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Formulation and *ex vivo* skin permeation of lidocaine HCl topical gels using dillenia (*Dillenia indica* L.) fruit gum

Formulación y permeación cutánea *ex vivo* de geles tópicos de lidocaína HCl usando goma de fruta dillenia (*Dillenia indica* L.)

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Abstract

Current work was endeavoured to formulate the topical gels of 4% lidocaine HCl by employing dillenia (*Dillenia indica* L.,) fruit gum (DG) extracted from ripen fruits (14.73% yield). Physicochemical properties such as colour, odour, taste, aqueous solubility, pH and viscosity of this extracted DG were evaluated. Occurrence of carbohydrates and mucilage was confirmed by phytochemical identification tests and was characterized by employing FTIR and ^{1}H NMR spectroscopy. Employing extracted DG along with Carbopol 940 (as gel-forming materials), propylene glycol (as plasticizer), methyl paraben (as preservative) and menthol (as permeation enhancer), topical gels containing 4% lidocaine HCl were formulated. The pH and viscosity of these gels were satisfactory. The *ex vivo* skin permeation of lidocaine HCl across excised porcine ear skin membrane from formulated and marketed topical gels containing 4% lidocaine HCl exhibited sustained permeation over 7 h. Highest lidocaine HCl permeation flux (1589.66 ± 13.36 μg/cm^2/h) was measured for the gel containing 0.1% menthol. Korsmeyer-Peppas model was observed as the best-fitting drug permeation model (R^2 = 0.9944-0.9992) with super case-II transport mechanism (n = 0.97-1.07). These topical gels of lidocaine HCl (4%) were found physically stable enough without syneresis in the freeze thaw cycling process.

Keywords: Dillenia fruit gum, topical gels, drug permeation, lidocaine HCl.

Resumen

El trabajo actual se hizo para formular los geles tópicos de HCl de lidocaína al 4% empleando goma de fruta dillenia (*Dillenia indica* L.,) extraída de frutos maduros (rendimiento del 14,73%). Se evaluaron propiedades fisicoquímicas tales como color, olor, sabor, solubilidad acuosa, pH y viscosidad de este DG extraído. La aparición de carbohidratos y mucílagos se confirmó mediante pruebas de identificación fitoquímica y se caracterizó por emplear espectroscopía de FTIR y ^{1}H NMR. Empleando DG extraído junto con Carbopol 940 (como materiales formadores de gel), propilenglicol (como plastificante), metil parabeno (como conservante) y mentol (como potenciador de permeación), se formularon geles tópicos que contenían 4% de HCl de lidocaína. El pH y la viscosidad de estos geles fueron satisfactorios. La permeación *ex vivo* de la piel de HCl de lidocaína a través de la membrana de la piel del oído porcino extirpado de geles tópicos formulados y comercializados que contenían 4% de HCl de lidocaína exhibió una permeación sostenida durante 7 h. El mayor flujo de permeación de lidocaína HCl (1589.66 ± 13.36 μg /cm^2/h) se midió para el gel que contenía 0.1% de mentol. El modelo de Korsmeyer-Peppas se encontró como el modelo de permeación de fármacos de mejor ajuste (R^2 = 0.9944-0.9992) con un mecanismo de transporte súper caso II (n = 0.97-1.07). Estos geles tópicos de lidocaína HCl (4%) se encontraron físicamente lo suficientemente estables sin sinéresis en el proceso de ciclo de congelación-descongelación.

Palabras clave: Dillenia, chicle de fruta, geles tópicos, permeación de drogas, lidocaína HCl.

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1 Introduction

The topical drug delivery is applied to any particular site/place onto the body surfaces (Leite-Silva *et al*., 2012; Waghule *et al*., 2019). Frequently, topical drug delivery systems in the forms of gels, creams, lotions, ointments, foams as well as nanoformulations are applied onto the body surfaces like skin, eye, mucous membranes, etc., for the cure of a number of diseases (Das *et al*., 2017; Jana *et al*., 2014a,b; Malakar *et al*., 2014; Hasnain *et al*., 2019). Amongst the body surfaces used for the topical drug delivery applications, skin is recognized as the most readily accessible topical drug delivery route (Benson, 2012). Since past few decades', a variety of topical deliveries through the skin are being tested to achieve the local and sustained actions of different drugs (Dua *et al*., 2010). The topical use of drugs encourages the safer and effectual drug deliveries with low doses as compared to that of the oral dosage formulations (Leite-Silva *et al*., 2012). Therefore, it is assumed that the topical deliveries of different drugs restrict the systemic drug absorption that stays away from the chances of hepatic metabolism, gastrointestinal irritation and systemic toxicities (Bachhav and Patravale, 2010; Nayak *et al*., 2010).

