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STUDIES OF OLEORESINS AS PENETRATION ENHANCER FOR TRANSDERMAL PATCH OF KETOPROFEN

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ABSTRACT

The study deals with the effect of oleoresins (nutmeg, capsicum, coriander and garlic) as penetration enhancer for transdermal patch of ketoprofen. The transdermal patch prepared by mercury substrate technique and evaluated. In vitro release was found to be more than 60% through rat skin and 70% through cellophane membrane in capsicum oleoresin. In vivo study indicated that capsicum had 80.56 edema inhibitions and transdermal patch without penetration enhancer had 65.84 edema inhibitions. This indicated that the formulation containing oleoresin capsicum had high % edema inhibition. The more skin permeability and greatest flux of ketoprofen was obtained significantly by capsicum oleoresin.

From Overall study, it can be concluded that to deliver the drug through the skin, penetration enhancer play a vital role. So to deliver the drug into systemic circulation at predetermined rate by transdermal therapeutic system of Ketoprofen, using natural oleoresins as penetration enhancer.

In nutshell the oleoresins like Oleoresin nutmeg, Oleoresin capsicum, Oleoresin garlic, Oleoresin coriander shows penetrability and drug with maximum flux. Amongst this oleoresin capsaicin shows better performance as penetration enhancer.

Keywords: Ketoprofen, penetration enhancer, transdermal drug delivery system and oleoresins

INTRODUCTION

Transdermal Drug Delivery System (TDDS) can deliver certain medication to systemic circulation in a more convenient and effective way that is possible with conventional dosage form. Main objective of transdermal drug delivery system is to delivery of drugs in systemic circulation at predetermine rate, with no or minimal inter or intra patient variation. Transdermal delivered drugs avoid first pass metabolism, decrease dose to be administered, decreases side or unwanted effects, decreases gastrointestinal side effects, easy to discontinue in case of toxic effects. [1]

Penetration enhancers or promoters are agents that have therapeutic properties of these own but can transport the sorption of drug from drug delivery system, on to the skin or their subsequent transdermal penetration through skin. The acceleration across the keratin to swell and leaches out essential structural materials from the stratum corneum, thus reducing diffusional resistance and increasing the permeability of drug through skin. An effective amount of penetration enhancer increases the skin permeability and correspondingly the desired depth of permeation rate and amount of drug delivered.[2]

Oleoresins are homogenous mixture of resins and volatile oils. Pharmaceutical oleoresins are derived from ginger, capsicum, nutmeg, coriander, garlic etc. Oleoresins also occur with gums, are called as oleo-gum resins include asafoetida and myrrh. Terpenes and terpenoids are usually the constituents of volatile oil. Their chemical structures consist of repeated isoprene (C_5H_8) units and classified according to number of isoprene units. Sesquiterpenes have three (C_{15}) and Di terpenes have four (C_{20}). Terpenes may also classified as acyclic/liner, monoterpenes and bicyclic. Terpenes have been utilized for number of therapeutic purpose such as antispas-

modic, carminatives, perfumes and others. But a few of them suggest their potential as penetration enhancers eg, menthol, capsaicin, 1, 8-cineol.[3]

Ketoprofen is Non Steroidal Anti – Inflammatory Drug (NSAIDs) and commonly used to treat pain and inflammation associated with various disorders. The effects of NSAIDs are due to the inhibition of the enzyme cyclooxygenase. With plasma half-life of 6-8 hrs, pKa 4.5 partition coefficient log P (octanol /buffer PH 7.4), 0.

The aim of the study is to compare natural drug permeation enhancers through skin at appropriate rate and for suitable time. In this way, we concentrated on the device and later examined for their percutaneous activity.

EXPERIMENTAL

Materials

Ketoprofen was gifted from BEC chemicals, Mumbai. Oleoresin nutmeg, capsicum, garlic, coriander were gifted by Kancor flavors and extracts Ltd., Kerala, India, Ethyl cellulose obtained from Colorcon Asia Pvt. Ltd., Goa, India. Disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium chloride were purchased from loba chemicals pvt. Ltd, Mumbai, India. All solvents used were of analytical grade.

