment of various ailments. The main disadvantage is that herbal medicines take too much time to act. The entire process is very slow. Large dose is required when compared to allopathic medicine to treat a particular disease. Now days, to overcome these disadvantages the herbal medicines are formulated using novel drug delivery system. Recently several technical advancements have been made and resulted in new techniques for drug delivery. Transdermal delivery constitutes one of the most important routes for new drug delivery system. It is non-invasive, convenient, can avoid gastrointestinal toxicity and hepatic first pass metabolism. These techniques are capable of controlling the rate of drug release. The transdermal drug deliver has advantage to deliver medicines via skin to systemic circulation at a predetermined rate and maintain therapeutic concentration for prolong period of time [2].

Solanum surettense (solanaceae) has many therapeutic uses mentioned in ayurveda. The plant has been used for inflammation and fever from ancient times[4]. This is mainly due to the alkaloid solasodine which is found in leaves and berries of the plant *S.surattense*. Leaves and berries are collected near local areas of Salem, Tamil Nadu and it is used for the isolation of Solasodine. The isolated compound is

characterized using IR, NMR and MASS spectroscopy. Transdermal patches are formulated using isolated Solasodine as two different formulations, Formulaion-1 containing Ethyl cellulose polymer and Formulation-2 containing HPMC polymer. Both the formulations are evaluated using various parameters. Formulation-2 which shows maximum drug release is used for evaluating the anti-inflammatory activity.

MATERIALS AND METHODS

Collection of plant material: The fresh leaves and berries of *Solanum surattense* were collected in the month of July – September from local areas of Salem and authenticated by Dr.P.Jayaraman, Director, Plant anatomy research center, Tambaram, Chennai. The part of plant was shade dried at room temperature and reduced to a coarse powder. It is used isolation purpose.

Isolation of Solasodine:

The dried leaves and berries are first powdered and processed to remove fats using petroleum ether to give greenish yellow oil. This is rejected. The defatted material is extracted three times using ethanol. The combined extracts are concentrated to one tenth of the volume. Concentrated hydrochloric acid is added and the solution is refluxed for about six hours to allow complete hydrolysis of glycol alkaloid. The mixture is made alkaline by adding ammonia and refluxed for one hour. The reaction mixture is cooled and filtered and the residue obtained is thoroughly washed with water till the pH become neutral; is dried. The dried material is dissolved in chloroform, where by solasodine mores into chloroform layer. The solution is filtered and the solvent is evaporated to yield residue containing solasodine. It is further purified by crystallizing from methanol.

Characterization of isolated compound:

TLC [6]:

1mg of the solasodine is dissolved in chloroform. 25µl of this solution was applied on Merck Aluminium plate pre coated with silica gel of 0.2mm thickness and the pate was developed using the mobile phase (10ml). The plate was dried and sprayed with Dragendroff's reagent. Plates are dried until it shows orange coloured spot.

TLC details:

Sample solution: 1 mg of solasodine in chloroform

Development system: Toluene: Ethyl acetate: Diethlamine (7:2:1)

Stationary phase : Silica gel 60 F254 TLC plate of 0.2mm thickness

Detection: Drogendroff's reagent

Spectral analysis [7]:

The isolated solasodine was spectroscopically analysed for confirmation of its structure. Instrumental spectral analysis such as

IR SPECTROSCOPY

NMR – ^{1}H &^{13}C

MASS SPECTROSCOPY

Methods for preparation of patches [8]:

METHOD 1: Ethyl cellulose, PVP was used as the skeletal material of preparation. Propylene glycol as penetration enhancer. PVP (1g) and ethyl cellulose (1g) were weighed in requisite ratios and mixed in 10ml distilled water, stirred the mixture over a hot water bath until dissolved. After the mixture was cooled down to 25^{o}c, solasodine 500mg (5g), propylene glycol (0.5ml), glycerol (0.5ml) were added. The mixture was then poured into glass moulds and dried at room temperature for 24 hrs. The patches were removed by peeling and cut into required size.

METHOD 2: Transdermal patches were prepared by solvent evaporation technique. The polymer (HPMC) and isolated compound of solasodine were weighed. PEG which acts as plasticizer and permeation enhancer, was used in the concentration of 30% v/v. ethanol was used as a solvent. PEG 2.68 ml (30% weigh of polymer) was dissolved in ethanol with stirring. The calculated amount of HPMC (1000mg) was dispersed in solvent ethanol. Isolated Solasodine 500mg was dissolved in ethanol; this solution was then added to polymer base and stirred continuously to get uniform solution. This solution was poured into Petri plate coated with liquid paraffin and then dried a room temperature. After during, patches were removed and cut into required sizes and used for further studies.