In general, local anaesthetics are administered through the intravenous (i.v.) or hypodermic injections (Reiz and Reiz, 1982). But, only a few of local anaesthetics are available in the commercial market. The topical anaesthetics are able to facilitate several benefits of local actions over the injectable-pathways like improvement of patient compliances, continuous drug releasing facility and avoidance of the side effects including nerve damage, hematoma, etc (Das *et al*., 2013; Wang *et al*., 2013). However, the slower penetration and reduced permeability rates of conventional topical anaesthetics into the skin are the two potential limitations of their clinical uses (Das *et al*., 2013; Lee *et al*., 2006). Thus, the local topical anaesthetics capable of faster penetration and increased permeability rates are required for the effective therapeutics of local anaesthesia.

Lidocaine is one of the effective local anaesthetics of hydrophilic nature (Reiz and Reiz, 1982; Smith *et al*., 1999). It has been extensively given as local anaesthetic in the therapeutic management of skin sores, skin lesions, etc., and also in various surgical methods (for example suturing of wounds, venipuncture, etc) (Lee *et al*., 2006; Smith *et al*., 1999). As a local anaesthetic, lidocaine presents several benefits such as faster onset, transitional action, lower incidences of systemic toxic effects, etc., which support the development of topical formulations of lidocaine as a favourable research avenue for achieving local topical anaesthetic action (Sarpotdar and Zatz, 1986). The already reported literature review reveals several topical formulations of lidocaine (Das *et al*., 2013; Wang *et al*., 2013; Lee *et al*., 2006; Sarpotdar and Zatz, 1986; Hasnain et al, 2017; Rowbotham *et al*., 1995; Mueller-Goymann and Frank, 1986; Shin *et al*., 2004). Amongst these formulations, some topical gels of lidocaine using natural gum have also been reported (Das *et al*., 2013; Hasnain *et al*., 2017).

Nowadays, naturally occurring polysaccharides are getting important role in the preparation of different type of drug delivery systems as the excepient and coating materials (Nayak *et al*., 2017, Nayak *et al*., 2018; Milivojevic *et al*., 2019; Hasnain *et al*., 2019; Nayak *et al*., 2019; Bera et al,, 2019; Dey et al, 2019; Samanta et al, 2019; Nayak et al, 2019; Ansari *et al*., 2019; Guru et al, 2018; Serrano-Niño *et al*., 2020; De la Mora-López *et al*., 2018; Flores-Martinez *et al*., 2016; Medrano de Jara et al, 2020; López-Hernández et al, 2018). Dillenia fruit gum (DG) is obtained from ripe dellinia (*Dillenia indica* L., family: Dilleniaceae) fruits (Sahu *et al*., 2011). DG is a water soluble polysaccharide which is biodegradable and biocompatible in nature (Sharma *et al*., 2013). The gummy mucilage of the dillenia fruit is typically employed to cleanse the hair for conditioning effect. It is reported as mucoadhesive gelling agents (Sahu *et al*., 2011; Ketousetuo and Bandyopadhyay, 2007). DG has been reoprted as drug delivery excipient in the preparations of mucoadhesive drug delivery (Sharma *et al*., 2009; Sharma *et al*., 2010). Recently, our team of researchers have reported the successful applications of DG as excipient raw material in the formulation of buccal patches (Hasnain *et al*., 2020a) and dental pastes (Hasnain *et al*., 2020b). Though in the literature, a number of investigations have been already reported on the utilization of DG as the excipients in the preparation of a number of drug delivery dosage forms by various groups (Sahu *et al*., 2011; Sharma *et al*., 2013; Sharma *et al*., 2009; Sharma *et al*., 2010), the investigation to formulate topical gels for local topical anaesthetic action using DG as gel-forming polymeric material is not reported till date. The aim of this research was to formulate topical gels containing 4% lidocaine HCl employing isolated DG along with Carbopol 940 as gel-forming

agents and to evauate *ex vivo* skin permeability of lidocaine from these newly prepared 4% lidocaine HCl topical gels.

