Original Article

Design, Development and Evaluation of Eucalyptol Transdermal Patch for Anti-Diabetic Activity

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Abstract

For almost two hundred years, diabetes mellitus has been a major health problem worldwide. While there has been progress in our knowledge of its pathophysiology and treatment, the therapeutic choices available today are woefully inadequate. The goal of transdermal medication delivery systems is to provide therapeutically effective doses of a medicine via the skin. The medicine is released at a steady, regulated pace thanks to the design of these devices. As a result, it is well suited for the management of long-term conditions like diabetes. Thus, the risks and inconvenient nature of the oral and parenteral routes are avoided. The purpose of this study is to develop and test eucalyptol transdermal patches for the treatment of diabetes. Drug distribution from different polymer-based transdermal patches might be enhanced by using oleic acid as a penetration enhancer. Matrix-style transdermal patches, composed of PVP K30, HPMC K100, and solvent, were created utilizing solvent casting procedures. By using Fourier transform infrared spectroscopy, researchers were able to examine the drug's and polymers' physicochemical compatibility. No evidence of physical or chemical incompatibility between the medication and the polymers was found. The patches also underwent a battery of physical tests in addition to the in-vivo research. The patches with the polymers, that is, PVP K30, HPMC K100, and oleic acid as the penetration enhancer, were regarded the best formulations for the transdermal distribution of eucalyptol based on the findings obtained from the physical assessment, ex vivo investigations, and in-vivo studies.

Keywords: Eucalyptol, PVP K30, HPMC K100, transdermal patches, Diabetes mellitus

INTRODUCTION

The chemical compound eucalyptol is a monoterpenoid. It is a colorless liquid bicyclic ether. Eucalyptol has a minty aroma and a pungent, cooling flavor. It is water-insoluble yet miscible with organic solvents. Although native to Tasmania, Eucalyptus globulus (eucalyptus; blue gum tree) is historically used to treat diabetes in South America and Africa. The medicinal component of the plant is the leaves used to make tea[1]. In 1902, Faulds recommended for the use of Eucalyptus globulus leaves in the treatment of diabetic mellitus. Diabetes is a chronic (long-lasting) disease that impairs your body's ability to convert food into energy. The body converts the majority of meals into glucose and releases it into the circulation. When blood sugar levels rise, the pancreas is signaled to produce insulin. Type 1 diabetes is believed to result from an autoimmune response (the body attacks itself by mistake). This response prevents the body from producing insulin. Type 1 diabetes affects around 5-10% of diabetics. Often, symptoms of type 1 diabetes develop rapidly. Typically, it is identified in children, adolescents, and young adults. Type 2 diabetes is characterized by insulin resistance and an inability to maintain normal blood sugar levels. About 90-95% of diabetics have type 2 diabetes. It develops over a long period of time and is often diagnosed in adults (but increasingly in children, adolescents, and young adults)[2]. If you are at risk for diabetes, it is imperative that you get your blood sugar checked. Changes in lifestyle, such as weight loss, good eating, and physical activity, may prevent or postpone the development of type 2 diabetes. TDD is a painless technique of systemic drug administration that involves putting a drug formulation to intact, healthy skin. The medication first enters the stratum corneum and then travels through the epidermis and dermis without accumulating in the dermis. When a medication enters the dermis, dermal microcirculation makes it accessible for systemic absorption. TDD provides several benefits over other traditional drug delivery methods. Large skin surface area and ease of access allow for a variety of transdermal absorption placement choices [3]. In addition, medication pharmacokinetic profiles are more consistent and have fewer peaks, reducing the possibility of harmful side effects. The goal of transdermal medication delivery systems is to reach therapeutic drug concentrations via the skin. These devices are meant to dispense the medication at a predetermined and regulated pace. This makes it especially suitable for treating chronic conditions such as diabetes[4].

2. MATERIALS AND METHODS

2.1. Materials

Euclyptol was received as a gift sample from LobaChemiePvt. Ltd. Hydoxy Propyl Methyl Cellulose K 100 was obtained from Department of Pharmaceutical Science, West Bengal . PVP K30 was obtained from SpectrochemPvt. Ltd., Mumbai .

Oleic Acid was obtained from LobaChemiePvt. Ltd . Menthol was used of Analytical grade and All other materials and chemicals used were of either pharmaceutical or analytical grade.

2.2. Methods

2.2.1. Formulation Of Transdermal Drug Delivery System Development of Blank Transdermal Patch

The formulations of drug free films were prepared by solvent casting technique employing glycerine as a substrate. The casting solution was prepared by dissolving appropriate polymers and plasticizers and these were incorporated in suitable solvents with the help of magnetic stirrer until a homogeneous mixture was formed. The solutions were then poured into the petridish and allow to dried for approx 1 day at room temperature. Problems faced like peel ability problem, bubble formed, easily gets teared. To overcome the problem, Oleic acid was added to the slurry to get the plasticizing property as well as lubricant. To control the solvent evaporation rate an inverted funnel over the petri dish was placed on it and left for one day without any disturbance at room temperature. The films could be retrieved intact by slowly lifting from the petri plate and packed in the aluminum foil and kept in the desiccators until used. A blank film layer was prepared and cut into even pieces of desired size (5cm x 5cm)[5].

2.2.2. Experimental Design

A 32 full factorial design was used in the present study. It is attractive to make up a suitable pharmaceutical formulation in shortest time using minimum amount of raw materials. The method is time consuming in nature and needs a lot of imaginative attempt. Two factors were evaluated, each at three levels, and experimental trials were performed at all nine possible combinations. Hence amount oleic acid and amount of HPMC were assumed as independent variables in a 32 full factorial design. The amount of oleic acid was taken as 1gm, 2gm and 3 gm while that of HPMC was taken as 1.50 gm, 2.25 gm and 3.00 gm which responded as -1, 0 and +1 levels respectively as shown in **table 1**. The dependent variables investigated were % of cumulative drug release (Y1), flux (Y2) and folding endurance of patches (Y3). A 32 full factorial design was employed for optimization of the formulations [6].

