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ANALGESIC AND ANTIPYRETIC ACTIVITY OF TRANSDERMAL PATCHES CONTAINING SOLASODINE ISOLATED FROM *SOLANUM SURATTENSE.*

R.Keerthana Devi*, R.Radha, N. Jayshree
Department of Pharmacognosy, College Of Pharmacy, Madras Medical College, Chennai- 600003

***Author for Correspondence-**
Email: keeravi89@gmail.com

ABSTRACT

The objective of the current study is to evaluate the Analgesic and Antipyretic activity of transdermal patches containing solasodine. Solasodine is isolated from leaves and berries of *Solanum surattense.* Solasodine has various therapeutic effects on different body systems. The isolated compound is subjected to TLC, IR, NMR and MASS spectroscopy. Transdermal patches are formulated using two different polymers Ethyl cellulose and HPMC. All the patches are subjected to various evaluation parameters like weight variation, folding endurance, drug content, % moisture content and drug release studies. The results obtained were found to be satisfactory and within the limits. The formulation containing HPMC shows maximum drug release at the end of 5th hour and it is selected for *in vivo* studies. Skin Irritation test for transdermal patches in rabbits shows no erythema or edema at the end of 14 days studies. Analgesic and Antipyretic activities were evaluated by acetic acid induced writhing method in mice and brewer's yeast induced pyrexia model. The test group treated with solasodine TDS shows 26.1% inhibition and 4.2% decrease in temperature, compared with standard treated group which shows 13.5% inhibition and 4.1% decrease in temperature for analgesic and antipyretic activity respectively.
KEWORDS: Isolation of solasodine, formulation of transdermal patches, analgesic and antipyretic activity.

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. Transdermal drug delivery systems (TDDS), also known as patches, are dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin. 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.

Solanum surettense (solanaceae) has many therapeutic uses mentioned in ayurveda. The plant has been used for fever and pain since ancient times. 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 analgesic and antipyretic 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:
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: 1mg of solasodine in chloroform
Development system: Toluene: Ethyl acetate: Diethyl amine (7:2:1)
Stationary phase: Silica gel 60 F254 TLC plate of 0.2mm thickness
Detection: Drogendroff's reagent
Spectral analysis:
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:
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 with sufficient amount of 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 2.68ml (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:
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.
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.
Percentage Moisture Content:
The prepared films were weighed individually and kept in a desiccators containing fused calcium chloride at RT for 24hrs. After 24hrs the films were reweighed and the percentage moisture content was calculated by the given formula,

$$\% \text{ Moisture content} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Drug Content Study:
Transdermal patches were taken (2 cm^2 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 (2ml) 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 Madras Medical College under the approval of Animal ethical committee MMC, Chennai-03.

Skin Irritation Study

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* evaluation for analgesic and anti pyretic activity**

Evaluation of antipyretic activity

The antipyretic efficacy of arceous extract was assessed using brewer's yeast induced pyrexia method. Pyrexia was induced by injecting 10 ml/kg of 20% w/v suspension of Brewer's yeast in normal saline subcutaneously 18 hours before the commencement of experiment. Only animals whose rectal temperature increased by at least 1°c after 18 hours of induced subcutaneous yeast injection were included in the study. The normal body temperature of each animal was measured using digital thermometer inserting into rectum. The experimental animals randomly selected were divided into 3 groups. The control group (A) was orally administered saline (10 ml/kg) while the standard group (B) was given (100 mg/kg) aspirin and group (C) treated transdermal patches. The rectal temperature was recorded at time interval of 1 hour, 2 hr, 3 hr, 4 hr and 5 hrs after drug administration.

Evaluation of analgesic activity:

The peripheral analgesic activity of test drug was evaluated in acetic acid induced writing experiment using mice. The abdominal constriction writings resulting from intraperitoneal injection of acetic acid (10 ml/kg of 0.6% V/V glacial acetic acid solution in water) were observed according to standard procedure. 10 ml/kg saline was orally administered to group A (control group) whereas standard aspirin (100 mg/kg) was prescribed for group (B) and transdermal patches are applied for group (C). Acetic acid solution was administered after 30 min and number of writings counted in each animal for 15 min.

Percentage inhibition response was calculated as the reduction in the number of abdominal constrictions between control and test treated groups as a percentage number of witness observed in case of control group.

