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Original Article

Formulation and Evaluation of Transdermal Gel of Ibuprofen: Use of Penetration Enhancer and Microneedle

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Abstract

The objective of the current study was to develop Ibuprofen (IBP) gel using different polymers individually and in combination and then to select best gel formulation based on various *in-vitro* evaluation parameters such as bioadhesive strength, gel strength, spreadability, viscosity and drug release study. The selected gel formulation was found to be composed of 1% (w/w) each of hydroxypropyl methylcellulose (HPMC K100M) and sodium carboxy methylcellulose (NaCMC). Two techniques such as chemical method using 1,8-cineole as chemical penetration enhancer (CPE) and physical technique using microneedle were employed to improve IBP permeation across the abdominal skin of rat. Out of the two techniques, the later technique showed higher (2.865-fold) permeation enhancement compared to control. Furthermore, a synergistic effect was also observed when both the techniques were used simultaneously with 3.307-fold increase in permeation enhancement. *In-vivo* anti-inflammatory study on rats induced with carrageenan paw oedema and analgesic activity investigation by tail flick method in rat model exhibited sustained effect up to 8 h compared to orally treated group. The stability study at room and accelerated conditions for three months did not show any sign of instability. Thus, the developed IBP gel is stable and have potential to illicit both anti-inflammatory and analgesic effect when administered transdermally.

Keywords: Analgesic activity, Anti-inflammatory activity, Ibuprofen, Microneedle, Penetration enhancers, Stratum corneum, Transdermal.

1. Introduction

The number of drugs and their formulations, which deliver the drug across the skin into systemic circulation, are increasing day-by-day since the approval of Transderm Scop® in 1979 by FDA. Furthermore, transdermal drug delivery (TDD) is increasingly important for drugs that need to

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be administered for disease of chronic nature such as cardiovascular agent [1], pain management in rheumatoid arthritis and osteoarthritis etc. It is reported that a worldwide transdermal patch market approaches £2 billion and is growing at a faster rate than ever [2]. In addition, some FDA approved transdermal formulations such as AndroGel® (testosterone gel), EstroGel®, and Gel-Andractim (Dihydrotestosterone gel) by Unimed Pharmaceuticals, Alprox-TD (Alprostadil Gel) by Nexmed, and Estradiol-MDTS (metered dose transdermal spray) by Acrux are widely used in clinical settings.

TDD can be defined as discrete and self-contained dosage form, when applied on the skin delivers the drug to the systemic circulation through the skin at a controlled rate [3]. These dosage forms are generally applied topically with systemic circulation as target and include gels, creams, sprays and patch systems. Among all, gels with bioadhesive property are widely used as they are capable of delivering the drug in controlled manner in addition to their excellent accessibility, and can easily be localized and terminated in the situation of adverse effect [3, 4]. A system can be called as gel when it surely satisfies following three criteria [5]: (i) have at least two components i.e. gelling agent and solvent component, (ii) show mechanical properties as exhibited by solid state, and (iii) each component is continuous throughout the system.

Based on the solvent used in the preparation, gels can be of two types; water based (aqueous gels or hydrogel) or organic solvent based (organogels) [6]. Hydrogels can be defined as three-dimensional polymeric network with the presence of a large number of hydrophilic groups or domain capable of imbibing large amount of water within [7]. In this case, the polymers may be of either natural or synthetic origin. Hydroxypropyl methylcellulose (HPMC) is widely used in several pharmaceutical formulations because of its nontoxic nature, ability to accommodate high levels of drugs and swelling properties [8]. Carbopol 934 (C-934) was selected on the basis of its good consistency [9]. Poly (ethylene oxide) (PEO) is a synthetic polymer which has FDA-approval for its negligible immunogenicity, absence of residual elements, forming strong solid gel and available in various grades [10]. Sodium carboxy methylcellulose (NaCMC) has wide range of pharmaceutical properties such as thickening, binding and stabilizing [11].

Ibuprofen (IBP), the most commonly used NSAID in the United States, is a propionic acid derivatives approved for the use in the symptomatic relief of pain related to various elements such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis etc [12]. IBP is a non-selective inhibitor of enzyme cyclooxygenase (COX), but its

pharmacological effects are believed to be due to inhibition of COX-2. This resulted in the decrease of prostaglandins synthesis involved in the mediation of inflammation, pain, fever and swelling. However, IBP undergoes extensive hepatic metabolism (90% of the administered dose metabolized to hydroxilate or carboxylate derivatives), which made it a poor choice for oral administration. In addition, like other NSAID it causes gastrointestinal adverse effect such as gastric irritation [12]. Molecular weight and log P values of IBP were 206.29 g/mol and 3.97, respectively. Furthermore, IBP has a half-life of 2 h and melting point of 76°C which make it suitable candidate for controlled release dosage form for transdermal delivery [13].