The medicated patches were prepared by solvent casting technique employing glycerine as a substrate.The casting solution were prepared by dissolving appropriate polymers, drug, plasticizers, and permeation enhancer (5% of the total weight of polymer) were incorporated in suitable solvents according to factorial designed and solution was mixed using magnetic stirrer till to get the clear homogeneous mixture.The solution was then poured into the petri dish and allows drying and solvent evaporation was controlled by placing an invert funnel over the petri dish.These were left at room temperature for one day. patches could be retrieved intact by slowly lifting from the petri plate and packed in the aluminum foil and kept in the desiccators until used.A film layer was prepared and cut into even pieces of desired size (5cm x 5cm)[7].

2.3. Preliminary studies

2.31. Determination of λmax

Determination of wavelength of maximum absorbance (λmax) of EO The maximum wavelength of absorption of EO was determined by scanning the concentration of 350 mcg/mL solution using a UV-visible double beam spectrophotometer within a wavelength range of 400-200nm against acetonitrile as blank [7]. The λmax was obtained at 233nm shown in **figure 1.**

2.3.2. Preparation of calibration curve for Eucalyptol

The standard stock solution was prepared by dissolving 500mg of EO in 100 mL of acetonitrile to obtain a concentration of 5mg/mL. Aliquots of 0.5mL, 1.0 mL, 1.5mL, 2.0mL, 2.5mL, 3.0 mL, 3.5 mL, 4.0mL, 4.5mL, 5.0 and 5.5mL were taken from stock solution and diluted with acetonitrile to 25ml separately to prepare series of concentration from 50-500 mcg/mL.**Figure no 2** shows standard calibration curves with a slope of 0.002 and a regression coefficient of 0.9981 . the curve was found to be linear in the range 50- 450 µg/ml[8] .

2.3.3 Drug-polymer compatibility studies

FTIR- The drug–polymer compatibility studies were carried out using FTIR-Spectrophotometer using SHIMADZU FTIR-8400S . the spectrum was recorded in the region of 4000-400 cm'1. There is no any interaction shown in study in **figure 3.**

Differential Scanning Calorimetry (DSC) – DSC was performed on pure drug, excipients and composition official formulation. DSC measurements were done on a Shimadzu DSC60 having TA60 software, Shimadzu, Japan. They are heated from temperature of 25°C to 300°C C at a heating rate of 10°C per minutes, under constant nitrogen purging and no any interaction shown in drug mixture[9].

Table 1: Formula for different transdermal patches

Ingredients (gm)	TDPE1	TDPE2	TDPE3	TDPE4	TDPE5	TDPE6	TDPE7	TDPE8	TDPE9
drug	0.0052	0.0052	0.0052.	0.0052	0.0052	0.0052	0.0052	0.0052	0.0052
HPMC	1.5	2.25	3.22	0.079	2.25	2.25	3	1.5	3
pvpk-30	1.00	1	1	1	1	1	1	1	1
oleic acid	3	2	2.00	2	1	3.25	3	0.78	0.78
glycerine	1	1	1	1	1	1	1	1	1
Distilled water	15	15	15	15	15	15	15	15	15

2.4. Evaluation of transdermal patches

2.4.1 Folding endurance

The folding endurance measured manually for the prepared patches. It is determined through folding a small strip of film (5cm×5cm) persistently at the identical area until it breaks and to develop visible cracks, gave the assessment of folding endurance[10]. Patch folding endurance was found to be satisfactory between 248 ±0.88 and 495±1.52 as shown in **figure 4.**

2.4.2 Weight Variation

Three patches from each batch were accurately weighed by using a digital weighing balance. The average weight and the standard deviation values were calculated from the individual weight[11]. The weight of Eucalyptol patches ranged from 178±0.01 to 390±0.036 as shown in **figure 5 .**

2.4.3 Thickness of the patches

The thickness of the transdermal films was measured at three different points using a screw gauge and the average thickness values and standard deviation were calculated for each formulation[12]. The thickness of Eucalyptol patches ranged from 0.200± 0.030 as shown in **figure 6 .**

2.4.4 Drug Content

A specific film area (1 x 1 cm^2) was cut and dissolved in a sufficient amount of phosphate buffer saline. The volume was made up to 10 ml and 1 ml was withdrawn from this solution and further diluted to 10ml. After adding suitable reagent and dilution the solution was filtered by Whatman's filter membrane, and the absorbance of the solution was found out at 251 nm by using UV- Vis spectrophotometer. From the absorbance and dilution factor, the drug content in the film was calculated[13]. Average drug content of three transdermal patches ranged from 91±0.50 to 98±0.50 percent, shown in **figure 7** indicating passable drug content in patches.