2 Materials and methods

2.1 Materials

Lidocaine HCl (Albert-David Pvt. Ltd., India), menthol (Qualigens Fine Chemicals, India), Carbopol 940 and propylene glycol (Loba Chemie Pvt. Ltd., India) were employed. Extraction of DG was done from mature and ripened dellinia fruits procured from the local Baripada market of Mayurbhanj district (Odisha) in the month of September. All other reagents and chemicals were analytical grade and were commercially available.

2.2 Extraction of DG from dillenia fruits

DG was extracted from mature and ripe dillenia fruits as said by the earlier mentioned technique by Kuotsu and Bandyopadhyay (2007) (Ketousetuo and Bandyopadhyay, 2007) with diminutive alterations. Firstly, collected fruits of dillenia were washed by means of water and then with the help of knife reduced into small pieces. Then 1 Kg of these fruits of dillenia were soaked in the demineralized water and afterthat, heated at 45 ± 1 ºC under intermittent agitation by means of an electrical water-bath until a bulky slurry was developed. After cooling, this was set aside in a refrigerator to settle down the undissolved segments for 24 h. The upper part of the clear solution was transferred and afterthat, centrifuged at a speed of 500 rpm for 20 min. The supernatant of this solution was then separated out. Subsequently, this separated solution at 50 ± 2 ºC was concentrated by employing an electrical water-bath until the solution volume reduces to 1/4 th of the initial volume, and cooled down to the room temperature. Then this solution was poured into 1/3 th volume of acetone by means of continuous stirring by employing a magnetic stirrer (Remi Motors, India). The precipitate obtained was washed frequently by means of acetone and consequently by means of demineralised water. This precipitate was then collected and dried for 12 h period at 45 ± 1 ºC in an oven. Fine powder was made by crushing the dried DG and passed through a mesh screen of 80 and stored in air-tight desiccators for further utilization.

2.3 Determination of yield

Yield was stated as %age of the dried extracted material mass in opposition to the total fresh crude material mass. The % yield of extracted material was obtained by employing the formula (Nayak *et al.*, 2015):

$$\%Yield = \frac{Dried\ extracted\ material\ mass}{Total\ fresh\ crude\ material\ mass} \times 100 \quad (1)$$

2.4 Phytochemical identification tests

These extracted materials were then tested for a few phytochemical tests for identification of carbohydrates (Molisch's test), starch (Iodine test), mucilage (Ruthenium red test), glycosides (Keller Killiani test), alkaloids (Dragendroff's test), steroids and sterols (Libermann-Burchard test), tannins ($FeCl_3$ test), proteins and amino acids (Ninhydrin test) (Nayak *et al.*, 2010; Nayak *et al.*, 2012).

2.5 Characterization of extracted DG

2.5.1 Physicochemical characterization

A number of physicochemical characteristics e.g., color, odor, taste, aqueous solubility, pH (at 37 ± 0.5 ºC in 1% solution) and viscosity (at 37 ± 0.5 ºC in 1% solution) of the extracted material were measured. The pH of extracted materials in 1% of the aqueous solution was assessed by means of a digital pH-meter (Systronics, India) whereas viscosity of this solution of extracted material was assessed by means of a cone and plate viscometer (Brookfield DV III ultra V6.0 RV, Brookfield, Middleboro, MA) having 100 rpm spindle rotation. The software employed for the estimation of viscosity was Rheocalc V2.6 (Nayak *et al.*, 2015).

2.5.2 Fourier transform-infrared (FTIR) spectroscopy analysis

Extracted material were mixed with KBr to prepare the KBr pellets and then with the help of a FTIR spectroscope (Perkin Elmer, USA), these pellets were analyzed. The spectral scanning of the pellet was done having a scan speed of 1 cm/sec and a resolution of 4 cm^{-1} in FTIR spectroscope.

Table 1. Formula of 4% lidocaine HCl topical gels containing extracted DG.

	Formulation codes			
Ingredients	**L1**	**L2**	**L3**	**L4**
Lidocaine HCl (%)	4	4	4	4
Extracted DG (%)	5.5	6	6.5	6.5
Carbopol 940 (%)	2	2	2	2
Menthol (%)	-	-	-	0.1
Propylene glycol (%)	5	5	5	5
Methyl paraben (%)	0.02	0.02	0.02	0.02
Purified water q.s. (gm)	10	10	10	10

2.5.3 ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy analysis

^{1}H NMR spectra of these extracted material samples in dimethyl sulfoxide (DMSO) were obtained with the help of a BrukerAvanceTM III 500 spectrometer (Bruker, Germany) functioning at 500.13 MHz employing a 4-mm CP-MAS probe head at 25 ºC.