It is always a challenge to develop a transdermal dosage form with appropriate drug penetration rate [14]. This is mainly due to formidable barrier nature of stratum corneum (SC), the outer most layer of epidermis [14]. This barrier must be reversibly altered to deliver drugs at a desired rate across the skin without permanent damage. In addition, penetration across skin is much more essential when a poorly penetrating drug is being selected for TDD. To create a reversible modification in the skin, various techniques are employed under either chemical or physical approaches. Various classes of chemical penetration enhancers (CPEs) are used in chemical approach. Among all, terpenes were widely used in number of studies and found to enhance percutaneous permeation of many drugs [15, 16], which is due to their non-toxic, non-irritating effect to the skin at low concentration (1-5%). In addition, US-FDA categorized them in generally recognized as safe (GRAS) category and also possesses transdermal favourable properties such as low melting point and low molecular weight [9]. In the present study we have used 1,8-cineole at 5% level as CPEs to enhance the permeation of IBP across skin. In recent year's physical technique like microneedles are successfully used to deliver not only drug molecules [17-19] but also vaccines [20-22] and toxoid [23] for immunization because of their ease of administration, well tolerability in the skin and great acceptance by the patient [24]. Therefore, microneedle technique is also used in this study with an intention to improve penetration of IBP across the skin.

The goal of the present study is to prepare IBP transdermal gel and to evaluate it for various parameters such as bioadhesive strength, gel strength, diffusion, *in-vitro* permeation and *in-vivo* analgesic and anti-inflammatory activity in rat model. 1,8-cineole as CPE and microneedle technique as physical method were used to improve permeation of IBP across rat skin. Finally, stability study was performed on selected gel formulation for three months at room condition and at accelerated condition.

2. Materials and Methods

2.1. Materials

IBP and polymers such as C-934, HPMC K100M, PEO and NaCMC were procured

from Loba Chemi Lab, Mumbai. Dialysis membrane was obtained from Himedia Laboratories Pvt. Ltd, Mumbai. 1, 8-cineole was procured from Merck Specialties Pvt. Ltd, Mumbai and MNR Microneedle roller system was obtained from Amazon, India. Other chemicals used in the experiment were of analytical grade and used as received from suppliers.

2.2. Pre-formulation Studies

2.2.1. Solubility Measurements

Solubility measurement of IBP in different media was carried out as per the method described in the literature [25]. The procedure is as follows; an excess amount of pure IBP was added to 10 ml of media such as phosphate buffer (PB) pH 7.4, PB pH 7.4 with PEG (80:20), PB pH 7.4 with PEG (60:40), PB pH 7.4 with PG (80:20) and PB pH 7.4 with PG (60:40) in glass vials followed by shaking in water bath shaker (REMI, Mumbai) for 24 h at 25°C and kept at rest for 1 h to attain the equilibrium. The solution was then filtered through a membrane filter and suitably diluted prior to the measurement of drug spectrophotometrically at 222 nm.

2.2.2. Partition Coefficient Determination

For the determination of partition coefficient, both n-octanol and distilled water (DW) were saturated with each other 24 h prior to the commencement of experiment. To the pre-equilibrated DW (10 mL), a known quantity of drug is dissolved. Then, 10 mL of octanol was added to equal volume of aqueous solution of drug in a separating funnel. The system was kept for 24 h with intermittent shaking. Finally, the aqueous layer was separated, clarified by centrifugation and assayed [26].

2.3. Preparation of the IBP Gel Using Various Polymers

Appropriate amount of polymers as specified in the Table 1 were soaked in 40 mL DW overnight and then, the swelled polymers were stirred using a mechanical stirrer to ensure their uniform dispersion. In case of C-934 based solution, the pH was adjusted till gel like consistent material was formed by using 1N sodium hydroxide solution. The required quantity of IBP (2.5g) was dissolved in 10 mL of ethanol. Thereafter, this resulted solution was added to polymer solution and stirred until uniformly dispersed gel of 5% (w/v) IBP concentration was formed. In case of gel preparation containing combination of polymers, both the polymers were separately soaked overnight in 20 mL each of DW and other steps are followed as per the above procedure.

2.4. In-Vitro Evaluation of Prepared IBP Gels

2.4.1. Drug Content Analysis

For IBP content analysis, a specific amount (500 mg) of prepared gel was placed in a volumetric flask containing combination of ethanol (5 mL) and phosphate buffer of pH 7.4 (q.s. to 100 mL). The flask having gel solution was shaken for 2 h on a water bath shaker (REMI, Mumbai, India) at 37°C to completely extract and solubilize the drug. This solution was filtered and estimated

spectrophotometrically at 222 nm using phosphate buffer pH 7.4 as blank after making appropriate dilutions [27].

2.4.2. pH Determination

pH of the 10% (w/v) gel solution in DW was measured by using digital pH meter (REMI, Mumbai) [28]. All the measurements were made triplicate and the mean was reported as the pH of the gel formulations.