2.4.5 Percentage Moisture content

The films were weighed accurately and placed in the desiccators containing 100ml of saturated solution of aluminium chloride. The individual films were weighed repeatedly and the patches were taken out, after 3 days, or until a stable weight of film was achieved. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight [14].

$$Percentage of moisture Absorption = Final weight - Initial weight Initial weight *$$

The moisture content in the patches ranged from 5.172±0.027 % to 9.389±0.28 % shown in **figure 8.**

2.4.6 Percentage Moisture Loss

The patches were weighted accurately and kept in desiccators containing activated silica. The in- dividual films were weighed repeatedly and the patches were taken out, after 3 days or until a stable weight of film was achieved. The percentage of moisture loss was calculated as the difference between initial and final weight with respect to initial weight[15].

$$Percentage of moisture loss = Initial weight - Final weight Initial weight * 100$$

The moisture loss in the patches ranged from 3.098±0.53 to 6.5131±0.5 % shown in **figure 9.**

2.5. In-Vitro Study of Transdermal Patches

In-vitro studies were performed by using a Franz diffusion cell with a receptor compartment capacity of 30 mL. in-vitro drug release studies were carried out using synthetic cellophane membrane. The prepared formulations 2cm2 were cut and fixed on to the membrane in the donor compartment and were uniformly spread onto the cellophane membrane. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 32 ± 0.5 °C. The samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically. The receptor phase was replenished with an equal volume of phosphate buffer pH 7.4 at each sample withdrawal[16]. The cumulative percentages of drug were reported in **table 2** and release kinetics were also seen for zero order, first order, higuchi model, korsmeyerpeppas model, hoxson- crowell model as shown in **table 3.**

2.6.1 Determination of Flux and Diffusion Coefficient

The flux (µg/cm2 /hr) of drug was calculated from the slope of the plot of the cumulative amount of drug permitted per sq.cm at steady state against time using linear regression analysis as shown in **table 4.** The drug diffused through the dialysis membrane can be considered to calculate the steady state permeability coefficient (Kp) using the following equation: Kp=J/C

Where, J = Flux at steady state. C = Drug concentration in donor compartment.

2.6.3 Optimization

Optimization of transdermal patch will be done on the basis of % of cumulative drug release , flux and folding endurance of patches[17] .

2.6 Stability Studies of Optimized Formulation

To perform the stability of optimized formulation, samples were sealed in aluminum foil and stored at room temperature[18]. The studies were conducted for six months at a temperature of 40℃ and 75% relative humidity were shown in **table 5**

2.7 In vivo studies

2.7.1 Determination of antidiabetic activity of Eucalyptol transdermal patches on rat model

The Albino rats weighing 150-200 gm were selected for in vivo preclinical studies. The rats were housed in propylene cages with free access to water and standard rat pellet diet. Water was given ad libitum during the entire period of the study. They were maintained at a temperature of 25±1℃ and a relative humidity of 45-55% with a 12 hr light or dark cycle. They were acclimatized to the laboratory conditions before carrying out experimental work in a well-ventilated animal house under natural photoperiod conditions for a period of 1 week. The protocol of in vivo experiments was approved by the Indian Animal Ethical Committee. The hair on the back side of the rat was removed completely on the previous day of the experiment.

2.7.2 Induction of diabetes mellitus

Rats weighing 150-200 g were selected and fasted for a 16 h period for experiments and allowed an excess ad libitum. Experimental Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (55 mg/kg) in 0.1 M citrate buffer (pH 4.5). After 4 days blood glucose level of each animal were determined and rats with blood glucose level above 200 mg/dl were considered as diabetic rats and the formulated transdermal patches were evaluated for anti-diabetic activity in overnight fasted diabetic rats [10].

2.7.3 Experimental design

Rats were divided into four groups, each group of six rats. (6 normal; 18 STZ-diabetic surviving rats) The treatment of rats was as follows shown in table 2 :

Group I was considered as a Normal group, received vehicle only Group II was considered as Disease control group, received 0.1 M citrate buffer (pH 4.5).in distilled water was used as a vehicle.
Group III was considered as Standard treatment group (Eucalyptol 15 mg/kg)
Group IV was considered as Test treatment group received Optimized transdermal patch (TDPE3)

The blood samples were collected by retro orbital method and the serum was separated by centrifugation at 3000 rpm after 30 min of stabilization. Biochemical parameters like glucose, cholesterol, Protein, HDL cholesterol, LDL cholesterol, and triglyceride were measured.

2.7.4. Glucose parameter

The blood glucose level was measured in the normal and experimental group at 0 days, 15th days and 30th days of treatment. A body glucose level for each group of rats was recorded for the last days during the period of experiment in glucometer (one touch select simple). The difference between mean glucose levels in each group was calculated to measure the change in the glucose level on the 30th day as shown in **table 7.**

2.7.5 Cholesterol parameter

The antidiabetic effect of Transdermal patches was observed through determination of lipid profile in serum. Body serum total cholesterol for each group of rats was recorded for the last days during the period of experiment. The difference between mean serum total cholesterol in each group was calculated to determine the change in the serum total cholesterol in 30th day shown in in results below in **table 8**

2.7.6 Protein level, Albumin, Urea parameters

The Total protein, Albumin, Urea levels in normal and streptozotocin induced diabetic rat was determined through body serum for the last days during the period of experiment. The difference between mean serum total Protein level, Albumin, Urea level in each group was calculated on the 30th day shown in results **table 9**

2.7.7 Skin irritation test

The pre-clinical study for skin irritation was conducted using male Albino rats weighing about 2.5-3.0 gms of 24 months of age. The animals were kept in animal cages for 24 hrs. Patches were withdrawn after 24 hrs and examined for erythema, eschar and edema. scale for erythema and edema formation. The results showed that the transdermal systems produced negligible erythema and edema as shown in **table 6.**

