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Development of non-invasive transdermal patch of *Emblica officinalis* for anti atherosclerotic activity

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Abstract

The present study was designed to formulate matrix type transdermal patches of a potent anti atherosclerotic botanical *Emblica officinalis* on a mercury substrate and evaluated for physicochemical parameters like thickness, % flatness, weight variation, moisture uptake, moisture content, folding endurance, elongation and drug content values. Further, in vivo drug release was also observed by HPLC in rabbit serum. Four formulations were prepared using different ratio of matrix forming polymers, plasticizer and penetration enhancers. Formulations E-1, E-2, E-3 and E-4 were composed of Ethyl cellulose (EC) and Hydroxypropyl Methylcellulose (HPMC) with the following ratios: 6:4, 7:3, 8:2 and 9:1. In vitro cumulative amounts of the permeated drug were observed 48.53, 55.46, 73.26 and 99.72% in 48 hrs from the four formulations. The release profile of the optimized formulation E-4; r^2 = 0.984 (Higuchi) showed that permeation of the drug controlled by a diffusion mechanism. The cumulative amount of the permeated drug after 48hrs from E-4 was 343.95mcg/cm^2. Permeability coefficient was calculated 7.16mcg/cm^2/hr. Based on physicochemical and in vitro skin permeation studies, E-4 was chosen for further in vivo studies. Blood plasma concentration of E-4 after 48 hrs was 0.2914mcg/cm^2. Skin permeation performance and scanning electron microscopic studies revealed that formulation E-4 was found to be better than other formulations and it was selected as the optimized formulation. The skin irritation tests showed negligible erythema and edema. The developed transdermal patches may increase the efficacy of *E. officinalis* for the therapy of atherosclerosis.

Keywords: Transdermal, polymers, permeation, atherosclerosis

Introduction

Many advances have been made in recent years in the area of biopharmaceutical technology especially in the field of Transdermal Drug Delivery Systems (TDDS). The systemic delivery of drugs through TDDS is one area in which significant changes and improvements have been made to achieve an adequate treatment of chronic diseases [1,2]. In a broad sense, all topically administered polymeric drug formulations deliver the active ingredient into the general circulation [3,4]. Increasing prevalence of chronic diseases is also pushing strong demand for TDDS.

WHO has also recommended the use of the herbal drugs which are safe and effective as compare to modern drugs. This leads to increasing demand for herbal products with anti-atherosclerotic activity [5]. Herbal transdermal formulation demonstrated significant anti-inflammatory activity against carrageenan-induced oedema in Wistar albino rats similar to standard formulation [6,7,8]. Currently synthetic drugs administered through transdermal patches form a major line of treatment in the management of chronic diseases like hypertension, atherosclerosis, diabetes etc. [9,10] but they may have several side effects. A targeted and safe drug delivery could improve the performance of some classical herbal medicines. Novel drug delivery technologies have increased the significance to attain modified delivery of herbal active bioingredients thereby, increasing the efficacy and therapeutic value as well as reducing toxicity. This is the fundamental idea behind integrating novel method of drug delivery in herbal medicines. Thus, it is important to integrate novel drug delivery system with medicinally important botanicals to fight against more serious diseases [11,12]. *Emblica officinalis* was selected on the basis of its synergistic action in suppressing hyperlipidemia. The plant *E. officinalis* commonly known as amla is highly valued in traditional Indian medicine [13]. It contains many active components i.e. ascorbic acid, tannins, trigalloylglucose, flavonoids, polyphenols (gallic acids, ellagic acid and phyllemblic acid), etc. which shows significant hypolipidemic activity [14,15].

The supplementation of gallic acid (Figure.1) may have a potential hypolipidemic effect on mice fed high-fat diet. Here, an attempt was

made to analyze the in vitro and in vivo permeation herbal drug through extracorporeal device in to the systemic circulation. The presence of this predominantly phenolic analyte, i.e. gallic acid was confirmed by HPLC. Therefore, aim of this study was to formulate and optimize topically administered herbal patches subjected to in vitro and in vivo evaluation for drug penetration in male rabbits.

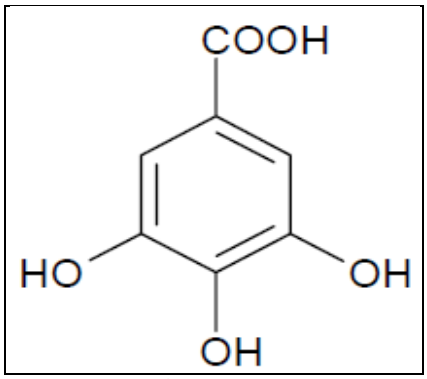

Figure. 1- Gallic Acid

Material and Methods

Emblica officinalis dry extract (B.No.- 108035) was procured as the gift sample from Sanat Product Ltd., U.P., India; Hydroxy Propyl Methyl Cellulose was kindly supplied by Cadila Pharmaceuticals Limited, Ahmadabad, Gujarat, India; Ethyl Cellulose gifted by Asha Cellulose (I) Pvt. Ltd., Valsad, India. All other chemicals used were of analytical grade. MilliQ water was used throughout the study.