2.4.3. Measurement of Gel Strength

Gel strength of the prepared gel was determined by modified apparatus as shown in the Fig. 1. The apparatus is consisting of two parts; (A) measuring cylinder and (B) device (20 g). The gel strength measurement is based on the time (s) required by the device to move 5 cm down through the IBP gel. Accurately weighed (50 g) gel was placed in the graduated cylinder at room temperature (25.5°C) and then the device was placed above it. The time in second required for the device to sink 5 cm down through the gel was measured [29].

2.4.4. Spreadability Study

Spreadability of developed gels was performed by using apparatus reported in the literature [30, 31] with suitable modification in the laboratory prior to its use in the experiment. The basic concept of spreadability in this apparatus is the 'Slip' and 'Drag' characteristics of gels. Briefly, the apparatus consists of two glass slides, one of which was fixed with the table top and another was placed above the gel kept on the first slide as shown in the Fig. 2. thread was tied to upper glass slide (placed above the gel) in one side and hook to hold weight on the other side. An excess of gel (about 2 g) under study was placed on the lower slide. The gel was then sandwiched between slides and 1 Kg weight was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top slide was then subjected to pull off with 100 g placed on the hook. The time (in seconds) required by the top slide to cover a distance of 10 cm was noted. Spreadability was then calculated using the following formula:

$$S = \frac{M \times L}{T}$$

Where, S = is the spreadability, M = is the weight in the pan, L = is the length moved by the glass slide and T = represents the time taken to separate the slide completely from each other.

2.4.5. Bioadhesive Strength Measurement of Gels Using Hairless Goat Skin

The measurement of bioadhesive strength of prepared gel was carried out using modified balance method [32, 33] and hairless skin of goat as model skin. Fresh goat skin was obtained from a local slaughter-house and cleaned by shaving. The underlying fat and loose tissues were separated. The skin samples were used within 2 h of collection otherwise fresh skin was collected. The fresh skin was cut into equal sizes (35.34 cm^2) and washed with PB pH 7.4 prior to the use. As seen in the

Fig. 3, one piece of skin was fixed to lower side of upper glass plate (fixed to wooden piece of left arm of balance) and another piece to the upper side of lower plate (fixed to platform) with the help of acrylic adhesive. Prior to the experiment the right and left pans were balanced by adding extra weight on the right-hand pan. Accurately, 1 g of IBP gel was placed between these two slides containing hairless skin pieces. Little pressure was applied on the left pan to sandwich two pieces of skin and to remove the air (if entrapped). Balance was kept in this position for 5 min. Weight was added slowly at 100 mg/min to the right-hand pan until the detachment of plates from skin surface in the left side. The weight in gram required to detach the gel from skin surface gave the measure of bioadhesive strength and then expressed in Dyne/cm^2. It was calculated by using following:

$$\text{Bioadhesive strength} = Wg/A$$

Where, Wg is weight required (g) and A is area (cm^2)

2.4.6. Rheology Study

The viscosity of the gel formulations was determined using Brookfield viscometer (Model DV I PRIME) with appropriate spindle numbers [25-27] suited for the gel at various rpm ranging from 0.3-12.0. Rheological studies of different formulations were performed at 25.5±1°C. Rheograms were generated by plotting rpm on X-axis and viscosity on Y-axis. The relationship between shear stress and shear rate of each formulation was determined using Power law equation given below:

$$\tau = k\gamma\, n$$

2.4.7. In-Vitro Drug Release and Kinetic Study

Vertical type of Franz diffusion cell (Murthy glasswares, Hyderabad, India) with receptor compartment capacity of 22 ml and exposed surface area of 3.8 cm2 was used to perform in-vitro IBP release from prepared gel formulations. PB of pH 7.4 was used as a receptor medium and dialysis membrane (HiMedia, India) was used as model membrane for the present study. The membrane was soaked in receptor medium for 1 h prior to placing on receptor compartment. The gel sample (1 g) was applied on the membrane uniformly and then it was fixed between donor and receptor compartment with the help of stainless steel clamp. The temperature of receptor medium was thermostatically controlled at 37 ± 1ºC by surrounding water in jacket and the medium was stirred by magnetic stirrer at 500±10 rpm. The samples (1 mL) were collected from the receptor compartment at pre-determined time intervals of 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, and 8 h and replaced by equal volume of fresh receptor solution to maintain sink condition throughout the experiment. The amounts of IBP in the sample were assayed spectrophotometrically at 222 nm against appropriate blank.