3. RESULT AND DISCUSSION

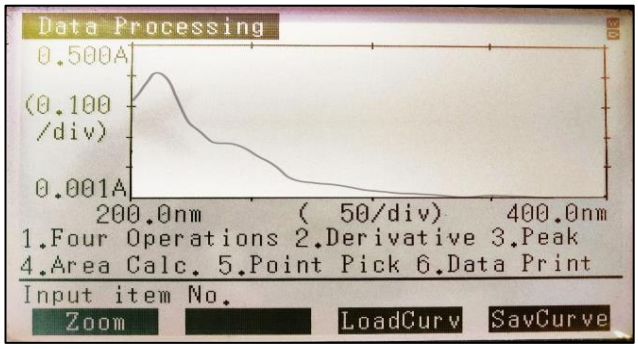

Figure 1 λ_{max} Value of Eucalyptol

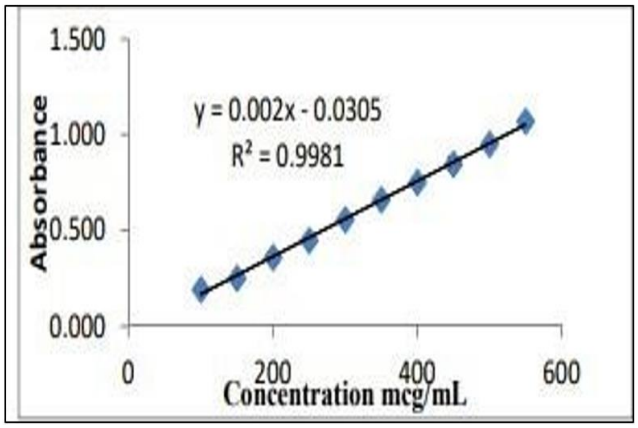

Figure 2: Calibration Curve of Cinnamaldehyde

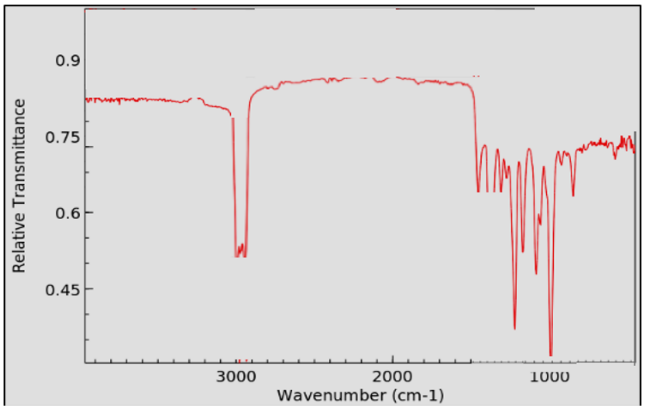

Figure 3 . FTIR Spectra of Mixture of drug and excipients

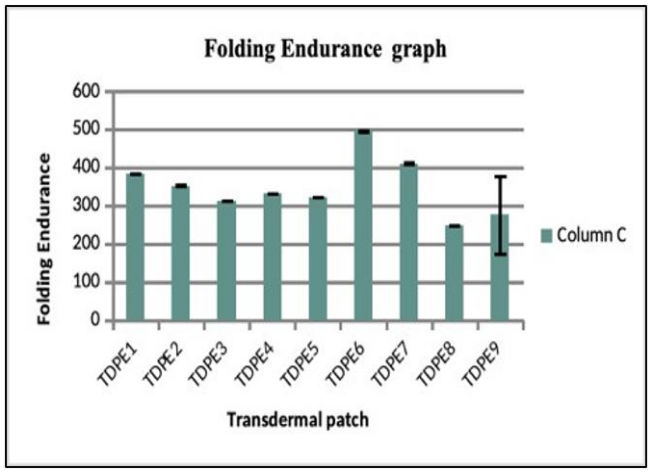

Figure 4: Graph of folding endurance of Eucalyptol

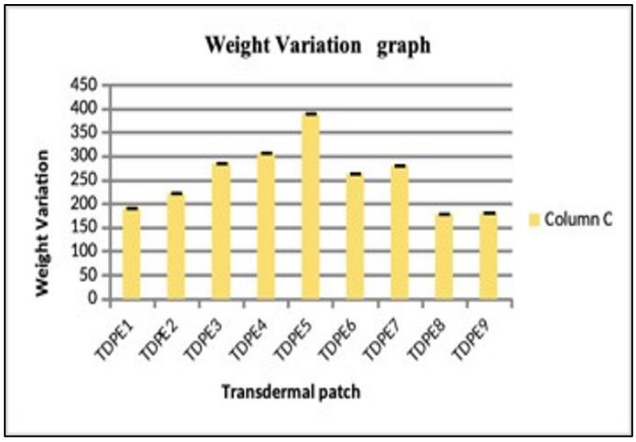

Figure 5: Bar graph of weight variation of Eucalyptol

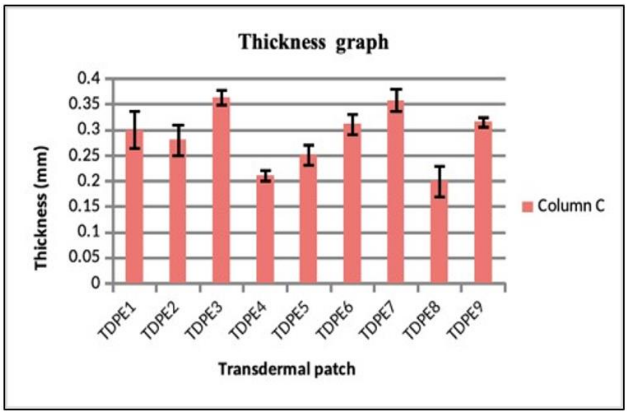

Figure 6: Graph of thickness of Eucalyptol

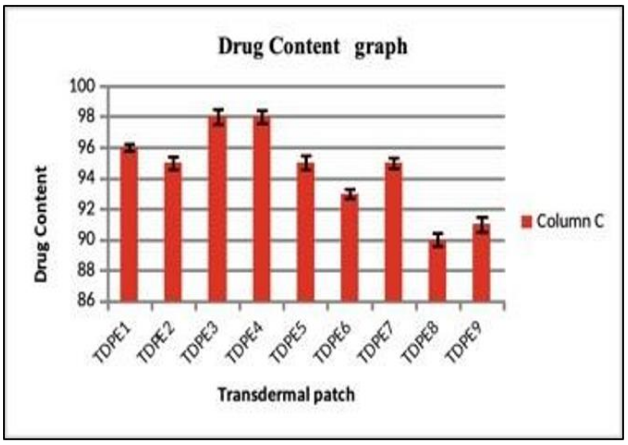

Figure 7: Graph of drug content

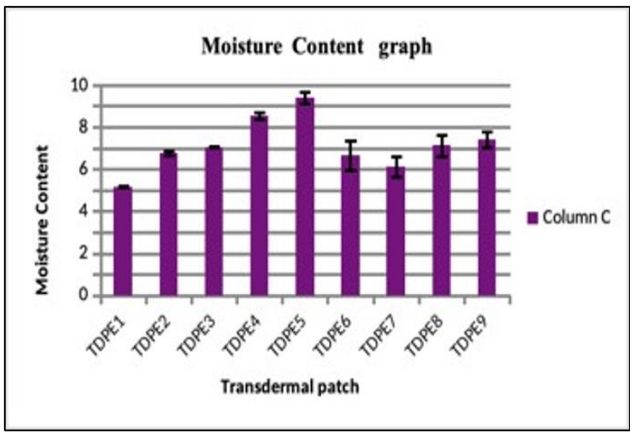

Figure 8: Graph of moisture content

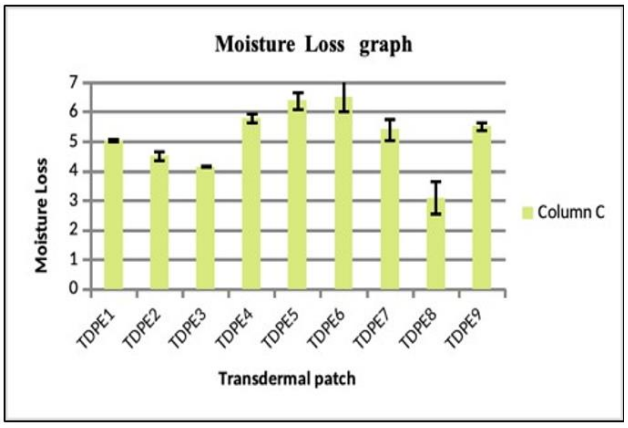

Figure 9: Graph of moisture loss

Table 2: In-vitro study of transdermal patch TDP1 to TDP9

Time (hrs)	CUMULATIVE PERCENT DRUG RELEASE								
	TDPE1	TDPE2	TDPE3	TDPE4	TDPE5	TDPE6	TDPE7	TDPE8	TDPE9
0	0	0	0	0	0	0	0	0	0
2	4.31 ± 0.11	6.18 ± 0.11	6.48±1.02	4.68±1.62	7.18 ± 0.11	9.18 ± 0.11	7.18 ± 0.11	5.08 ± 0.08	6.18 ± 0.11
4	18.42± 0.42	12.68 ± 0.15	15.60±1.08	17.42±0.42	15.18 ± 1.01	23.68 ± 0.14	13.08 ± 0.41	17.14 ± 0.08	13.18 ± 1.01
6	22.78 ±0.01	20± 0.43	28.18±0.8	27.78±0.62	22.08 ± 0.21	36.08 ± 0.51	20.18 ± 0.11	28.15 ± 0.08	23.08 ± 0.21
8	32..05±0.62	37.09±00.02	38.82 ± 0.04	32.84±0.08	30.18 ± 0.1	42.78 ± 0.47	31.18 ± 0.61	33.82 ± 0.08	30.18 ± 0.1