Transdermal patch preparation

The patches were formulated by incorporating dibutyl phthalate (10% w/w of dry polymer) as a plasticizer and PEG 400 (10% w/w of dry polymer) as a permeation enhancer. The polymeric casting solutions were prepared by dissolving EC and HPMC (ratios 6:4, 7:3, 8:2 and 9:1 in formulations E-1, E-2, E-3 and E-4 respectively), plasticizer, penetration enhancer in 10 ml blend of chloroform and ethanol (90%) using a magnetic stirrer to get uniform solution. The dry extract of *E. officinalis* (5 mg/cm^2) was added slowly to the solution and dissolved by continuous stirring for 30 min. This polymeric solution was poured on to the mercury surface (25 cm^2) contained in the laboratory fabricated moulds with raised edges [16]. The moulds were kept on a horizontal surface. The rate of evaporation was controlled by inverting a funnel over the mould. After 24 h, the dried cast films were then detached from the mercury substrate and cut to generate transdermal patch of 1.0 cm^2 diameter (Figure. 2). The formulated patches were stored in dessicator until further evaluation. A thin layer of drug-compatible, hypoallergenic pressure sensitive adhesive polymer silicone adhesive applied on the external surface of transdermal patches to provide intimate contact of TDDS with the hair cleaned skin of rabbit [17].

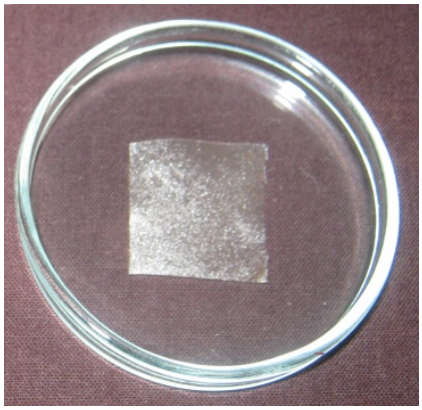

Figure.2: Transdermal patch of *Emblica officinalis*, E-4 (1.0 cm^2) formulated by EC and HPMC (9:1)

Statistical analysis

Statistical evaluation was carried out with SPSS 14.0 (SPSS Inc. Chicago, Illinois, USA). All values were expressed as mean + SEM. The kinetics of drug release from the patches was explained by zero-order, first-order, Higuchi's.

Evaluation of patches

Physical characterization

The thickness of the dried films was measured at five different places using thickness gauze (Instrumentation, India) and their mean ± S.E.M. values (µm) were calculated [18]. The six dried patches of 1cm^2 were cut and weighed on electronic balance to check the uniformity of weight and then mean ± S.E.M. (mg/cm2) was calculated [19]. Folding endurance of the film was determined by repeatedly folding a small strip of at the same place till it broke [20]. Flatness of patches was observed by cutting strips of patches from center, left and right position followed by measuring the length of each strip. The variation in length of strip omitted percent constriction, with 0% constriction equivalent to 100% flatness [21].

Constriction (%) = $(l_1-l_2)/l_1$ 100

Where l_1= initial length of each strip; l_2= final length

Uniformity of drug content of the patches estimated by taking three patches (1cm^2) in separate stoppered conical flasks containing 25 ml. of casting solvent, stirred vigorously for 4 hours on a magnetic stirrer. The above solutions were filtered (0.22 µ) and their drug content was estimated from the standard curves by using gallic acid reference standard (Sigma) at 273nm. Casting solvent of free film (non-medicated) was taken as blank. The patches for each drug were tested for their water absorption capacities. The prepared patches are weighed individually and placed in a desiccators containing activated silica at room temperature for 24 h. Each patch was weighed repeatedly after a specified interval until they showed a constant weight. The percent moisture content is calculated using following formula [21,22].

% Moisture content Initial weight – Final weight

$$= \frac{\text{Initial weight}}{} \times 100$$

A weighed patch kept in desiccators at room temperature for 24 hours was taken out and exposed to 84% relative humidity (saturated solution of potassium chloride) in desiccators until a constant weight for the film was obtained. The % moisture uptake is calculated as given below [21].