Evaluation of medicated transdermal patches [9]:

1) Weight Variation Test: The study was carried out on 9 films. The mean weight of

the film as well as deviation from the mean was obtained.

2) Determination of Folding Endurance:

A patch was repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance.

3) Percentage Moisture Content:

The prepared films were weighed individually and kept in a desiccators containing fused calcium chloride at RT for 24 hrs. After 24hrs the films were reweighed and the percentage moisture content was calculated by the given formula,

Percentage moisture content = initial weight - final weight x 100 Initial weight

5) Drug Content Study:

Transdermal patches were taken (2 cm2 areas) individually, crushed and taken in a 100ml volumetric flask (pH 7.4 phosphate buffer). The medicine was stirred with Teflon – coated magnetic bed for 24hrs. The contents were filtered using wahtmann filter paper and the suitable phosphate buffer pH 7.4. Absorbance of dilutions was measured by using UV – VIS spectrometer at 206nm against phosphate buffer pH 7.4 as a blank.

***In vitro* evaluation of transdermal patches:**

The *in vitro* permeation experiments were conducted using Franz diffusion cell (Receptor compartment capacity: 20ml). Cellophane membrane is used. The receiver compartment was filled with 20ml of 10% hydroalcoholic phosphate buffer, pH7.4. The transdermal patch was firmly pressed into the center of the membrane and then the cellophane membrane is mounted to the donor compartment. The donor compartment was then placed in position such that the surface of the membrane touches the receptor fluid surface. The whole assembly was kept on a thermostatically controlled magnetic stirrer set at 37 ± 2°c and the content in the receiver compartment was continuously stirred at a constant speed (100 rpm) using a magnetic bead. The samples (2ml) were withdrawn at the intervals of half an hour up to 6hrs and replaced with same amount (2 ml) of 10% hydro alcoholic phosphate buffer to maintain the membrane condition.

The samples were analyzed for drug content using UV – VIS spectrophotometer at 206nm. The cumulative % drug release from the transdermal patch of solasodine was calculated.

***Invivo* evaluation of transdermal patches:**

Required animals are procruded from Animal house, Madras Medical College under the approval of Animal ethical committee MMC, Chennai-03.

Skin Irritation Study [9]

Healthy male albino rabbits weighed 1.5 – 2.5 kg are divided into 2 groups containing 3 animals each. On the test day the dorsal surface of each rabbit is shaved prior to the experiment. Group I (control) treated with the transdermal patches without drug. Group II (test) treated with transdermal patches containing test drug. The patches are applied to intact skin for hours. The patches are then removed after hours of exposure period and the formation of any erythema or edema is observed at 24, 48 and 72 hours. The observation was made for 14 days to determine any persistent or delayed effects.

***In vivo* studies for anti inflammatory activity (Carrageenan induced paw edema in rats) [10]:**

The paw edema was induced by 0.1ml of 1% (w/v) carrageenan suspension into the sub-planter region of right hind paw of rats. The control group (A) was orally administered saline (10ml/kg) while the standard group (B) was given indomethacin (5mg/kg) and group (C) patches were applied topically one hour before carrageenan injection. The inhibitory activity was calculated according to the formula,

$$\% \text{ inhibition} = \frac{(Ct-Co)conrol-(Ct-Co)treated)}{(Ct-C0)control} \times 100$$

Where, C_t is the paw circumference at time t, C_o is the paw circumference before carrageenan injection and ($C_t - C_o$) is edema or change in paw size after time t.