10	39.05± 0.62	45.12±0.01	58.60±1.02	48.83±1.05	41.18 ± 0.01	48.18 ± 0.11	44.218 ± 0.11	42.81 ± 0.08	40.78 ± 0.01
12	51.05± 0.62	58.79±0.08	67.68 ± 0.94	54.08 ± 0.08	52.18 ± 0.18	53.18 ± 0.31	58.18 ± 0.47	50.08 ± 0.08	52.55± 0.18
24	86.05± 0.62	88.68 ± 1.18	95.68 ± 0.94	85.08 ± 0.08	93.18 ± 0.11	90.58 ± 0.11	89.88 ± 0.11	82.08 ± 0.08	90.18 ± 0.18

n=3, values are average of three reading ±standard deviation

Table 3: Drug release kinetics (Eucalyptol)

S.no.	Formulation	Zero order (R^2)	First order (R^2)	Higuchi model (R^2)	Korsmeyerpeppas model (n) (R^2)		Hixson- crowell model (R^2)
1	TDPE1	0.9808	0.938	0.9706	0.591	0.9658	0.7623
2	TDPE2	0.9882	0.9337	0.9869	0.504	0.9525	0.7757
3	TDPE3	0.9836	0.9745	0.9754	0.612	0.9735	0.8147
4	TDPE4	0.9422	0.9614	0.991	0.698	0.96	0.774
5	TDPE5	0.9837	0.999	0.9733	0.544	0.944	0.6232
6	TDPE6	0.9904	0.9707	0.994	0.519	0.9958	0.9813
7	TDPE7	0.9737	0.9193	0.9697	0.483	0.9918	0.8163
8	TDPE8	0.9869	0.9583	0.9986	0.273	0.9541	0.9167
9	TDPE9	0.9925	0.9432	0.9805	0.346	0.9798	0.942

Table 4: Determination of Flux and Diffusion Coefficient

S. No.	Formulation	Drug Release (%)	Flux ($\mu g/cm^2$ /h × 10^{-2})	Diffusion Coefficient (cm^2 /h × 10^{-2})
1	TDPE1	86.05	41.78	0.82
2	TDPE2	88.68	45.46	0.72
3	TDPE3	95.68	56.74	0.95
4	TDPE4	85.08	38.75	0.81
5	TDPE5	93.18	53.32	0.90
6	TDPE6	90.58	50.12	0.84
7	TDPE7	89.88	48.49	0.78
8	TDPE8	82.08	35.18	0.64
9	TDPE9	90.18	50.61	0.86