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

The tensile strength of patches was determined by gradually increasing the pulling force till the patch broke. The percentage elongation was calculated as Kg/mm^2 by fabricated tensile. Accelerated stability study of patches was performed for 3 months.

In vitro skin permeation studies

In vitro permeation study was carried out to evaluate active ingredient permeability through excised abdominal skin of rabbit using Modified Franz diffusion cell consisting diffusional area of 3.14 cm^2 [23,24]. Hair on the abdominal area of Male New Zealand white rabbits weighing 1.2-1.8 kg were clipped off by applying depilatory for 10min and washed with distilled water one day before the experiment. The rabbits were anaesthetized with ether and the abdominal skin was excised followed by stitching and antiseptic cream was applied for healing. The epidermis of dissected skin was peeled from the dermis and rapidly rinsed with hexane and water to remove surface lipids. The skin was mounted immediately between the donor and receptor compartments of the diffusion cell with the epidermis facing upward to the donor compartment. The film to be tested was placed on the skin [25]. Isotonic phosphate buffer solution (pH 7.4) (22.5ml) was used as receptor phase and agitated with a magnetic stirrer at a speed of 600 rpm and the temperature was maintained at 37±1ºC. Samples (100µl) were withdrawn at regular intervals through the sampling port and fresh receptor fluid was added to maintain the constant volume of the receptor phase. The amount of drug permeated is quantified by using spectrophotometer [26]. The cumulative release was plotted against time and the permeation flux values were calculated from linear portion of the plot. The patches which had shown better permeation flux values were considered for in vivo studies on male albino rabbits [27].

Kinetics of Release

The intrinsic rate of drug release from TDDS is defined by Zero-Order Model, First Order Model and Higuchi Model i.e.

Zero-Order Model

% Released = $K_o t$, Where K_0 is the zero order release constant,

First Order Model,

Log (fraction unreleased) = $(K_1/2.303)t$, Where K_1 is the first-order release constant,

Higuchi Model

$M_t = M_o + K_H \times t_{1/2}$, Where M_t is the amount of drug released at time t and K_H is the Higuchi release rate [28].

In vivo skin permeation studies

The amount of permeated drugs in blood samples after 48 hrs of patch (E4) application on rabbit abdominal skin was quantified by HPLC. The blood samples were collected from marginal ear vein and kept for 1 hour at 37 C to allow it to clot followed by separation of serum by centrifugation at 4000 rpm for 20 minutes at 4 C. Drug separations were performed on a Waters RP HPLC using gradient elution equipped with w600 pump, Waters 2489 UV/VIS detector in combination with Empower2 software. Column used was C-18, (5 µm column having 4.6X250 mm length and 4.6 mm internal diameter was used). Water-acetonitrile- acetic acid (88:10:2; v/v/v) was used as mobile phase delivered at a rate of 1 mL/min. 20µl of the sample solution was spiked with universal injector (Hamilton) on to the HPLC column. Effluent was monitored at 273 nm and compared with gallic acid reference standard (Sigma) [29,30].

The patches applied for in vivo studies were also tested for their skin irritation potential. Patches were applied to the intact skin of rabbits occluded for 24 hrs and then removed for screening of irritancy, erythema or oedema [31,32].

Scanning Electron Microscopy (SEM) studies

The shape and surface morphology of the drug dispersed patches before and after in vivo studies were investigated using scanning electron microscopy (JEOL JEM-100 CX II with ASID). This facility was kindly provided by Electron & Confocal Microscopy Lab, Cancer Pharmacology Division, IIIM, Jammu. Prior to mounting the patches onto the stubs, they were cut in to less than 3mm in length and stubs were than cleaned with polish (Pikal Metal Polish, Tokyo, PIKAL), followed by cleaning with diethyl ether, than double sticky cellophane tape was used to mount patches onto a stub. Silver paint was used to fix the sample on stubs. Stub was placed on a sample disc carrier (3-mm height, 10-mm diameter). Graphite coating was done by vacuum evaporator (vacuum 0.25 Torr) to enhance the better quality of picture and to improve secondary emission of samples. Interaction of the electron beam (40 kV) with the specimen produces a variety of physical phenomenon that detected, are used to form images and provide information about the specimens [33,34].

Results and Discussion

The results of the physicochemical characterization i.e. thickness, % flatness, weight variation, moisture uptake, moisture content, folding endurance, elongation and drug content values of the the formulation are shown in Table 1.