2.5.4 Preparation of 4 % lidocaine HCl gels

Gels of lidocaine HCl (4%) were formulated with various concentrations of extracted DG along with Carbopol 940, lidocaine HCl, propylene glycol (as plasticizer) and methyl paraben (as preservative). All these excipients and required amount of lidocaine HCl were mixed via the geometric mixing procedure. In Table 1, the formula of 4% lidocaine HCl gels is described and these formulated gels were kept at a cool place until further application.

2.6 Characterization of 4% lidocaine HCl gels

2.6.1 pH measurement

pHs of these formulated gels of lidocaine HCl were measured with the help of digital pH meter (Systronics India Pvt. Ltd., India) by introducing glass electrode into the tested gels, entirely (Hasnain *et al*., 2017).

2.6.2 Viscosity measurement

The viscosities of these 4% lidocaine HCl gels were measured at 25 ± 0.3 ºC with the help of a cone and plate viscometer. For the estimation of viscosities, Rheocalc V2.6 software was employed (Hasnain *et al*., 2017).

2.7 Ex vivo studies

2.7.1 Preparation of skin for ex vivo experiment

For the study of *ex vivo* permeation, excised porcine ear skin membrane was utilized (Das et al, 2017), which were collected subsequent to sacrificing the animal from slaughter house within 1 h. With the help of a hair clipper, the hair onto the surface of skin was removed and complete thickness of the skin membrane was obtained. With the help of a surgical scalpel, layers of fat sticking to the dermis-side of the skin were removed. Lastly, these excised porcine ear skin membrane were cleaned thoroughly by using distilled water and subsequently utilized in the *ex vivo* experiments.

2.7.2 Ex vivo skin permeation experiment of 4% lidocaine HCl gels

With the help of Franz diffusion cell, *ex vivo* skin permeation experiment was performed for prepared gels and marketed gel containing 4% lidocaine HCl. Each Franz diffusion cell contains 2 separate chambers, i.e., the donor as well as the receptor chambers (Malakar *et al*., 2011; Malakar *et al*., 2012). The Franz diffusion cell used in this study comprises 0.79 cm^2 of diffusion area. At the top, the donor chamber of Franz diffusion cell was opened and exposed to atmosphere. In between the chambers of the cell, the collected excised porcine ear skin membrane was lifted with stratum corneum in front of the donor chamber and fixed into the position. Within the receptor chamber, a magnetic stirrer bar was fixed and filled by phosphate buffer saline (pH 7.4) as the media of receptor phase. To avoid the chances of microbial growth, little quantity of sodium azide (0.0025% w/v) was supplementary integrated into the system (Malakar *et al*. 2011). The whole system was kept over a magnetic stirrer maintaining the temperature, 37 ± 1 ºC. In the beginning, the

collected excised porcine ear skin membrane was kept for 2 h in the Franz diffusion cell so as to hydrate it. After the hydration, 4% lidocaine HCl gels (prepared and marketed) of 1 g were placed onto the surface of excised porcine ear skin membrane. From the receptor chamber, 1 mL of receptor phase media was withdrawn at the predestined time-breaks and after that, the identical volume of fresh receptor phase medium was immediately substituted to receptor chamber. With the help of Whatman® filter paper (No. 42), collected samples were filtered. Lidocaine permeated through the excised porcine ear skin membrane was assayed by employing UV-VIS spectrophotometer (Shimadzu, Japan) at 274 nm wavelength (λmax) in opposition to a blank.

2.7.3 Ex vivo skin permeation data analysis

2.7.3.1 Permeation flux

The quantity of lidocaine permeated via the excised porcine ear skin membrane from 4% gels of lidocaine HCl (prepared and marketed) were plotted in opposition to the time function. Slope as well as intercept of the linear segment of the plot were computed through the regression analysis. Drug permeation fluxes from 4% lidocaine HCl gels were derived through dividing the slope by the used skin membrane's surface area (Malakar *et al*., 2014):

$Jss = (dQ/dt)ss \bullet 1/A$, where Jss is the steady-state permeation flux (μg/cm^2/h), A is the area (cm^2) of excised porcine ear skin membrane uncovered to the Franz diffusion cell, and $(dQ/dt)ss$ is the permeated drug amount across the excised porcine ear skin membrane per unit time at the steady state condition (μg/h).