2.3.1. Optimization of Formulation

In vitro release of Eucalyptol across dialysis membrane from TDPE1 to TDPE9 formulation was only 86.05, 88.68 , 95.68 , 85.08, 93.18 , 90.58, 89.88 ,82.08 , 90.18 % at the end of 24hrs. The flux was calculated from the slope of linear graph, and it was found to be 41.78, 45.46,,56.74, 38.75, 53.32, 50.12, 48.49, 35.18, 50.61 µg/cm2 /h × 10-2, diffusion coefficient was 0.82, 0.72, 0.95, 0.81 , 0.90, 0.84, 0.78 , 0.64, 0.86 cm2 /h × 10-2 respectively. It was evident from the above result that there was a lower flux and lower diffusion rate through the dialysis membrane for all formulation except TDPE3. However, at the end of 24h, in vitro release of eucalyptol across dialysis membrane from formulation. It was revealed from the above results that the TDPE3 showed prolonged release of drug from the patches. The formulation of TDPE3 containing HPMC and PVP-K 30 in the amount of 3.22, 1.00 showed 95.68% of eucalyptol release at the end of 24h study. The flux and diffusion coefficient was found 56.74 µg/cm2 /h × 10-2 and 0.95 cm 2 /h× 10-2 respectively. The physicochemical

properties of the formulation TDPE3 depicted suitable formulation for the transdermal delivery. From the above criteria, TDPE3 was hand-picked as an optimized formulation.

Table 5 : Stability evaluation of optimized formulation of Eucalyptol (TDPE3)

Eucalyptol Formulation (TDP3)					
S. No	**Parameter**	**Initial(0days)**	**1 Months**	**3 Months**	**6 Months**
1	Moisture loss	4.152±0.030	4.152±0.030	4.052±0.030	4.052±0.030
2	folding endurance	342±0.15	342±0.15	342±0.15	342±0.15
3	Drug content	98±0.50	98±0.50	98±0.50	98±0.50
4	Drug release	95.68 ± 0.94	95.68 ± 0.94	94.68± 0.64	94.68± 0.124
5	Weight Variation	286±0.012	286±0.012	286±0.012	286±0.012
6	Tensile Strength	0.612±0.030	0.612±0.030	0.632±0.030	0.632±0.030

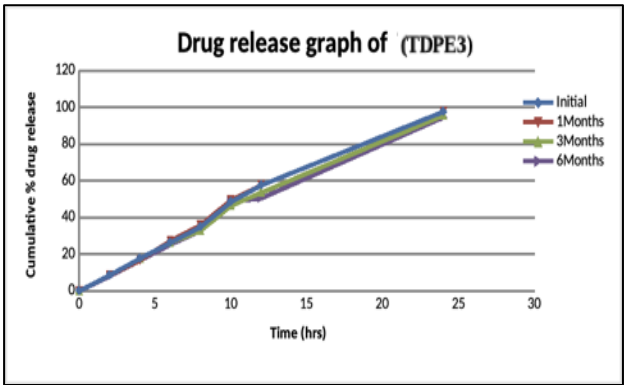

Figure 10: Drug release graph of TDPE3 after 6 month

Stability studies of selected formulations of eucalyptol transdermal patch (TDPE3) were carried out according to ICH guidelines to establish the structural integrity of matrix transdermal film. The results revealed no change or little change in the physical appearance of the formulations after 6 months of study. Optimised selected formulations were subjected for stability study and observed for change in colour, appearance, flexibility and physicochemical parameters like thickness, folding endurance, moisture loss , drug content as well as drug release. The results after the stability period are given in tables. There was no difference in the results of the stability study. The data, after the stability period, of evaluation parameters of transdermal films were found nearly the same as those of patch, before the stability period. It was concluded that the release of drug after the stability period was the same as before. Hence, stability study indicates that the formulation is quite stable.

3.2. In -Vivo Studies

The optimized formulations TDPE3 were further evaluated in vivo i.e. skin irritation, and pharmacodynamic studies.

3.2.1. Skin irritation studies :

The pre-clinical study for skin irritation was conducted using male Albino rats weighing about 2.5-3.0 gms of 24 months of age. The rats were divided into 5 groups (each group consisted of six rats). The hair on the dorsal surface of the rat was removed, on the day prior to that of the experiment. The animals constituting the group I served as control, without any treatment. Animals comprising group II were treated with the commercially available adhesive tape (official adhesive tape in USP). Group III, group IV were treated with optimized formulations TDPE3. The animals were kept in animal cages for 24 hrs. Patches were withdrawn after 24 hrs and examined for erythema, eschar and edema.

The skin irritation study was carried out since severe skin irritation may affect the safety and efficacy of transdermal patch. The optimized formulations have not shown any significant sign of erythema or edema formation. The results were shown in **Table 6.**

Taking the obtained results into consideration it was concluded that none of the animals showed any remarkable irritation of skin, recommending that the developed transdermal patches of cinnamaldehyde and eucalyptol will be well tolerated.