Table 1: Physical Characterization of transdermal patches of *Emblica officinalis*

Code	EC:HPMC	Thickness (µm) ± SD (n=3)	% Flatness ± SD (n=3)	Weight (mg/cm2) ± SD (n=3)	Moisture Uptake (%) ± SD (n=3)	Moisture content (%) ± SD (n=3)	Folding endurance (%)± SD (n=3)	Elongation at break (%) ± SD (n=3)	Drug content (%) ± SD (n=3)
E-1	6:4	195.27±35	99.87	11.85±0.02	6.54 ± 2.3	5.35 ± 2.9	134± 1.8	0.708 ±0.13	98.3 ± 0.4
E-2	7:3	191.42±14	99.97	12.05±0.002	5.58 ± 2.7	4.38 ± 3.5	145± 1.6	0.710 ±0.5	98.2 ± 0.3
E-3	8:2	189.13±0.24	99.99	12.15±0.02	4.94 ± 2.5	5.14 ± 2.3	148± 1.4	0.881± 0.24	99.0 ± 0.4
E-4	9:01	177.25±0.23	100	12.34±0.03	4.31±0.74	3.14 ± 1.5	144± 1.5	0.878 ±0.4	99.98± 0.6

All the formulations found uniform in thickness ranging from 177.25 to195.27 µm. The weights of prepared films ranged between 11.85 mg and 12.34 mg, indicated that the films were uniform in weight. The flatness study revealed that E4 formulation had the same strip length before and after their cuts, further, E4 did not constrict with time indicating 100% flatness (Table1). The drug content of the prepared films ranged from 98.2% to 99.98% showed that the polymers, plasticizer and penetration enhancer used to prepare the films is capable of giving uniform drug content and minimal patch variability. Moisture uptake was observed more in patches having high content of HPMC (E1) with polymer ratio of EC: HPMC as 6: 4 as compared to 9: 1 (E4) (Table 1). The results also conferred the work of Mosquera et al. [35] Formulation E4 had shown minimum moisture content (3.14± 1.5) as compared to other. Formulation E3 and E4 had shown maximum folding endurance i.e. 148 and 144, indicated that these patches would not break and would maintain their integrity when applied on skin.

The results of in vitro skin permeation studies of gallic acid from *Emblica officinalis* patches (E1,E2, E3 and E4) are shown in Figures 3. The % cumulative amount of drug released in 48 hrs from formulations (1 cm^2) E1, E2, E3 and E4 was 48.53 and 55.46, 73.26 and 99.72%)

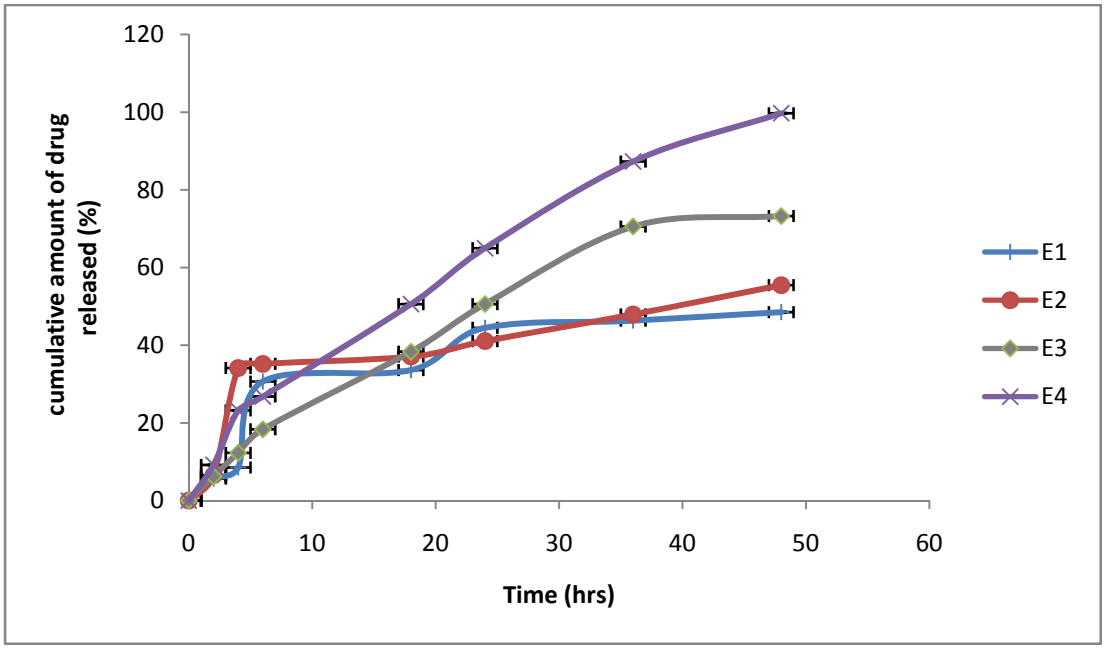

Figure. 3: *In vitro* drug permeation after 48 hrs from E1,E2, E3 and E4 formulations. Each point represents the mean ± SEM value (n=3)

When the cumulative amount of drug permeated per square centimetre of E4 patches through rabbit skin was plotted against time, the permeation profiles of the drug followed mixed zero-order/Higuchi's kinetics (Figure 4. a,b and c).