Methods

Preparation of film

The free film comprise of 3 % w/v ethyl cellulose in chloroform containing 30% w/w of Dibutylpthalate and 6.66 % w/w of ketoprofen (20 mg) of polymer composition. In this solution four different oleoresin of various concentrations were incorporated, like 5%, 10%, 20%, 30%, and 40% evaluated for optimization of concentration for all oleoresin. From the result by taking 30% as common concentration for all oleoresin further study were carried out.53, 68, 70 The composition of formulation were shown in table No 1.

Development of patch

The breaking membrane was glued to an adhesive tape keeping matrix side upward. The wax paper was used to give a protective covering.

Evaluation of free film [4, 5, 6]

Thickness uniformity

Transverse sections of the film at 10 different points were taken and thickness of each five film was measured individually by screw gauge. (Naugra Export, Haryana, India)

Weight variation test

Weight variation was performed by weighing each of five films. The average of film was taken as weight of films.

Tensile strength

Tensile strength was evaluated by using laboratory-developed apparatus. Film strip in dimension 50 ? 10 mm and free air bubbles or physical imperfection, were held between two clamps, one attached to fix position and other attached to pulley by which load was applied .A cardboard was attached on the surface of the clamp via adhesive tape to prevent film cut by the grooves of the clamp. During measurement, the strips were pulled by the clamp attached to pulley.

Content uniformity

The content uniformity of the film were determined by dissolving the film in 10 ml of methanol, filtered and again diluted after dilution with 25 ml methanol, analyzed at 261 nm. And an amount was calculated from standard calibration curve method.

Drug release studies [1, 3]

Drug Release studies through free films were performed in modified Keshary Chien cell (cell capacity 20 ml, cross sectional area 3.14 cm2). A portion of film was cut, measured and placed on the donor compartment facing drug matrix side to the membrane and backing membrane is upward. The receptor medium was saline phosphate buffer pH 7.4 with a content of 10% v/v methanol to maintain sink condition. The samples were withdrawn periodically from receptor compartment over a period of 6 hr. The amount of drug release in the receptor was determined spectrophotometrically (Double Beam UV Visible Spectrophotometer, Model: UV 2401 (PC) S 220V, Shimadzu Corporation, Singapore) at 261 nm.

In vitro penetration [6, 7, 8]

1) Drug through cellophane membrane

In vitro penetration was carried out with the cellophane membrane .The membrane was boiled in water for 2 hrs to remove glycerin which was included as humectants in the membrane. Then the membrane was soaked in 90 % ethanol for 24 hrs for removal of sulphur from membrane. For in vitro penetration study was performed in modified Keshary Chien cell, using dialysis membrane;

film was cut, measured and placed on the donor compartment. The receptor medium was saline phosphate buffer pH 7.4 with a content of 10% v/v methanol to maintain sink condition. Films without oleoresins and with oleoresin as penetration enhancer were studied. The receptor compartment was stirred by using magnetic stirrer at 100 rpm and whole assembly maintains at 37 ± 1^0C. At regular interval the concentration of drug over a period of 6 hrs was determined spectrophotometrically at 261 nm.

2) Drug through rat skin membrane [9, 10, 11, 12]

Male wister rat weighing 130-160 g and free from any visible sign of disease were selected for, in vitro permeation study. The hair on abdominal region was removed using depletory preparation one day prior to experiments. On the day of experiment, animals were sacrificed by cervical dislocation and abdominal skin was excised. The fatty material adhered to the dermis was carefully peeled off. Freshly excised rat skin of thickness of 2 mm was mounted on donor compartment. The receptor medium was saline phosphate buffer pH 7.4 with a content of 10% v/v methanol to maintain sink condition. Films without oleoresin and with oleoresins were studied. The amount of drug permeated in the receptor was determined spectrophotometrically at 261 nm.