RESULTS

Physicochemical parameters of Transdermal patches of solasodine isolated from *S.suratense*:

In the present study, solasodine is isolated from *S.suratense* and it is subjected to TLC studies. The R_f value was found to be 0.62 compared with authenticated compound of R_f 0.6 [Table 1]. Isolated compound is also characterized using IR, NMR and MASS spectroscopy. Transdermal patches of Solasodine were prepared using Ethyl cellulose and HPMC. Poly ethylene glycol is used as a plasticizer and penetration enhancer. The patches were evaluated for their physical characteristics, such as weight variation, folding endurance, % moisture absorbance, % moisture loss, drug content study and release characteristics. All the physicochemical properties were within the limits. There is no sign of erythema or edema for formulated transdermal patches in albino rabbits were observed in 14 days study. *Invivo* evaluation of solasodine patches for anti-inflammatory activity performed by carrageenan induced rat paw edema technique shows 57.1 % inhibition compared with standard Indomethacine treated group which shows 52.3 % inhibition at the end of 4th hour. Paw edema is measured using plethysmograph [**Table 2**].

DISCUSSION AND CONCLUSION

The leaves and berries of *Solanum surattense* is used for the isolation of solasodine and formulation of transdermal patches. The patches which show maximum drug release in *invitro* studies were selected for pharmacological evaluation. Transdermal patches containing solasodine shows better anti-inflammatory activity when compared to synthetic standard indomethacin. Thus these patches may be beneficial for the treatment of inflammation but detailed preclinical and clinical studies are required to establish the use of solasodine transdermal patches as anti-inflammatory formulation.

Tables and Figures

TLC:

Table 1: TLC of solasodine

Sample	Mobile Phase	Ratio	R_f value	Detection	Spot colour
Solasodine	Toluene:ethyl acetate:diethyl amine	(7:2:1)	0.62	Dragendroff's reagent	Orange

Evaluation parameters:

1. Uniformity in weight:

Table 2: Uniformity in weight

Code	Formulation 1	Formulation 2
Polymers	E.C + PVP	HPMC
Weight in g	0.0159	0.0161
	0.0158	0.0158
	0.0162	0.0157
Mean ± SD	0.0159 ±0.0014	0.0159 ±0.0017

2. Percentage drug content:

Table 3: Percentage drug content

Code	Formulation 1	Formulation 2
Polymer	E.C + PVP	HPMC
% Drug Content	92.912	98.758
	93.019	98.448
Mean ±SD	92.858	98.811
	92.929± 0.0211	98.775± 0.0249

3. Percentage moisture absorbance:

Table 4: Percentage moisture absorbance

Code	Formulation 1	Formulation 2
Polymer	EC + PVP	HPMC
% Moisture absorbance	2.4162	2.4540
	2.4155	2.4691
	2.4150	2.4539
Mean±SD	2.4155 ±0.002	2.459 ±0.001

4. Percentage moisture loss:

Table 5: Percentage moisture loss

Code	Formulation 1	Formulation 2
Polymer	E.C + PVP	HPMC
% Moisture Loss	1.205	1.198
	1.199	1.197
Mean±SD	1.200	0.988
	1.201 ±0.004	1.127 ±0.17

5. Folding endurance

Table 6: Folding endurance

Code	Formulation 1	Formulation 2
Polymer	E.C + PVP	HPMC
Folding Endurence	>200	>200

IN VITRO Evaluation of TDS:

Formulation 1:

Table 7: *in vitro* evaluation of Formulation-1

	Abs (nm)	Con. In µg/ml	Concentration in 5ml (µg)	Cumulative concentration in 5ml (µg)	Cumulative concentration in 200ml (µg)	Cumulative % release
1 hr	0.183	0.9320	0.00466	0.00466	0.0932	9.3%
2 hr	0.281	1.4005	0.00700	0.01166	0.2232	22.3%
3 hr	0.324	1.6061	0.00830	0.01996	0.3992	39.9%
4 hr	0.486	2.3804	0.01190	0.03186	0.6372	63.72%
5 hr	0.701	3.4080	0.01704	0.04890	0.9781	97.81%

Formulation 2:

Table 8: *in vitro* drug release of Formulation-2

Time (hrs)	Abs (nm)	Con. in µg/ml	Concentration in 5ml (µg)	Cumulative concentration in 5ml (µg)	Cumulative concentration in 200ml (µg)	Cumulative % release
1 hr	0.326	1.615	0.0080	0.008	0.16	16%
2 hr	0.401	1.974	0.0098	0.1078	0.356	35.6%
3 hr	0.421	2.069	0.0103	0.1181	0.562	56.2%
4 hr	0.440	2.160	0.0108	0.1289	0.778	77.8%
5 hr	0.454	2.227	0.0111	0.1400	0.998	99.8%

Skin Irritation Studies:

Table 9: Skin irritation studies in Albino rabbits

Treatment Group	On 7th day	On 14th day
Group I	0	0
Group II	0	0

Table 10: Anti inflammatory efficacy of transdermal patches of solasodine by carrageen induced paw edema

Treatment group	0 hour	1 hour	2 hour	3 hour	4 hour	% inhibition at 4th hour
Control (10mg/kg)	0.0072 ±0.001	0.0072 ±0.001	0.028 ±0.002	0.024 ±0.005	0.021 ±0.005	12.5%
Indomethacin (5mg/kg)	0.0072 ±0.011	0.0072 ±0.002	0.021 ±0.005	0.021 ±0.001	0.010 ±0.005	52.3%
Solasodine TDS (30mg/kg)	0.0081 ±0.005	0.0082 ±0.002	0.024 ±0.005	0.017 ±0.002	0.010 ±0.002	57.1%

Note: The results are given as mean ± standard deviation; $p < 0.01$ compared to control (n=6)

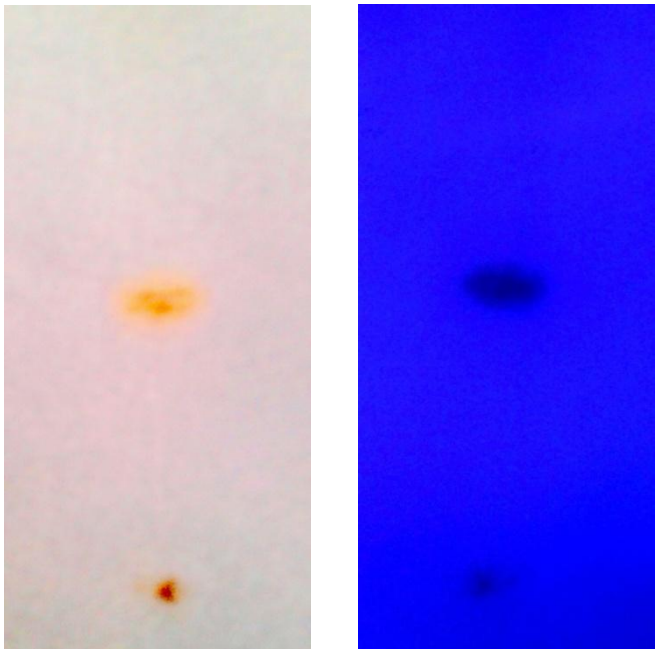

Figure 1: TLC of Solasodine

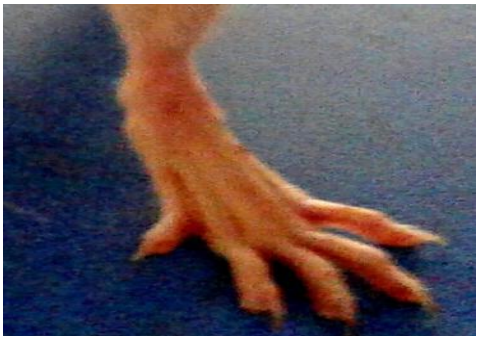

Fig 2: Rat paw before treatment

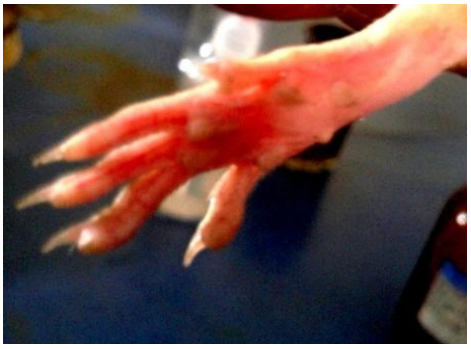

Fig 3: Animal treated with indomethacine at the end of 4th hr

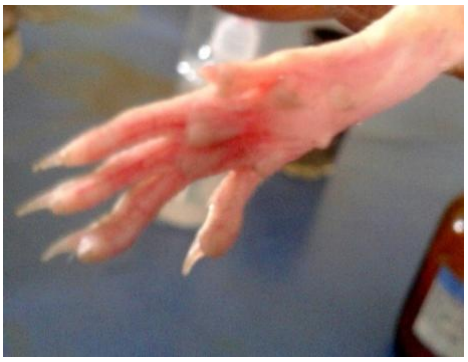

Fig 4: Animal treated with solasodine TDS at the end of 4th hr

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