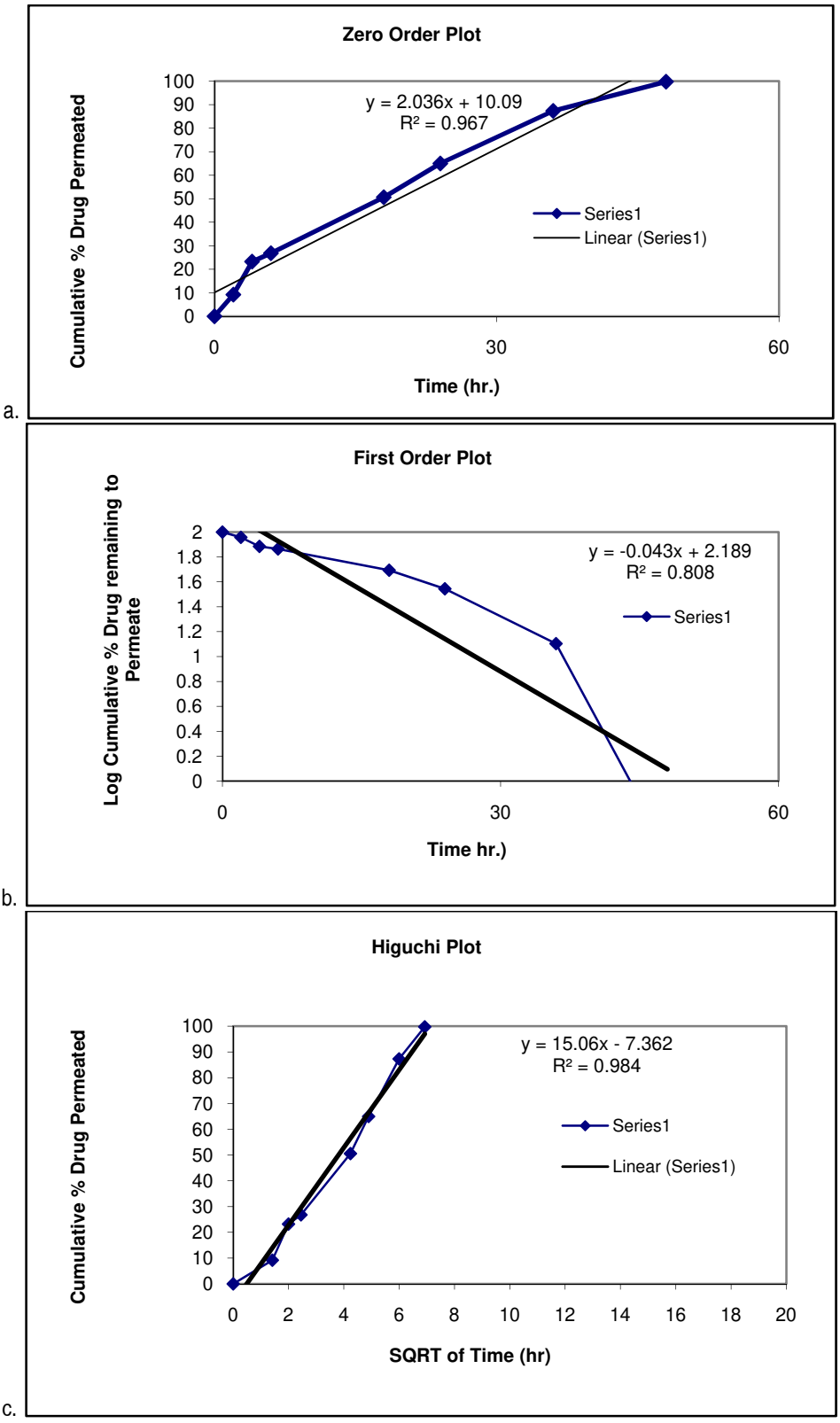

Figure. 4: (a,b,c) Graphical representation of drug release kinetics (Zero order, First order and Higuchi's order) for transdermal formulation of E-4.

The in vitro release profiles of the formulations did not optimally fit into first-order kinetics (r^2 = 0.808). However, the release profile of the formulated patches followed zero-order (r^2= 0.967) and Higuchi's equation (r^2 = 0.984), which indicates that the permeation of the drug from the E4 formulation was governed by a diffusion mechanism (Table.3). Graf of cumulative % drug released vs. square root of time was found linear also suggesting the Higuchian matrix diffusion from the E4 formulations (Figure. 4c). Maximum amount of drug released (99.72%) from formulation E4 was found whereas in case of E3 it was found 73.26% after 48 hrs of study (Table 3 and 2).