In- vivo **anti-inflammatory study (rat paw edema)** [13]

In the present study, 24 albino rats of either sex weighing between (100-150 g) were used. Animal were divided in 4 groups of 6 animals each .The initial paw volume up to the ankle to joint of each animal of each group were measured. In all groups, acute inflammation was produced by sub–plantar injection of carrageenan in normal saline in left hind paw of each rat. The respective patch formulation 2 cm^2 was applied once to the inflamed paw of each animal immediately after carrageenan injection. The edema volume was measured by plethysmograph at 1, 2, 3 up to 8 hrs. after patch application. The average paw edema volume in all the groups was compared with that of control group.

Stastical significance was calculated by following equation.

% edema inhibition = (1-vt/vc) ×100

Where, vt and vc are mean edema volume in treated and control group.

Stability test [14]

Stability studies were conducted by storing the medicated transdermal films of ketoprofen at 40 ± 2^0C and 75 ± 5% RH for 3 months. The samples were withdrawn at different time intervals and analyzed for drug content by UV spectrophotometrically at 261 nm.

Drug interaction test [15]

Interaction study was conducted on medicated TDDS for mutation by comparing them with the pure drug and placebo formulation on the basis of UV- Spectrophotometer, FTIR- Spectrophotometer and thin layer chromatography (TLC).

FTIR analysis:- The IR absorption spectra of pure drug and oleoresins formulation were taken in the range of 400-4000 cm-1using potassium bromide disc method by using (FTIR Spectrophotometer, Model: 84005, Shimadzu Asia Pacific Pvt Ltd.,Singapore)

Skin irritation test [9, 10]

A primary skin irritation test was performed since skin is a vital organ through which drug is transported. The test was carried out on 6 healthy guinea pig weighing 400 to 500 g. Drug free polymeric film of diameter of 4.1 cm were used as control. The dorsal surface of guinea pig was cleaned well and hair was removed by using depletory preparation. The skin was leaned with rectified sprit. Transdermal patch of Ketoprofen placed over the skin with the help of adhesive tape film and patch were removed after 24 hrs, and skin was examined for erythema for 7 day. The entire experimental protocol involving laboratory animal was approved by the IAEC.

Histopathological studies [9]

The patches were applied on the freshly isolated hairless rat skin. The film was removed after 24 hr. and then vertical section of the skin were taken. Each section was dehydrated using ethanol .The tissue were cut into pieces of 5 µg, stained with hematoxyline and eosin and observed under microscope at 100X magnification.

RESULTS AND DISCUSSION

Thickness uniformity

The low S.D. value is thickness measurements ensure the uniformity of the film prepared by mercury substrate method. The thickness was found to be high with the film prepared with Nutmeg as penetration enhancer than other oleoresins, may be due to its high viscosity in polymer solution. Results as shown in Table 2.

Weight variation test

The weight of film containing nutmeg oleoresins was more than other oleoresins. It may also be due higher viscosity of nutmeg in polymer solution (Table 2). The rate of weight variation tests was decreased in order of oleoresins from capsicum> garlic > coriander> nutmeg.

Tensile strength

The incorporation of dibuty1 phthalate (30%) resulted the flexible film. The tensile strength of film decreased in the order coriander > capsicum > garlic > nutmeg. This may due to presence of excess concentration of the plasticizer than required to plasticize the polymer. The results (Table 2) indicate the thickness is directly proportional to the tensile strength.

Content uniformity

The drug content uniformity of the formulation varies from 97.5 % to 99.1%.

Drug release studies

The oleoresins did not interfered with UV spectrophotometric method used for the analysis as showed γ – max other than observed for drug. The % drug release of drug from formulated film in 6 hr was found to be highest with capsaicin oleoresin, as penetration enhancer (87.31 ? 0.0758) compared to other oleoresins nutmeg, garlic, coriander and drug Ketoprofen (Figure 1). The release of drug through the film were decreased in the order KTN> KTCA> KTG > KTP this may be due to terpenes may causes the loosening of stratum cornea. All terpenes increased the activity coefficient of ketoprofen in the skin. As all the terpenes disrupted the lipid bilayer and extracted the lipids.