The drug release kinetics was analyzed by fitting data obtained from the *in-vitro* release experiment into different mathematical models such as zero order (% of drug release vs time), First order (log % drug release vs time,

Higuchi model (% drug release vs √time) [34]. Further, in order to ascertain the type of diffusion, data were fed to Power law or Peppas equation;

$$\text{Log } (Mt/M\infty) = \text{LogK} + n \text{ Logt}$$

Where, $Mt/M\infty$ is fraction of drug release in time t, k and n are the rate constant and drug release exponent, respectively. The n value is an indicative of drug release mechanism and the values varies based on the geometry of formulation such as sphere, tubular and film. When, n value ≤ 0.5 the release mechanism is Fickian diffusion (i.e. only diffusion), n value >0.5 and <0.89 the release mechanism is Anomalous transport (i.e. both diffusion and swelling), and n value ≥ 0.89 the release mechanism is based on polymer swelling [35].

2.5. Ex-Vivo Permeation Studies

2.5.1. Skin Preparation

Wister rats of either sex weighing between 200 and 250 g were sacrificed by cervical dislocation. The abdomen portions of rats were cleaned with hand razor from tail to head direction without damaging SC. The abdominal skin was separated from rat surgically and subcutaneous fats were removed carefully with forceps and cotton swab. Then, the skin pieces were sized appropriately and washed thrice with PB pH 7.4 prior to wrapping in aluminum foil. Thereafter, these wrapped skin pieces were stored in refrigerated condition and were used on very next day [36]. Two groups of skin samples were tested as follows: intact skin and microneedle porated compromised skin. For the microneedle poration technique, microneedle array (0.5 mm length) was rolled over the skin pieces for 1 min to form microchannels. All groups studied were n = 3 [37].

2.5.2. Ex-Vivo Permeation Study

Ex-vivo permeation study was performed by using the same diffusion cell mentioned in *in-vitro* release study with same medium (PB pH 7.4). The temperature of the receptor medium was maintained at 32±0.5°C with continuous stirring at 500±10 rpm [38]. Thawed skin piece (intact as well as microporated) was equilibrated in receptor medium for 1 h prior to fixing between donor and receptor compartment with a clamp. A fixed quantity of 0.5 g of IBP gel equivalent to 25 mg of IBP was placed above the skin sample. At pre-determined time intervals of 1, 2, 3, 4, 6, 8, 22 and 24 h, aliquots of 0.5 mL were withdrawn from the receptor medium and the concentration of IBP was analyzed by UV spectrophotometric method. By using IBP amount, parameters such as flux (Jss), permeability coefficient (Kp) and enhancement ratio (ER) were calculated as described in our previous paper [39]. Total of four experiments were conducted: gel without PE applied on intact as well as on microporated skin sample and gel with PE applied on intact as well as on microporated skin sample.

2.6. In-Vivo Study

After obtaining approval from Institutional Animal Ethical Committee (IAEC) (Reg. No. 1287/ac/09/CPCSEA and protocol No. IAEC/GIP1287/SN/11/2013-14), in-vivo study was conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Total of 12 Wistar rats (150±20 g) were randomly divided into three groups, each containing 4 rats. The first group served as control, second group was administered with oral drug suspension (1 % w/v of IBP in 1%w/v of carboxy methyl cellulose suspension in water) and the third group was treated with IBP transdermal gel. IBP gel (equivalent to 35 mg of IBP) containing 1,8-cineole was applied on the microporated dorsal side of rats (third group) 30 min prior to carrageenan injection. The first group was the control group. Paw oedema was induced across the three groups by an intradermal injection of 0.1 ml of a 1% w/v homogenous suspension of carrageenan in normal saline in to the subplanter surface of the right hind paw of rats. The paw oedema volume (mL) was measured immediately (0 h) and at ¾, 1.5, 3, 4, 5 and 6 h after injection using digital plethysmometer (PLM-01 Plus, Orchid Scientific, Chennai, India). The percentage inhibition of paw oedema was calculated using following formula [41]:

$$\%\ \text{inhibition} = \frac{\text{Mean increase in paw volume in control group} - \text{Mean increase in paw volume in test group}}{\text{Mean increase in paw volume in control group}} \times 100$$

The rats were provided with free access to food and water ad libitum. The dorsal sides of rats belong to third group were shaven 12 h prior to starting of experiments and microchannels were created on the shaven skin just before the application of IBP gel.

2.6.1. Evaluation of Anti-Inflammatory Activity

The anti-inflammatory activity and its sustaining action of the IBP loaded gel was performed using carrageenan-induced right hind paw oedema method [40]. IBP suspension (10 mg/kg body weight) was administered orally to rats of second group and