Table 2: In vitro skin permeation of the E3 formulation

Time(hr)	Concentration of withdrawal samples (mcg/ml)	Cumulative amount released (mcg)	Cumulative % Drug Released	Cumulative %Drug remaining to Released
0	0	0	0	100
2	13.856	311.76	6.2352	93.7648
4	27.316	614.61	12.2922	87.7078
6	40.8	918	18.36	81.64
18	85.2	1917	38.34	61.66
24	112.4	2529	50.58	49.42
36	156.8	3528	70.56	29.44
48	162.8	3663	73.26	26.74

(* Dissolution factor = 40)

Table 3: In vitro skin permeation of the E4 formulation

Time (hr)	Concentration of withdrawl samples (mcg/ml)	Cumulative amount released (mcg)	Cumulative %Drug Released	Cumulative % Drug remaining to Released
0	0	0	0	100
2	20.4	459	9.18	90.82
4	51.6	1161	23.22	76.78
6	59.6	1341	26.82	73.18
18	112.4	2529	50.58	49.42
24	144.4	3249	64.98	35.02
36	194	4365	87.3	12.7
48	221.6	4986	99.72	0.28

(* Dissolution factor = 40)

The diffusion studies revealed that 9:1 ratio of EC: HPMC (E4) maintain the rate of drug diffusion. Increased percentage of EC in E4 formulation may restrict the instant drug release, due to hydrophobic nature. It may also restrict the formation of gel layer around the patches. High percentage of HPMC polymer may cause rapid hydration and swelling of patches to form viscose gelatinous layer which may cause rapid drug release at the initial hours of patch application on skin (Figure. 3) but low amount of HPMC provide a consistent release of drug with out the loss of patch integrity [36]. Varying the ratio of polymer composition of formulation, the permeability coefficient and thickness of rate controlling membrane can alter the drug release rate. Formulation E-4 prepared with hydrophobic and hydrophilic copolymer containing dibutyl phthalate (10% w/w of dry polymer) as a plasticizer and PEG 400 (10% w/w of dry polymer) as a permeation enhancer showed best in vitro skin permeation through rabbit skin as compared with all other formulations. This may occur due to presence of both hydrophobic and hydrophilic polymer which allows little swelling but did not allow rapid diffusion of the drug from the patches.

Among all formulations, E4 patches considered for in vivo studies on male rabbits. The blood plasma concentration of the drug observed 0.291mcg/ml after 48 hrs (Figure. 5). Figure 4 also showed sustained release of the drug from formulation E4. HPLC analyses also indicated that gallic acid is present in the serum along with some more undetected compounds. During HPLC analysis 1 peak had shown almost same retention (t_R = 4.39min) as shown by reference standard (t_R = 4.42min) of gallic acid (Figure 6 and 7).

Scanning electron microscopic studies revealed that patch (E4) had given smooth morphology with uniform distribution of drug particles before in vivo studies (Figure.8) but after in vivo study (48hrs) physical properties of E4 patch was unchanged and but it converted in to a porous membrane (Figure.9).

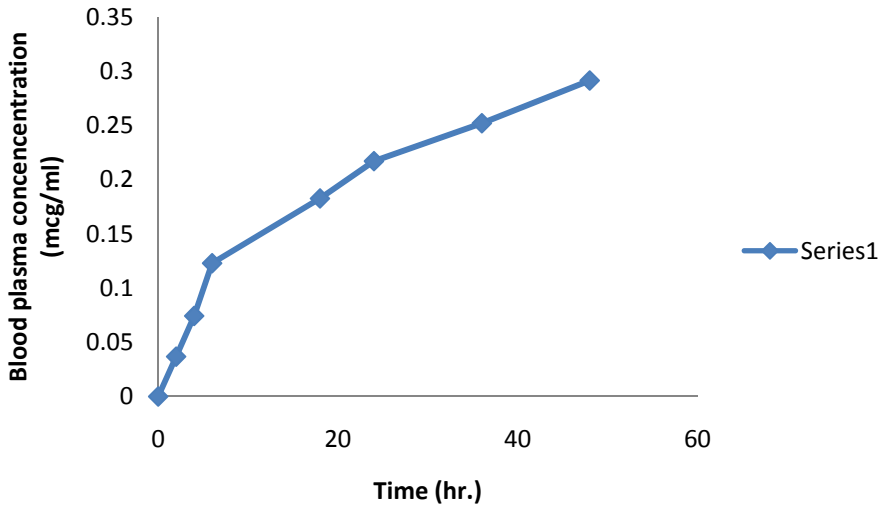

Figure.5: Blood plasma concentration (mcg/cm^2) profile of drug after trasdermal application of formulation E-4 (48hrs) on hair cleaned dorsal abdominal area of rabbit.