2.7.3.2 Ex vivo permeation kinetics

Result of *ex vivo* lidocaine permeation across excised porcine ear skin membrane from 4% lidocaine HCl gels was estimated kinetically by fitting by means of diverse mathematical models (Malakar *et al*., 2012; Malakar *et al*., 2014):

Zero order model: $Q = k_0 t + Q_0$
First order model: $Q = Q_0 e^{k1t}$
Higuchi model: $Q = kHt^{1/2}$
Korsmeyer-Peppas model: $Q = k_{KP} t^n$

Q and Q_0 refers the amount of lidocaine permeation at time, t and 0, correspondingly; k_0, k_1, k_H and k_{KP} refer lidocaine permeation rate constants. Beside this, n indicates the diffusion exponent entailing permeation mechanism (Das *et al*., 2017). When the value of n is $\leq$ 0.5, Fickian diffusion (non-steady) phenomenon controls and when the value of n is $\geq$ 1, case-II transport (zero order) controls. The value of n in-between 0.5 and 1 indicates to (anomalous) non-Fickian diffusion (Das *et al*., 2017).

2.8 Stability testing

The prepared 4% lidocaine HCl gels were tested for the stability. The stability testing of these gels was performed using freeze-thaw cycling process using a freeze (Godrej Lab Refrigerator, India) (Hasnain *et al*., 2017). For the stability testing, the storage temperatures were altered in every 24 h in-between 25 ℃ to - 5 ℃ for 5 complete cycles. The physical stability as well as syneresis of these gels was noted.

2.9 Statistical analysis

All data was tested by means of plain statistical analyses. Simple statistical analysis was done by employing MedCalc software version 11.6.1.0.

3 Results and discussion

3.1 Yield of extracted DG

DG extraction was done from full-grown and ripe dillenia fruits as said by the earlier mentioned process by Kuotsu and Bandyopadhyay (2007) (Ketousetuo and Bandyopadhyay, 2007) with diminutive alterations. The yield (%) was observed as 14.73% for extracted DG.

3.2 Phytochemical identification of extracted DG

These tests of the extracted DG are summarized in Table 2 and the outcomes suggested the existence of carbohydrates as well as mucilage within the extracted material. A violet-colored ring was appeared at the middle of two liquids when Molisch's reagent added, which indicated the existence of carbohydrates. On the addition of extracted DG solution to ruthenium red, a red-colored ring was formed demonstrating the occurence of mucilage in the extracted DG. The tests for the occurance of starches, glycosides, alkaloids, steroids and sterols, tannins, proteins and amino acids

were found negative without any evidences of changes suggesting the absence of these within extracted DG.

3.3 Characterization of extracted DG

3.3.1 Physicochemical characterization

Physicochemical characterization of the extracted gum of dillenia was depicted in Table 3. This DG powder was of white in color, odorless as well as tasteless and was reported soluble in water at the room temperature, less soluble in cold water and more soluble in hot water. The pH of 1% aqueous solution of extracted DG at a temperature of 37 ± 0.5 ºC was observed as 6.18 ± 0.17; while, viscosity was observed as 14.27 ± 1.22 cps for the identical solution at 37 ± 0.5 ºC and 100 rpm spindle rotation.

3.3.2 Fourier transform-infrared (FTIR) spectroscopy analysis

The FTIR images of extracted DG is presented in Fig. 1 which demonstrates broader band in the region of 3610.74 and 3526.66 cm^{-1} due to −OH groups stretching. In addition, it exhibits some peaks at 2881.65 cm^{-1} owing to −CH stretching, at 1514.12 cm^{-1} owing to aromatic $-NO_2$, and at 1134.14 cm^{-1} due to −C−O−C linkage of polysaccharide (glycosidic linkage). Hence, the outcomes of FTIR analysis demonstrated the presence of polysaccharide in the extracted samples.

3.3.3 ^1H nuclear magnetic resonance (^1H NMR) spectroscopy analysis

The ^{1}H NMR spectrum of extracted DG is presented in Fig. 2 which indicates characteristic signals of polysaccharides that are packed in a tapered region in-between 3.517 to 4.707 ppm (^{1}H NMR signals in-between 3 to 5 ppm is reported as an indicative of polysaccharides) (Cui, 2005; Hasnain *et al*., 2018a,b). Therefore, the results of the ^{1}H NMR spectroscopy analysis suggested the presence of polysaccharide in the extracted samples.