The release study indicates that formulation KTCA AND KTN containing oleoresins capsaicin and nutmeg having the higher flux rate and permeation coefficient (Table 5) for each film of ketoprofen in following order KTCA> KTN> KTG > KTCO> KTP. Also release of all he film follows Higuchi pattern (Table 4).

In vitro **penetration**

In vitro penetration of drug through the rat skin was slightly lesser than cellophane membrane (artificial membrane). The effect of oleoresins in the film of ketoprofen on permeability rate of drug through rat skin was shown in figure 2. Oleoresins as penetration enhancer, those penetrate into stratum cornea and decomposed this layer and hence reduce its resistance to drug penetration. Oleoresins can accumulate with in the lipid bilayer of stratum cornea cell and hence increase their flow ability and permeation ability. After 6 hrs in vitro penetration of drug through the rat skin were 40.85, 59.51, 64.38, 54.19 & 50.32 respectively (Figure 2) and in following order, KTCA > KTN> KTG > KTCO> KTP.

As KTCA and KTN film with capsaicin and nutmeg appear to have better permeability coefficient when compared with KTCO:KTG : KTP film. These data supports that higher drug release the formulation should posses relatively high partition coefficient value (Table 5)

In-vivo **anti-inflammatory study (rat paw edema)**

Depict that comparison of percentage edema inhibition after application of patches KTP, KTN, KTCA, KTG & KTCO patch was found very effective in term of inhibiting carrageenan induced edema as inhibition after 8 hrs. of carrageen challenge (Table 6). Application of KTCA had 80.56 edema inhibitions and application of KTP HAD 65.84 edema inhibitions (Figure 3). This indicated that the formulation KTCA containing Oleoresin capsicum had high % edema inhibition, due to multiple action of drug along with Oleoresin capsicum and drug ketoprofen that shows synergism effect.

Stability test

No significant variation in the thickness, content uniformity and release at mentioned condition was observed for release and penetration study. Hence films are stored at the 40° ± 2°C at RH 75 ± 5% with shelf life at ambient condition was found to be 180 days.

Skin irritation

Free film containing nutmeg, garlic and coriander as oleoresin shows negative test but only capsaicin showed patchy edema for 4 days. Capsaicin exhibited a relatively to skin deposited enhancing activity was obtained (Table No. 7 and figure 5).

Drug interaction test

Study shows that, oleoresins and drug ketoprofen having no interaction with each other (Fig.4)

Histopathological studies

Histopathological changes in the upper layer of rat skin after application of transdermal delivery system with or without penetration enhancer. In this evaluation epi-

dermal liquefaction, oedema of collagen fibers in sub epidermal tissue were considered as an important criteria for distinguishing the effect of penetration enhancer on layers of skin and changes were noted. There is no apparent changes in the skin morphology were observed after application of patch containing blank. Further, epidermal liquefactions containing Ketoprofen and Capsicum Oleoresin with slight to marked sub epidermal

Table 1: Film containing different ratio of oleoresins.

Sr.No	Film	Oleoresins (polymer composition)
1.	KTP	Without oleoresin
2.	KTN	Nutmeg Oleoresin 30% (w/w)
3.	KTCA	Capsicum Oleoresin 30%(w/w)
4.	KTG	Garlic Oleoresin 30% (w/w)
5.	KTCO	Coriander Oleoresin 30% (w/w)

Table 2: Evaluations of free film

Studies	KTP	KTN	KTCA	KTG	KTCO
Thickness (cm)*	0.0125 ± 0.084	0.0136 ± 0.076	0.0127 ± 0.074	0.0127 ± 0.074	0.0121 ± 0.07
Weight (g)*	0.216 ± 0.0131	0.231 ± 0.0124	0.195 ± 0.0133	0.207 ± 0.0210	0.215 ± 0.0214
Tensile strength (kg/mm2)*	0.36 ± 0.0394	0.33 ± 0.0352	0.38 ±0.0364	0.31 ± 0.045	0.42 ± 0.0398
Percent content*	97.5 ± 0.582	98.2 ± 0.572	98.7 ± 0.485	99.1 ± 0.457	98.3 ± 0.481