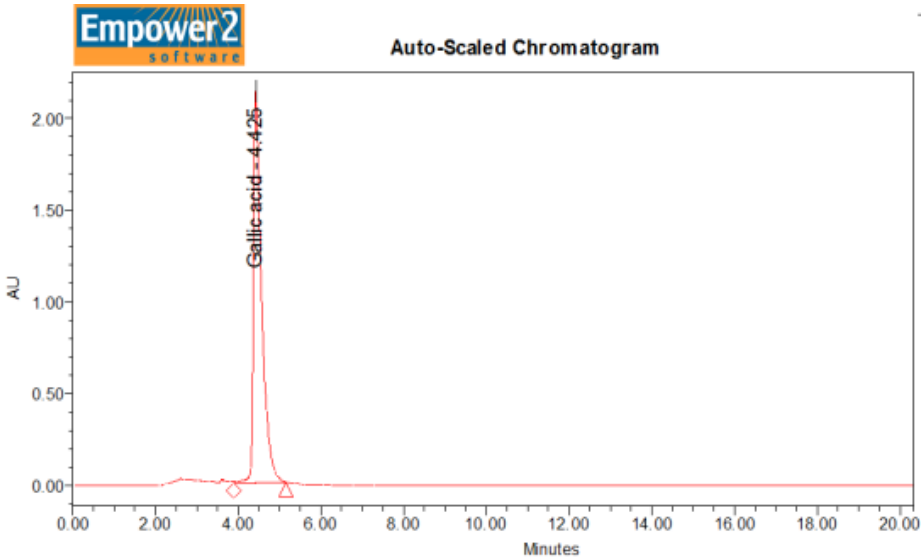

Figure.6: HPLC chromatogram of gallic acid reference standard (Sigma) by Waters RP HPLC using gradient elution equipped with w600 pump. (t_R = 4.42min, Run time = 20 min)

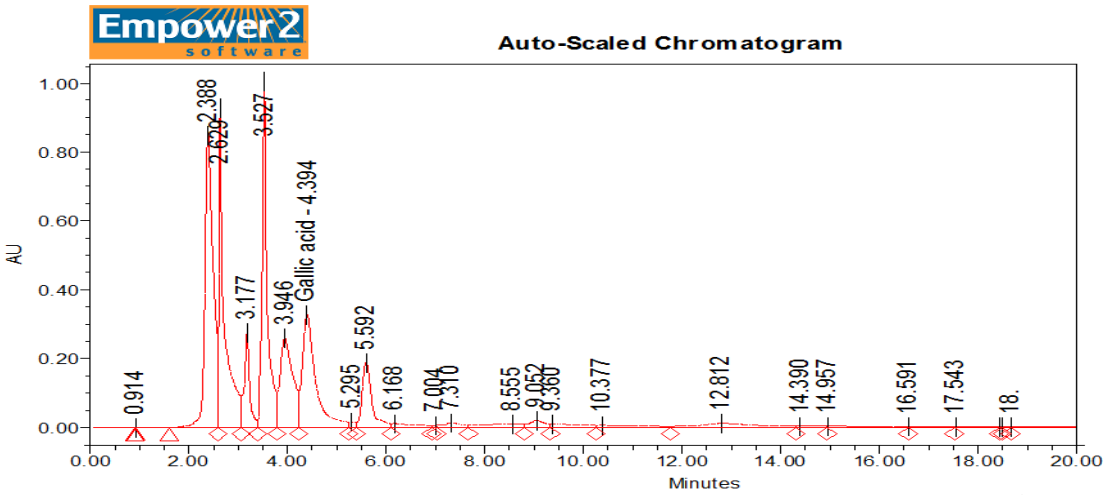

Figure.7: HPLC chromatogram (at 273 nm) of *E. officinalis* patch (E4) after 48 hrs in rabbit serum by Waters RP HPLC using gradient elution equipped with w600 pump. (t_R = 4.39 min, Run time = 20 min).