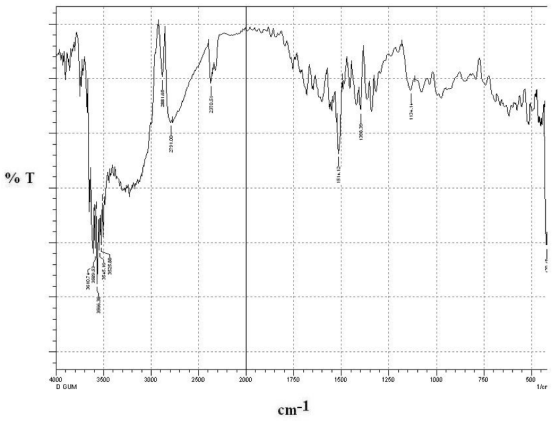

Fig. 1. FTIR spectrum of extracted DG.

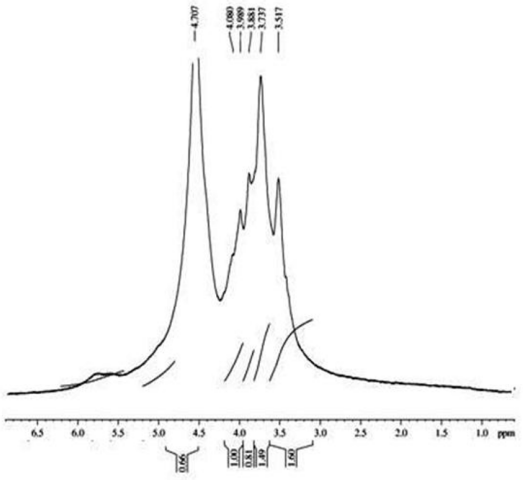

Fig. 2. ^{1}H NMR spectrum of extracted DG.

Table 2. Phytochemical identification tests on extracted DG.

Identification tests	Name of tests	Observations[$]
Test for carbohydrates	Molisch's test	+
Test for proteins and amino acids	Ninhydrin test	-
Test for mucilage	Ruthenium red test	+
Test for starches	Iodine test	-
Test for alkaloids	Dragendroff's test	-
Test for glycosides	Keller-Killiani test	-
Test for tannins	Ferric chloride test	-
Test for steroids and sterols	Libermann-Burchard test	-

[$] + indicates positive; - indicates negative.

Table 3. Physicochemical properties of extracted DG.

Physicochemical properties	Results
Colour	White
Odour	Odourless
Taste	Tasteless
Solubility in water	Soluble in water at room temperature; also soluble in cold water (less) and hot water (more)
pH (1 % solution at 37 ± 0.5 ºC)	6.18 ± 0.17 (Mean ± S.D.; n = 3)
Viscosity (1 % solution at 37 ± 0.5 ºC)	14.27 ± 1.22 cps (Mean ± S.D.; n = 3)

3.4 *Preparation of 4% lidocaine HCl gels*

Lidocaine HCl (4%) gels were formulated with various amounts and ratios of isolated DG and Carbopol 940. In these gel formulations, extracted DG and Carbopol 940 were added as gel-forming agents. Beside these, in the formula of 4% lidocaine HCl gels, methyl paraben and propylene glycol were incorporated as preservative and plasticizer, respectively (Table 1). In the gel formulation L4, menthol (0.1%) was incorporated as permeation enhancer.

3.5 *Characterization of 4% lidocaine HCl gels*

3.5.1 *pH*

pH plays important role in topical gel formulations. Highly acidic or alkaline pH of the topical gel formulations may alter the skin environment, which might cause the irritation of skin when applied (Das *et al*., 2013). The 4% lidocaine HCl gels containing extracted DG and Carbopol 940 showed the pH within the range of 6.12 ± 0.03 to 6.38 ± 0.03 (Table 4). The pH results demonstrated that the pHs of these newly produced topical gels were approximate to the skin pH and can be safely applied as topical formulation.

3.5.2 *Viscosity*

Viscosities of the newly produced 4% lidocaine HCl gels containing extracted DG and Carbopol 940 were measured at 25 ± 0.3 ºC. The 4% lidocaine HCl gels containing extracted DG and Carbopol 940 exhibited the viscosity values within the range of $4.40 \pm 0.03 \times 10^6$ to $4.77 \pm 0.05 \times 10^6$ cps (Table 4). A pseudoplastic flow (i.e., indicative of share thinning) was shown by these newly prepared gels, which is considered as ideal for the topical gel applications (Hasnain *et al*., 2017).