*** Values are represented as Mean ± S.D. (n = 3)**

Table 3: Estimated values of n and k by regression of log (Mt/M8) on log (t)

Formulation	n	k	R2
KTP	0.7210	0.245	0.9326
KTN	0.7684	0.1993	0.9658
KTCA	0.5933	0.3056	0.8917
KTG	0.7634	0.0296	0.9742
KTCO	0.7719	0.0635	0.9954

Table 4: Kinetic treatments to in vitro release data (R2) of KTP, KTN, KTCA, KTG and KTCO films

Order of reaction	KTP	KTN	KTCA	KTG	KTCO
Zero Order	0.9207	0.9648	0.8670	0.9478	0.9841
First Order	0.7165	0.7714	0.6579	0.8045	0.8250
Higuchi	0.9790	0.9871	0.9468	0.9763	0.9864

Table 5: Calculation of flux and permeability coefficient

Study	Formulation	Flux (mcg/cm2 /min)	Permeation coefficient (cm/min)	Enhancement ratio
Penetration through cellophane membrane	KTP	99.038	0.0495	1.000
	KTN	134.16	0.0671	1.385
	KTCA	138.27	0.0691	1.379
	KTG	135.36	0.0677	1.344
	KTCO	128.15	0.0641	1.273
Penetration through rat skin membrane	KTP	76.081	0.0380	1.000
	KTN	118.05	0.0590	1.541
	KTCA	122.9	0.0615	1.596
	KTG	97.664	0.0488	1.2615
	KTCO	94.618	0.04730	1.233

Table 6: Percentage edema inhibition of oleoresins patches

Sr. No	Formulation	Percentage edema inhibition							
		1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr
1	KTP	9.04	18.98	20.42	25.09	26.47	55.45	59.00	65.84
2	KTN	9.50	11.39	19.23	31.90	50.47	61.18	65.24	78.28
3	KTCA	11.76	20.25	23.04	39.68	54.09	60.44	68.98	80.56
4	KTG	4.97	8.86	10.92	36.38	58.28	62.84	66.66	67.60
5	KTCO	9.04	17.97	23.75	49.80	55.42	65.24	68.80	69.52

Table 7: Skin irritation test

Sr. No	Treatment	Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Control	0	0	0	0	0	0	0
2	KTP	0	0	0	0	0	0	0
3	KTN	0	0	0	0	0	0	0
4	KTCA	0.5	0.5	0.5	0	0	0	0
5	KTG	0	0	0	0	0	0	0
6	KTCO	0	0	0	0	0	0	0

Scores: 0- No reaction, 0.5- Slight, patchy erythema, 1- Slight but confluent or moderate but patchy erythema, 2-Moderate erythema, 3- sever erythema with or without edema