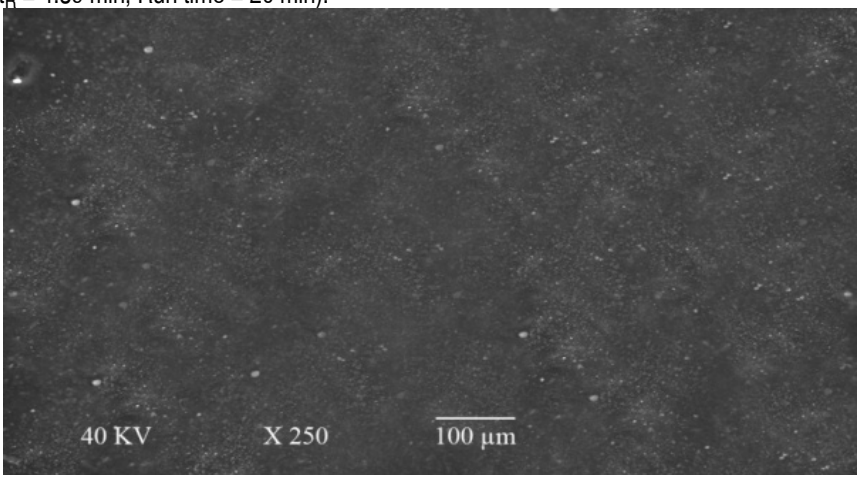

Figure. 8: Scanning electron photomicrograph of the transdermal patch of *Embilica officinalis* (E4), EC: HPMC (9:1) before in vitro application showed film was smooth, discrete, uniform distribution of drug and polymer

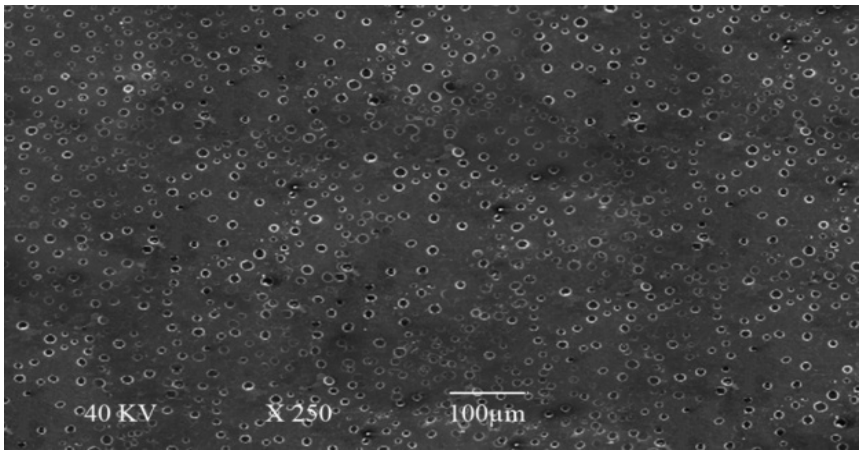

Figure.9: SEM of E4, after 24 hrs in vivo permeation studies did not show change in the morphology of film. It indicated that the ratio of EC: HPMC as 9:1 for developing patches will maintain its physical properties even application on skin. Large no of holes also observed after permeation study indicated that sufficient amount of drug have been released during patch application on skin.

Formation of small pores after in vivo application indicated that there may be some release take placed during in vivo study and permeation study determined that what actual amount of substances (drugs) had permeated via skin. Skin irritation test were performed after in vivo studies to check any irrational effect of patches on rabbits. It was observed that E4 patch was free from skin irritant effect and there was no any sign of erythema after 48 hrs. application on abdomen of rabbit.

Conclusions

Our study revealed that rate controlling membrane of *Emblica officinalis* (E4) have great utility and may be a viable option for effective and controlled release of drug in to the systemic circulation to manage atherosclerosis. The study confer that transdermal patches of *Emblica* are a promising prolonged delivery system for gallic acid with other substances and have reasonably good stability characteristics. In conclusion, on correlating all the formulations studied, E4 was found to be an efficient penetrative extracorporeal device. Incorporation of herbal drugs in novel drug delivery system may lead to an excellent result. Present study may also open up an attempt to utilize herbal drugs through TDDS. Transdermal patch of Emblica penetrates gallic acid successfully and it may be beneficial for topical use to reduce hyperlipidemia after successive preclinical and clinical trial to bring them in market via a suitable drug delivery system for mankind.

Authors' contributions

ND Jasuja, SC Joshi and S Sharma conceived and designed the study and prepared the manuscript. ND Jasuja carried out all the experimental work and statistical analysis to draft the manuscript. PR Sharma carried out the SEM studies; ND Jasuja isolated the compounds by using HPLC. All authors read and approved the final manuscript.

List of abbreviations

HPMC- Hydroxypropyl methyl cellulose; EC- Ethylcellose; PEG- Poly ethylene glycol; DBP- Dibutyl phthalate; TDDS-Transdermal Drug Delivery Systems

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