Table 6: Skin irritation score of transdermal patches

Group	**Erythema**	**Edema**
I (Control)	0.00±0.00	0.00±0.00
II (Adhesive tape)	1.82±0.021	1.58±0.22
III (Cinnamaldehyde patch)	1.56±0.20	1.27±0.11
IV (Eucalyptol patch)	1.47±0.21	1.28±0.08

No erythema=0, Very slight erythema=1, Well-drained erythema=2, Moderate to severe erythema=3, Severe erythema=4, No edema=0, Very slight edema=1, Slight edema=2, Moderate edema=3, Severe edema=4

Table 7: Effect of Eucalyptol on plasma glucose levels in normal and streptozotocin-induced diabetic male Albino rats

Groups	Plasma glucose levels (mg/dl)		
	0^{th} days	15^{th} days	30^{th} days
Normal(Liquid))	85.67± 5.3	89.27±6.20	90.01±4.10
Disease control (Simple buffer)	281.4±2.10	288±1.60	310 ± 2.20
Standard treatment (Liquid + Eucalyp- tol 15 mg/kg)	214±2.10	192±2.50	137.26±6.15
Test treatment (Liquid + Optimized transdermal patch TDPE 3)	317.56±2.20	285.6±4.37	183.30±1.43

n=6, values are average of three of 6 reading ±standard deviation

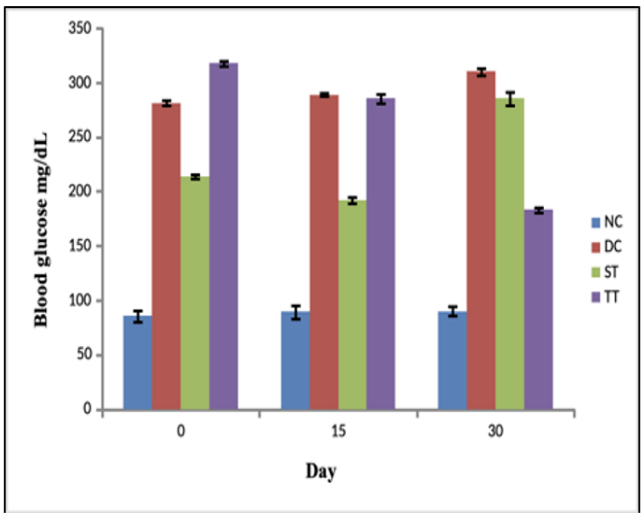

Figure 11. Graph of plasma glucose level

3.2.2. Cholesterol parameter

The antidiabetic effect of Transdermal patches was observed through determination of lipid pro- file in serum. Body serum total cholesterol for each group of rats was recorded for last days dur - ing the period of experiment. The difference between mean serum total cholesterol in each group was calculated to determine the change in the serum total cholesterol in 30th day **(Table 8).**

Table 8. Effect of Eucalyptol on serum total cholesterol, triglyceride, HDL cholesterol levels in normal and streptozotocin induced diabetic rats

Groups	**Lipid Parameter**			
	Total choles- terol (mg/dl)	**Triglyc- erides (mg/dl)**	**HDL-cholesterol (mg/dl)**	**LDL cholesterol (mg/dl)**
Normal(Liquid))	64.5± 4.3	101.2±4.20	69.2.±4.10	12.34 ± 3.10
Disease control (Simple bufer)	205.4±2.10	187.88±4.60	32.10 ± 2.20	179± 2.45
Standard treatment (Liquid + Eu- calyptol 15 mg/kg)	63.1±2.0	89.58±2.50	65.26±1.15	56.8 ± 2.45
Test treatment (Liquid + Op- timized trans-dermal patch TDPE 3	78.56±2.20	73.58±4.37	61.30±1.43	72 ± 3.45

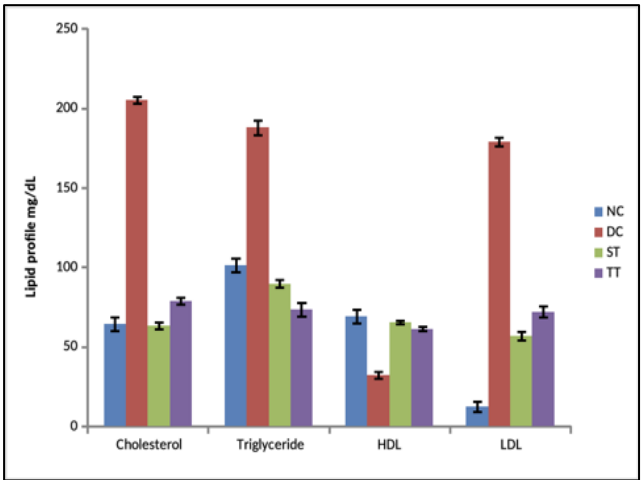

Figure 12. Effect of Eucalyptol on cholesterol level in normal and streptozotocin induced diabetic rats

Table 9. Effect of Eucalyptol on Total protein, Albumin, Urea levels in normal and streptozotocin induced diabetic rats

Groups	Total Protein (g/dl)	Albumin (g/dl)	Urea levels (mg/dl)
Normal(Liquid))	7.68.± 0.3	4.86±0.20	36.24±1.10
Disease control (Simple buffer)	2.69±0.10	2.48± 0.60	74.67 ± 1.20
Standard treatment (Liquid +Eucalyp- tol 15 mg/kg)	7.2±1.0	4.58±1.01	34.26±1.15
Test treatment (Liquid + Opti- mized transdermal patch TDPE 3)	6.13.±0.04	3.57±0.37	30.30±1.43

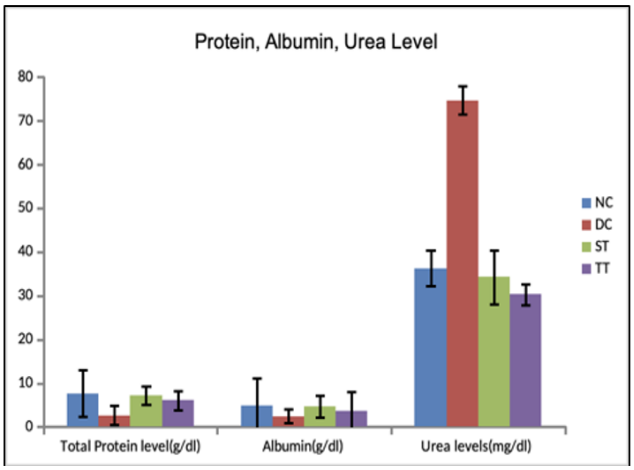

Figure 13: Effect of Eucalyptol on Total protein, Albumin, Urea levels in normal and streptozotocin induced diabetic rats