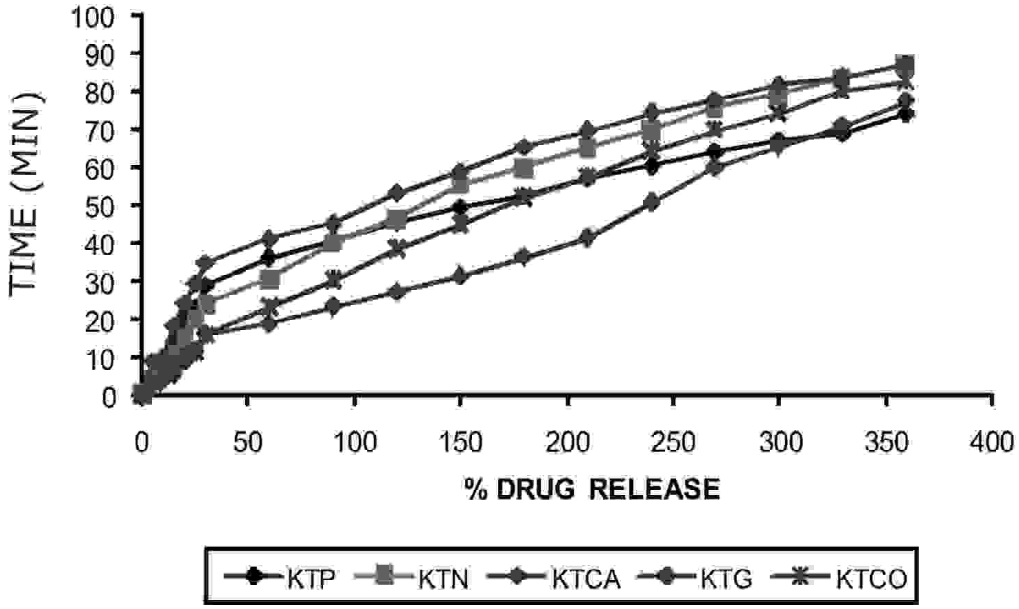

Figure 1: Comparative drug release study of Ketoprofen through different oleoresins with different oleoresin

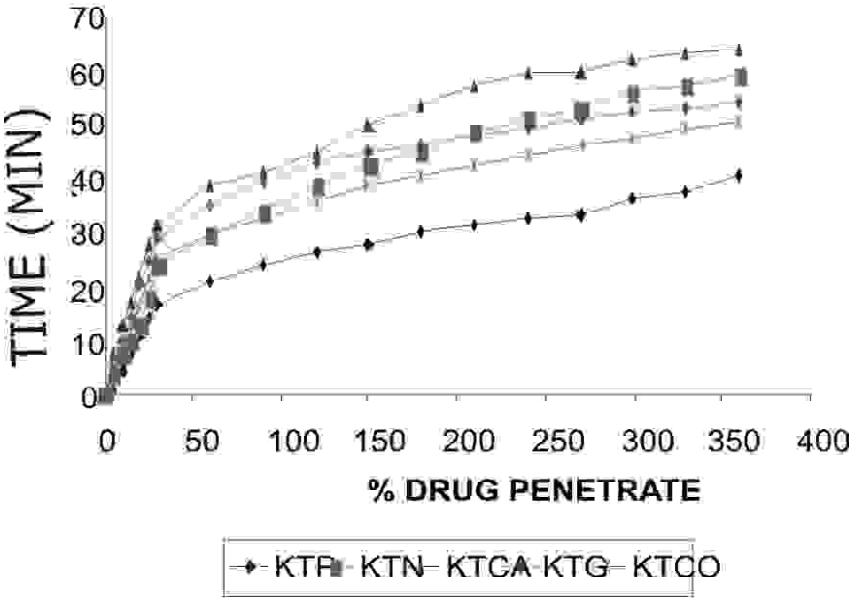

Figure 2: Comparative penetration of Ketoprofen with different oleoresins through rat skin