2.6.2. Evaluation of Analgesic Activity

Analgesic activity was assessed in rats using tail flick method employing analgesiometer [42]. Second group of rats were administered orally with IBP suspension (10 mg/kg body weight) and IBP gel (equivalent to 35 mg of IBP) containing 1,8-cineole was applied on the microporated dorsal side of rats (third group). The first group was the control group. Time in seconds between placing the tail of the rat on the radiant heat source and sharp withdrawal of the tail was measured as reaction time. Reaction time was considered as the unit for the measurement of pain and increase in

reaction time was indicative of analgesic activity. Maximum latency (cut off time) of 10 seconds was fixed for all groups of animals in order to exclude thermal injury while recording the reaction time. Tail-flick measurement was carried out for all the groups prior to drug administration and at ½, 1, 2, 3, 4, 6, and 8 h after the drug administration. The reaction time at each time point was recorded and percentage of analgesic activity was calculated using following formula [43]:

$$\text{Analgesic activity (\%)} = \text{M. P. E.} = \frac{\text{T. L.} - \text{B. L.}}{\text{M. L.} - \text{B. L.}} \times 100$$

Where, M.P.E. is maximum possible effect, T.L. is total latency at the end of particular period of time of testing, M.L. is maximum latency or cut off time and B.L. is basal latency or control latency.

2.7. Stability Studies

The stability study on the selected gel formulation was performed for 3 months [44] at room and accelerated conditions (at room temperature 40±2°C temperature and 75±5% relative humidity (RH)) using a stability chamber (Model no: BIT-2U, Bio Technics-India). Visual inspection, drug content, permeation study was performed after 3 months to assess the stability of selected gel formulation of IBP.

2.8. Statistical Analysis

Statistical analysis on the data obtained from the experiments was performed using one-way analysis of variance (ANOVA). Turkey-Kramer multiple comparison test was employed using trial version of GRAPHPAD INSTAT 3 software (Graph-Pad Software Inc., San Diego, CA) to compare different formulations with fixed significant level of $P<0.05$.

3. Results and Discussion

3.1. Pre-formulation Study

Saturation solubility is useful in determining the solubility of the drug under normal temperature conditions. In addition it is helpful in selecting appropriate medium with sink condition for subsequent studies. In current study the solubility of IBP was found to be highest (5.09±0.04 mg/mL) in PB pH 7.4. Therefore, PB pH 7.4 has been selected as the medium for further studies. The partition coefficient was determined in the n-octanol and water systems. The n-octanol-water partition coefficient serves as a parameter of lipophilicity. The value was found to be 3.96±0.01. This shows that the drug is highly lipophilic and is having low aqueous solubility.

3.2. In-Vitro Evaluation of Ibuprofen Gels

Prepared IBP gels were visually inspected for colour and phase separation. All the gels were found to be translucent, off white colour with no sign of phase separation. This indicates that drug is solubilzed in the solvent system and dispersed throughout the gel matrix uniformly.

3.2.1. Determination of Drug Content

The results of drug content are presented in Table 2. All the formulations are showing drug content in the range of 100±10%. Therefore, all the formulations are considered for further studies.

3.2.2. Determination of pH

The pH of the transdermal or topical formulation has a strong influence on the patient acceptance as more acidic or more basic pH may alter the skin environment leading to skin irritation [45]. pH of the 10% w/w gel in DW was determined and the obtained results are mentioned in Table 2. The pH values observed are in the range of 5-6, which is close to pH of normal human skin. Thus, the developed gels are expected to avoid the risk of irritation to the skin upon application on the skin surface.

3.2.3. Study of Gel Strength

The gel strength is measured by modified apparatus as explained in the procedure and the obtained results are presented in Table 2. The higher the weight used for passing the apparatus 5 cm through the gel higher is the gel strength. The highest and lowest gel strength of 239±3.61sec and 131±4.58 sec was observed for formulation IF2 and IF4, respectively. This indicated that C-934 has more gel strength compared to HPMC K100M at same concentration level of 1%.

3.2.4. Study of Spreadability

Spreadability term is used to define the ability of the gel to readily spread on application to skin surface. As it is related to the area, therefore it is not only influence the therapeutic efficacy of gel formulation but also the patient compliance [46]. A well developed gel will spread uniformly in short time and obviously will have high spreadability value [9, 47]. It was found that formulation IF-6 is showing maximum spreadability of 16.67±2.83 g/cm.sec followed by IF-1 (13.16±1.46 g/cm.sec) (Table 2). These results indicated that the above gel formulations were easily spreadable with less amount of shear.

3.2.5. Bioadhesion Study

Bioadhesion is the ability of the gel to bind to the surface of the biological membrane when applied on it. This test is conducted by using modified balance method and the results are noted in Table 2. If the strength required in separating the two surfaces of skin from each other is more the bioadhesive character of the gel is more and it won't be easily removed from the surface of the skin [47]. Bioadhesive strength was found to be maximum for IF-4 (9157.28±679.58 Dyne/cm^2) followed by IF-3 (8324.80±524.79 Dyne/cm^2) demonstrating HPMC K100M has better bioadhesive property compared to the mixture of C-934 and HPMC K100M at equal concentration.