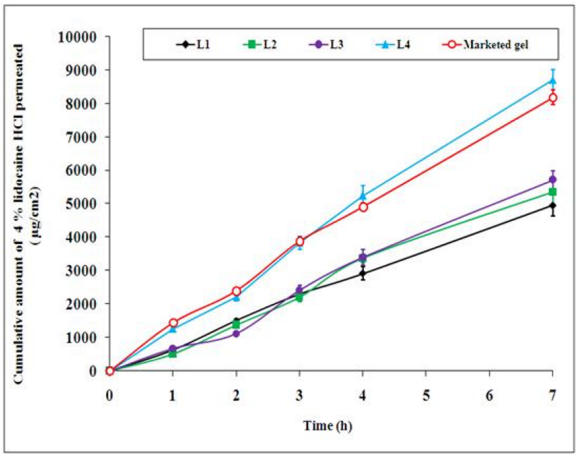

Fig. 3. *Ex vivo* permeation profile through excised porcine ear skin membrane per unit area from newly prepared 4% lidocaine HCl gels (containing extracted DG and Carbopol 940) and marketed 4% lidocaine HCl topical gel across the excised porcine ear skin membrane (mean ± standard error, n = 3).

3.6 *Ex vivo permeation*

The newly formulated 4% lidocaine HCl gels (containing extracted DG and Carbopol 940) and marketed 4% lidocaine HCl topical gel preparation were assessed for the *ex vivo* permeation across the excised porcine ear skin membrane. The *ex vivo* skin permeation of lidocaine HCl from the prepared and marketed topical gels were found to be sustained over 7 h (Fig. 3). The result of *ex vivo* skin permeation experiment of these 4% lidocaine HCl gels containing extracted DG and Carbopol 940 demonstrated the permeation fluxes in the range of 864.71 ± 9.24 to 1589.66 ± 13.36 $\mu g/cm^2/h$ (Table 4). The permeation fluxes were observed to be enhanced as the quantity of extracted DG augmented within the gel-formula.

Table 4. pHs and viscosities of 4% lidocaine HCl topical gels containing extracted DG and Carbopol 940.

	Formulation codes			
	L1	**L2**	**L3**	**L4**
pH*	6.12 ± 0.03	6.16 ± 0.02	6.38 ± 0.03	6.29 ± 0.03
Viscosity x 106 (cps)*	4.40 ± 0.03	4.72 ± 0.04	4.77 ± 0.05	4.76 ± 0.04

*(mean ± standard error, n = 3).

Table 5. *Ex vivo* permeation fluxes (J,μg/cm^2/h) of newly prepared 4% lidocaine HCl gels (containing extracted DG and Carbopol 940) and marketed 4% lidocaine HCl topical gel across the excised porcine ear skin membrane.

Formulation code	**Permeation flux (J, μg/cm^2/h)***
L1	864.71 ± 9.24
L2	1088.36 ± 10.38
L3	1273.87 ± 11.53
L4	1589.66 ± 13.36
Marketed gel	1422.18 ± 12.76

*(mean ± standard error, n = 3)

The highest *ex vivo* permeation flux (1589.66 ± 13.36 μg/cm^2/h) was noticed for gel formulation L4, which contained 0.1% menthol. Methanol (a terpene material) is reported as an effective permeation enhancer (Pathan and Setty, 2009). It has widely been incorporated in several topical gels to enhance drug permeations (Leite-Silva *et al*., 2012). The incorporation of 0.1% menthol in the formula of gel formulation L4 has enhanced the drug permeation across the skin membrane. Substances which abetting the absorption of drugs across the skin barrier through elevating permeability of the skin are known as skin permeation enhancers. These skin permeation enhancers are mostly functioning through one or more of these three probable mechanisms: (i) enhanced separation of solvent or drug into the stratum corneum, (ii) through the interaction of intracellular proteins and (iii) via disrupting highly ordered lipidic composition of the stratum corneum (Pathan and Setty, 2009). Menthol preferentially dispenses into the intercellular gaps of the stratum corneum, as an effectual enhancer of permeation across the skin barrier (Jain *et al*., 2002). Additionally, menthol possibly capable of producing a kind of reversible disruption of stratum corneum lipidic domains and therefore, enhances skin permeation (Das *et al*., 2013). In contrast, the marketed 4% lidocaine HCl topical gel exhibited 1422.18 ± 12.76 μg/cm^2/h of *ex vivo* permeation flux (Table 5), which was lesser than that of the gel formulation L4 (containing 0.1% menthol).