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. Isolated compound is also characterized using IR, NMR and MASS spectroscopy. Transdermal patches of

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

Table 2: Uniformity in weight

Code	Formulation 1	Formulation 2
POLYMERS	E.C + PVP	HPMC
WEIGHT in g	0.0159	0.0161
MEAN ± SD	0.0158	0.0158
	0.0162	0.0157
	0.0159 ±0.0014	0.0159 ±0.0017

Table 3: Percentage drug content

Code	Formulation 1	Formulation 2
POLYMER	E.C + PVP	HPMC
% DRUG	92.912	98.758
CONTENT	93.019	98.448
MEAN±SD	92.858	98.811
	92.929± 0.0211	98.775± 0.0249

Table 4: Percentage moisture absorbance

Code	Formulation 1	Formulation 2
POLYMER	EC + PVP	HPMC
% MOISTURE ABSORBANCE	2.4162	2.4540
MEAN±SD	2.4155	2.4691
	2.4150	2.4539
	2.4155 ±0.002	2.459 ±0.001

Table 5: Percentage moisture loss

Code	Formulation 1	Formulation 2
POLYMER	E.C + PVP	HPMC
% MOISTURE LOSS	1.205	1.198
MEAN±SD	1.199	1.197
	1.200	0.988
	1.201 ±0.004	1.127 ±0.17

Table 6: Folding endurance

Code	Formulation 1	Formulation 2
POLYMER	E.C + PVP	HPMC
FOLDING ENDURENCE	>200	>200

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

Time (hrs)	Abs (nm)	Conc. in µg/ml	Conc. in 5ml (µg)	Cumulative conc. in 5ml (µg)	Cumulative conc. 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%

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

Time (hrs)	Abs (nm)	Conc. in µg/ml	Conc. in 5ml (µg)	Cumulative conc. in 5ml (µg)	Cumulative conc. 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%

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: Analgesic effect of transdermal patches containing isolated compound of solasodine

Treatment group	Average number of writhings/ 15 min	%inhibition
Control (10ml/kg)	55.5±2.12	0 %
Standard (Aspirin 100mg/kg)	48±1	13.5%
Solasodine TDS (30mg/kg)	41±1	26.1%

Table 11: Anti pyretic effect of transdermal patches containing isolated compound of solasodine

Treatment group	Initial rectal temper-ature (˚C)	Rectal temperature in ˚C after 18 hrs of yeast injection						% Reduction in temperature after 4 hour
		0 hour	1 hour	2 hour	3 hour	4 hour	5 hour	
Control (10ml/kg)	35.6 ±1.02	37.2 ±0.42	37.8 ±0.92	37.8 ±0.84	37.6 ±0.94	36.9 ±0.92	36.7 ±0.70	1.3 %
Standard (Aspirin 100mg/kg)	35.3 ±0.42	37.7 ±2.34	37.8 ±1.13	37.7 ±1.27	37.5 ± 1.20	36.5 ±0.28	36.15 ±0.49	4.1 %
Solasodine TDS(30mg/kg)	35.8 ±0.42	37.4 ±1.34	37.6 ±1.13	37.15 ±0.94	37.1 ±0.56	36.5 ±0.28	35.8 ±0.28	4.2%

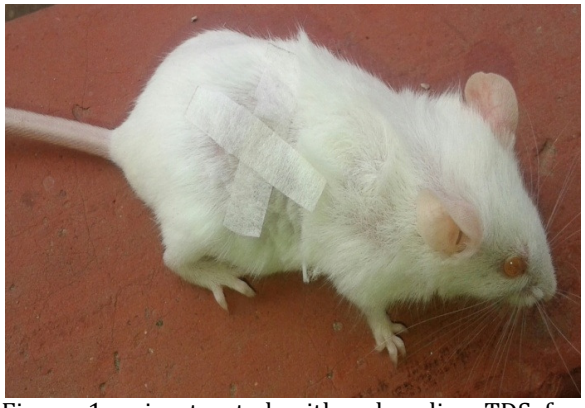

Figure 1: mice treated with solasodine TDS for analgesic activity

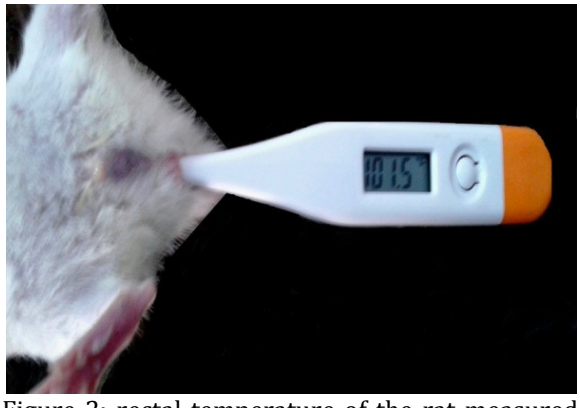

Figure 2: rectal temperature of the rat measured using digital thermometer for antipretic activity

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 analgesic and antipyretic activity were performed by acetic acid induced method in mice and Brewer's yeast induced pyrexia model in rats respectively. The test group treated with solasodine TDS shows 26.1% inhibition and 4.2% decrease in temperature, compared with standard treated group which shows 13.5% inhibition and 4.1% decrease in temperature.

DISCUSSIONS 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 analgesic and anti-pyretic activity when compared to synthetic standard Aspirin. Thus these patches may be beneficial for the treatment of pain and fever but detailed preclinical and clinical studies are required to establish the use of solasodine transdermal patches as analgesic and antipyretic formulation.

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