3.2.6. Rheology Study of Gels

Viscosities of the prepared gels were determined by using Brookfield viscometer and the values were illustrated in Fig. 4. The gels show decrease in the viscosity on increment of the shearing stress which shows that the gels are showing pseudo plastic flow.

This implies that the gel behaves as a solid at rest and flows easily on application of a small amount of shearing force due to decrease in viscosity. Hence, their extrudability from the tube and spreadability will be more on application on skin [47].

3.2.7. In-Vitro Drug Release and Kinetic Study

Drug diffusion studies are performed to find out not only the amount of drug diffusing through unit area of membrane but also the amount of drug is being released from the gel matrix in to the dissolution medium. The result of *in-vitro* drug release study is illustrated in Fig. 5. It is useful in determining what kind of release kinetic model is followed by the gel formulation and to know how the drug release rate is being affected by the polymeric matrix. The highest and lowest percentage of IBP release at the end of 8 h of diffusion study was found to be 59.55±2.46% and 15.57±1.49% in case of IF-6 and IF-2, respectively. The diffusion result of formulation IF-6 was highly significant ($P<0.001$) compared to formulation F4 and it is significant compared to IF-3 ($P<0.01$), when the diffusion results between 3 to 8 h were compared. The other formulation pairs showed significant difference in results were IF-1 with IF-2 and IF-1 with IF-5.

From the Table 3, it was clear that formulation F2 demonstrated zero order drug release with r^2 value of 0.9873. But formulation IF-6 with r^2 value of 0.991 exhibited Higuchi model as best fit model indicating diffusion is the main mechanism of drug diffusion. The drug release exponent 'n' value was between 0.55 to 0.89 except for the formulation IF-3 (n=0.45). These results indicated that drug release from the gel followed anomalous transport i.e. diffusion along with swelling are the drug release mechanisms. Formulation IF-6 containing 5% of IBP, 0.5% each of HPMC and NaCMC was selected as the best formulation based on *in-vitro* results and r^2 value (0.991) obtained from Higuchi model along with the other evaluation parameters (formulation IF6 exhibited highest spreadability of 16.67±2.83 g/cm.sec and the values for other parameters such as drug content, pH, gel strength and bioadhesive strength are within the highest and lowest values). This formula was used in subsequently studies.

3.3. Ex-Vivo Permeation Studies

Ex-vivo permeation studies were carried out on IF6 gel formulation alone and its combinations with 1,8-cineole (chemical method) and microneedle (physical method) to assess the individual effect of PE and microneedle on the permeation enhancement of IBP across rat skin. In addition, combination of 1,8-cineole and microneedle was also used to study if there is any synergistic effect. The obtained results were illustrated in Fig. 6 and Table 4. The cumulative percentage of IBP permeated (CPP) across the intact skin after 24 h of study was 26.989±2.8%. Whereas, intact skin with 1,8-cineole in gel resulted in 50±3.76% of CPP and 142.543±5.471 µg/cm^2/h of Jss. 1,8-cineole, a hydrophilic terpene due to the

presence of alcohol group, shows its penetration ability by disrupting bilipid layer of SC. This is because of its competitive hydrogen bonding ability with ceramides which resulting in the loosening of tightly packed lipid layer in the SC. In addition to above mechanism, 1,8-cineole may increase partition coefficient and drug solubility, thereby increasing thermodynamic activity and molecular orientation of terpenes molecule within the lipid bilayer [9]. Valsartan gel containing 1% of 1,8-cineole exhibited a transdermal flux of 143.51±9.2 µg/cm^2/h and an flux enhancement of 4.53-folds [46]. In the present study, we observed a flux enhancement of 1.926-folds with 5% 1,8-cineole. This may be due to the fact that the hydrophilic terpenes are often enhancing the penetration of hydrophilic drugs more compared to the lipophilic drugs [48]. IBP is a lipophilic drug with practically insoluble in water and logP value of 3.97. In one of our previous study, hydrophilic drug metoprolol succinate with 5% of 1,8-cineole showed a flux enhancement of 5.95 compared to gel without it [49].

Microneedle as a physical technique has been widely investigated in last decade by various research groups to enhance drug delivery into skin as well as across the skin. It involves non-invasive means of creating microchannels in SC and thereby allowing drugs to move across skin in a higher rate [37, 50]. Skin compromised with microneedle followed by the application of gel without and with 1,8-cineole significantly ($p<0.05$) increased CPP (76.084±6.94% and 89.263±8.64%, respectively) compared to control formulation. The permeation across skin increased in order: IF6> IF6 with 1,8-cineole> IF6 + Microneedle > IF6 with 1,8-cineole + Microneedle. The highest flux and permeability coefficient (Kp) of 244.682±9.456 µg/cm^2/h and 9.787±0.942 $\times 10^{-3}$ cm/h, respectively, was shown by gel formulation containing 1,8-cineole with microneedle. A similar result was reported in the literature where Kp value for diclofenac diethylamine was almost 3-fold in case of microneedle compromised skin compared to intact human skin [37]. We observed a synergistic effect of 1.717-fold increase in permeation enhancement of IBP when both the chemical and physical techniques were employed compared to only chemical method. This result is attributed to the different mechanism of action of 1,8-cineole and microneedle technique.