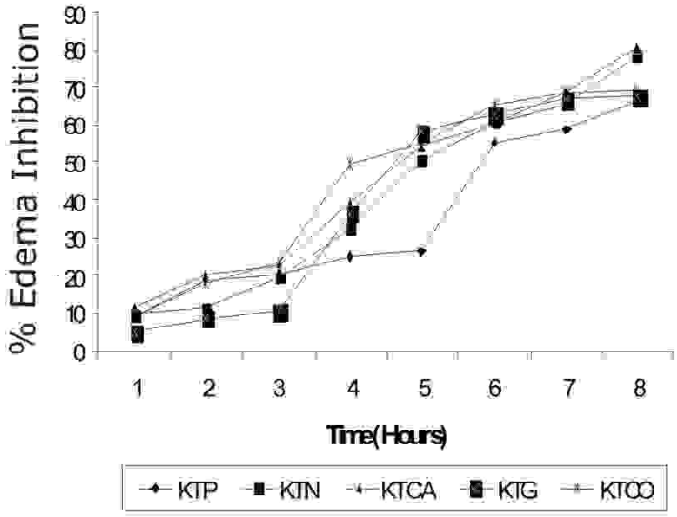

Figure 3: Percentage edema inhibition of oleoresins patches

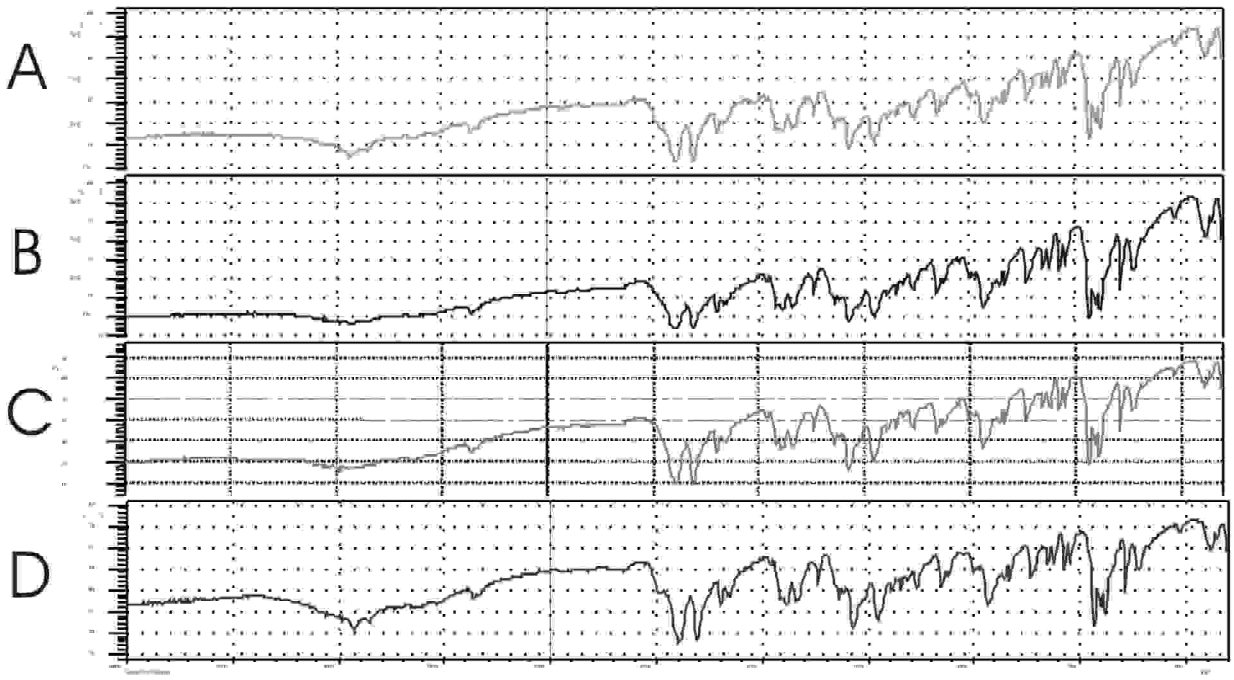

Figure 4 : Drug interaction study for drug Ketoprofen and different oleoresin

A. Ketoprofen with Nutmeg Oleoresin

B. Ketoprofen with Capsicum Oleoresin

C. Ketoprofen with Garlic Oleoresin

D. Ketoprofen with Coriander Oleoresin

oedema and redness was observed (Figure 6).

CONCLUSION

From Overall study, it can be concluded that to deliver the drug through the skin, penetration enhancer play a vital role. So to deliver the drug into systemic circulation at predetermined rate by transdermal therapeutic system of Ketoprofen, using natural oleoresins as penetration enhancer.

In nutshell the oleoresins like Oleoresin nutmeg, Oleoresin capsicum, Oleoresin garlic, Oleoresin coriander shows penetrability and drug with maximum flux. Amongst this oleoresin capsaicin shows better performance as penetration enhancer.

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