Transdermal patch containing eucalyptol were prepared by hydrophilic polymer of HPMC K100M,. The patches containing permeation enhancer oleic acid, which also influences the particle permeation through the skin layer by enlarging the skin pore and also to maintain elasticity for eucalyptol patch. After formulation of transdermal patch evaluation study was done for weight variation test, thickness, folding endurance , moisture loss, moisture absorption, drug content and drug release. transdermal patch was practices for in-vivo release studies by using Franz diffusion cell. In this experiment, the patches showed significant release due to HPMC and oleic acid present in surface. After drug release kinetics of drug release pattern were seen by different kinetic model like, zero order, first order, higuchi model, korsemeyerpeppas model, hoxson- crowell model. In vitro release of Eucalyptol across dialysis membrane from TDPE1 to TDPE9 formulation was only 86.05, 88.68 , 95.68 , 85.08, 93.18 , 90.58, 89.88 ,82.08 , 90.18 % at the end of 24hrs. The flux was calculated from the slope of linear graph, and it was found to be 41.78, 45.46,,56.74, 38.75, 53.32, 50.12, 48.49, 35.18, 50.61 µg/cm2 /h × 10^{-2}, diffusion coefficient was 0.82, 0.72, 0.95, 0.81 , 0.90, 0.84, 0.78 , 0.64, 0.86 cm2 /h × 10^{-2} respectively. Folding endurance was found to be 383, 352, 313, 332 , 322 , 495, 410, 248.6, 276. and optimum folding endurance were seen for TDP3 patch. It was evident from the above result that there was a lower flux and lower diffusion rate through the dialysis membrane for all formulation except TDPE3. However, at the end of 24h, in vitro release of eucalyptol across dialysis membrane from formulation. It was revealed from the above results that the TDPE3 showed prolonged release of drug from the patches. The formulation of TDPE3 containing HPMC and PVP-K 30 in the amount of 3.22, 1.00 showed 95.68% of eucalyptol release at the end of 24h study. The flux and diffusion coefficient was found 56.74 µg/cm2 /h × 10-2 and 0.95 cm 2 /h× 10-2 respectively. Stability studies of selected formulations (TDP1) & (TDPE3) were carried out according to ICH guidelines to establish the structural integrity of matrix transdermal film. The results revealed no change or little change in the physical appearance of the formulations after 6 months of study. Optimised selected formulations were subjected for stability study and observed for change in colour, appearance, flexibility and physicochemical parameters like thickness, folding endurance, moisture loss , drug content as well as drug release. The results after stability period are given in table 5, and figure 10.. There was no difference in results of stability study. The data, after stability period, of evaluation parameters of transdermal films were found nearly same as those of patch, before the stability period. It was conclude that the release of drug after stability period was same as before. Hence, stability study indicates that the formulation is quite stable . The skin irritation study was carried out since severe skin irritation may affect the safety and efficacy of transdermal patch. The optimized formulations have not shown any significant sign of erythema or edema formation.

The data of reduction of blood glucose levels by the formulated transdermal drug delivery systems in comparison with orally administered system in both normal and diabetic rats was shown in Tables 7. A significant blood glucose lowering effect was identified in oral and transdermal system treated animal groups within 30 days. Eucalyptol when administered orally produced a percentage reduction of 200.26±6.15 in diabetic control $p<0.05$ compared to control group with no treatment and 210.30±1.43 reduction in blood glucose levels in 30 days in diabetic control with optimized formulation. The blood glucose lowering response was gradual in case of transdermal system. A significant reduction in blood glucose levels were produced by the transdermal patch up to 30 days when compared to control ($p<0.05$). The data of reduction of cholesterol levels by the formulated transdermal drug delivery systems in comparison with orally administered system in both normal and diabetic rats was shown in Tables 8. A significant reduction in cholesterol level was identified in oral and transdermal system of eucalyptol treated animal groups within 30 days. 63.1±2.0 and 78.56±2.20 reduction in total cholesterol (mg/dl) was identified. Triglycerides (mg/dl) level was identified 148.58±2.5 with oral treatment of eucalyptol.

and 182.58±4.37 with optimized transdermal patch. 65.26±1.15 and 61.30±1.43 reduction in HDL level with oral and transdermal treatment was identified. 56 ± 1.45 and 72 ± 3.45 level was identified in LDL level in 30 days with oral and optimized formulation. Total Protein level (g/dl) in diabetic control with optimized formulation 6.13.±0.04 was identified for eucalyptol. Albumin level was 3.57±0.37 , and urea level was 30.30±1.43 identified for transdrmal route of eucalyptol. But from oral route total protein, albumin, urea, it was found to be 7.2±1.0 , 4.58±1.01 , 34.26±1.15 for eucalyptol.

4. CONCLUSION

Transdermal patch of eucalyptol have been successfully formulated by solvent casting technique. Evaluation of the prepared patch in terms of, weight variation, folding endurance, drug content, thickness, moisture absorption and moisture loss, suggest that the method employed for formulation of the transdermal patches was reproducible and ensured excellent quality and uniformity in patch characteristics with minimum variability. Further, in vitro and ex vivo drug release studies for all the formulations showed that drug release was good. nearly complete release (95%) was achieved in 24 h. These results show that transdermal delivery of eucalypol can have good potential applications in therapeutic arena offering advantages in terms of reduced dosing frequency, improved patient compliance, non-invasive characteristics, improved bioavailability, and easy termination of therapy. The required chronic administration of eucalyptol transdermal patch should further accentuate the aforesaid advantages.

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