Correlation was studied between cumulative percentage of IBP release and CPP of formulation IF6. The correlation coefficient was found to be 0.982 (Figure 7). This indicated a better correlation between *in-vitro* drug diffusion across dialysis membrane and *in-vitro* permeation of IBP across abdominal skin of rat.

3.4. In-Vivo Studies

3.4.1. Evaluation of Anti-Inflammatory Activity

The anti-inflammatory activity of the selected IBP gel was performed on Wister rats after carrageenan induced paw oedema and the obtained result was compared with control and

orally treated group of rats (Figure 8). It was observed that there are high significant differences ($p < 0.001$) between treated and control group as assessed with Turkey-Karmen multiple comparison method. However, there was no significant difference ($p > 0.05$) observed between two treated groups. It was also observed from the Fig. 8 that there is an abrupt decrease in paw volume of rats belongs to orally administered groups from 0.5 h to 4 h of study. The same was not seen in case of transdermally treated group, instead there was a steady decrease in paw volume from 0.5 h until end of the study.

3.4.2. Evaluation of Analgesic Activity

Tail flick method was used to study the analgesic activity of the selected gel on the rats of the three groups and the results are illustrated in the form of analgesic latency (Figure 9a) and MPE (Figure 9b). When the latency of three groups of rats were compared, both the orally administered and transdermally treated groups exhibited statistical significant ($p<0.05$ and $p<0.001$, respectively) compared to control group. The graph also showed that latency period of transdermally treated group is statistically significant ($p<0.01$) compared to orally administered group. The above results indicated that the selected transdermal gel of IBP is more efficient exerting analgesic activity compared to orally treated group. Fig. 9b showed the comparison of MPE (%) or maximum analgesia (%) between oral and transdermally treated groups. Compared to orally treated group, transdermally treated group demonstrated more uniform analgesic effect after 3rd h of the study. This indicated that the selected transdermal gel of IBP showed sustained drug release after 3rd h of study to the end (8 h) of the study.

3.5. Stability Studies

Stability potential of the drug in the formulation is generally assessed by stability study. The selected gel formulation was found to be translucent and off-white colour without phase separation as fresh gel. Drug content of the selected gel after 3 months of storage at room and accelerated condition were found to be 98.23±1.93 % and 97.63±3.18 %, respectively. When permeation was conducted after the stability period, the CPP value of 88.193±5.78 and 87.90± 6.62 was observed for the gel formulations stored at room and accelerated conditions, respectively, which were insignificant ($p>0.05$) compared to fresh gel. Usually the variation in physical appearance and permeation rate were observed when there is formation of small crystals due to accelerated conditions. In our study, we did not observe such deviation in physical appearance and permeation of IBP across the skin. This is attributed to the presence of well-known crystallization inhibitor HPMC, which might have prevented the formation of IBP crystals even after 3 months of storage [51].

4. Conclusion

The present work indicated that transdermal gels of IBP can be developed with individual (such as C-934 and HPMC K100M)

and combination of polymers (viz. C-934 with HPMC K100M, HPMC K100M with NaCMC and HPMC K100M with PEO). The selected gel was composed of 1% w/w each of HPMC K100M and NaCMC. Higher ER of 2.865 was observed when microneedle technique was employed. There was a synergistic effect with ER of 3.307 observed when both 1,8-cineole and microneedle technique were used simultaneously. *In-vivo* anti-inflammatory study on carrageenan induced paw oedema in rats demonstrated that IBP gel with both CPE and microneedle technique had sustained effect for 8 h compared to orally treated group. Tail flick method with the same formulation treatment in rats indicated higher analgesic latency compared to orally treated group along with better sustained MPE values. Hence, transdermal gel of IBP with 1, 8-cineole and simultaneous application of microneedle technique holds promise for safe delivery of desired amount of IBP transdermally.

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Tables:

Table 1. Gel formulations of IBP (50 g).

Formulation Code	IBP (g)	Ethanol (mL)	C-934 (g)	HPMC K100M (g)	PEO (g)	NaCMC (g)
IF-1	2.5	10	0.25	0.25	---	---
IF-2	2.5	10	1.0	---	---	---
IF-3	2.5	10	0.5	0.5	---	---
IF-4	2.5	10	---	1.0	---	---
IF-5	2.5	10	---	0.5	0.5	---
IF-6	2.5	10	---	0.5	---	0.5

Table 2. Results of physico-chemical parameters of IBP gels. (Mean±SD, n=3).