The *ex vivo* permeation result of newly formulated 4% lidocaine HCl gels (containing extracted DG and Carbopol 940) and marketed 4% lidocaine HCl topical gel across the excised porcine ear skin membrane was estimated kinetically by fitting with different mathematical models (Table 6). When the relevant correlation coefficients (R^2) of models were assessed and judged, Korsmeyer-Peppas model was observed as the best-fitting model (R^2 = 0.9944 to 0.9992) over 7 h of permeation study. Further, it was also found that the zero order model (R^2 = 0.9873 to 0.9932) was also reported approximately nearer to the best-fitting Korsmeyer-Peppas model. The diffusion exponent (n) of 4% lidocaine HCl topical gels containing extracted DG and Carbopol 940 (L1 to L4) were found in the range of 0.97 and 1.07 (Table 6).

Table 6. Curve fitting results of the *ex vivo* skin permeation of newly prepared 4% lidocaine HCl gels (containing *extracted* DG and Carbopol 940) and marketed 4% lidocaine HCl topical gel across the excised porcine ear skin membrane.

Formulation code	**L1**	**L2**	**L3**	**L4**	**Marketed gel**
Zero order model	0.9932	0.9873	0.9894	0.9912	0.9926
First order model	0.8643	0.8935	0.9314	0.8444	0.8839
Higuchi model	0.7712	0.6837	0.5274	0.6353	0.7844
Korsmeyer-Peppas model	0.9974	0.9992	0.9976	0.9944	0.9982
n (diffusion exponent)	0.97	0.98	1.07	1.05	0.88

Therefore, this can be expained that the drug permeation from these topical gels was occured with progress of time. In contrast, the marketed topical gel of 4% lidocaine HCl exhibited the n value of 0.88. The outcomes designated that *ex vivo* skin permeation of lidocaine HCl from these produced 4% lidocaine HCl topical gels containing extracted DG and Carbopol 940 (L1 to L4) followed the mechanism of super case-II transport.

3.7 Stability

The stability of these newly formulated 4% lidocaine HCl gels containing extracted DG and Carbopol 940 (L1 to L4) was tested by means of freeze thaw cycling process. These gels were found physically stable even the variation of storage temperatures were maintained in every 24 h in-between 25 ºC to - 5 ºC for 5 complete cycles. In addition, even after completion of 5 complete freeze thaw cycling process, the syneresis of these gels was not experienced.

Conclusions

In this study, DG was extracted from ripe dellinia (*Dillenia indica* L., family: *Dilleniaceae*) fruits (14.73% yield). Various physicochemical properties e.g., colour, odour, taste, aqueous solubility, pH as well as viscosity of extracted DG were evaluated. Phytochemical tests demonstrated the occurence of carbohydrates as well as mucilage within the extracted DG. Using the extracted DG along with Carbopol 940 (as gel-forming materials), topical gels of lidocaine HCl (4%) was formulated. In these topical gels, propylene glycol and methyl paraben were incorporated as plasticizer and preservative, correspondingly. In the gel formulation L4, menthol (0.1%) was incorporated as permeation enhancer. The pH and viscosity of these topical gels were found satisfactory. The *ex vivo* skin permeation of lidocaine HCl from the prepared topical gels (having extracted DG and Carbopol 940) and marketed topical gels exhibited a sustained drug permeation over 7 h. The permeation fluxes of lidocaine HCl were observed to be enhanced as the quantity of extracted DG augmented within the gel-formula. The highest *ex vivo* lidocaine HCl permeation flux was measured in case for the formulation L4 containing 0.1% menthol as skin permeation enhancer. Korsmeyer-Peppas model was found as the best-fitting drug permeation kinetic model with super case-II transport mechanism (n = 0.97 and 1.07) over 7 h of *ex vivo* skin permeation study. In the stability analyses, these topical gels of lidocaine HCl (4%) were found physically stable enough without syneresis even after completion of 5 freeze thaw cycling process. These 4% lidocaine HCl topical gels can be used for local topical anaesthesia in the management of skin lesions, skin sores, etc., and also in various minor surgical practices such as venipuncture, suturing of wounds, etc.

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