Formulation Code	Drug content (%)	pH	Gel strength (sec)	Spreadability (g/cm.sec)	Bioadhesion strength (Dyne/cm^2)
IF-1	100.63±7.86	5.39±0.07	231±9.01	3.16±1.46	3052.43±208.36
IF-2	96.37±2.53	5.04±0.05	239±3.61	4.57±0.68	4717.39±416.34
IF-3	104.83±3.56	5.32±0.04	196±6.08	3.77±1.01	8324.80±524.79
IF-4	90.13±1.91	5.27±0.03	131±4.58	3.46±0.94	9157.28±679.58
IF-5	96.60±4.59	5.58±0.07	167±7.55	5.59±1.57	5549.87±506.31
IF-6	99.00±2.85	5.49±0.04	175±4.36	16.67±2.83	6104.85±389.18

Table 3. The r^2 and n values obtained from different kinetic models.

Formulation Code	Zero order	First order	Higuchi model	Korsmeyer-Peppas model	
			r^2		n
IF-1	0.9316	0.8782	0.8576	0.9558	0.65
IF-2	0.9873	0.9827	0.968	0.9108	0.73
IF-3	0.9604	0.9638	0.9569	0.9705	0.45
IF-4	0.9227	0.9184	0.8749	0.9246	0.57
IF-5	0.9135	0.9326	0.9707	0.883	0.55
IF-6	0.9799	0.9830	0.991	0.8521	0.75

Table 4. Effect of different techniques on the permeation of IBP across abdominal skin of rats.

Formulations	Jss (µg/cm^2/h)	Kp (×10^{-3} cm/h)	ER
Control (IF6)	73.98±1.89	2.959±0.721	----
IF6 with 1,8-cineole	142.543±5.471	5.701±0.426	1.926
IF6 + Microneedle	212.017±10.452	8.48±1.586	2.865
IF6 with 1,8-cineole + Microneedle	244.682±9.456	9.787±0.942	3.307

Figures:

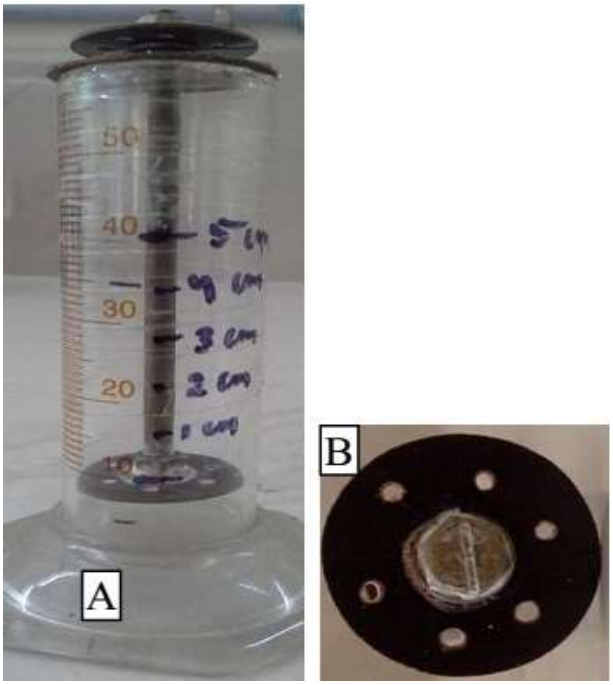

Figure 1. Gel strength measurement device. (A) Measuring cylinder with device inside; (B) Device.

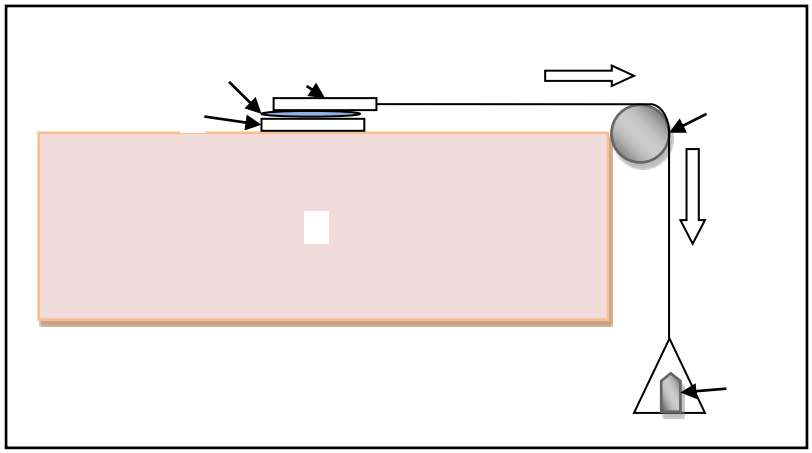

Figure 2. Modified spreadability testing apparatus. (A) Table; (B) Upper glass slide; (C) Lower glass slide; (D) Thin layer of IBP gel; (E) Nylon thread; (F) Glass rod; (G) Weights.

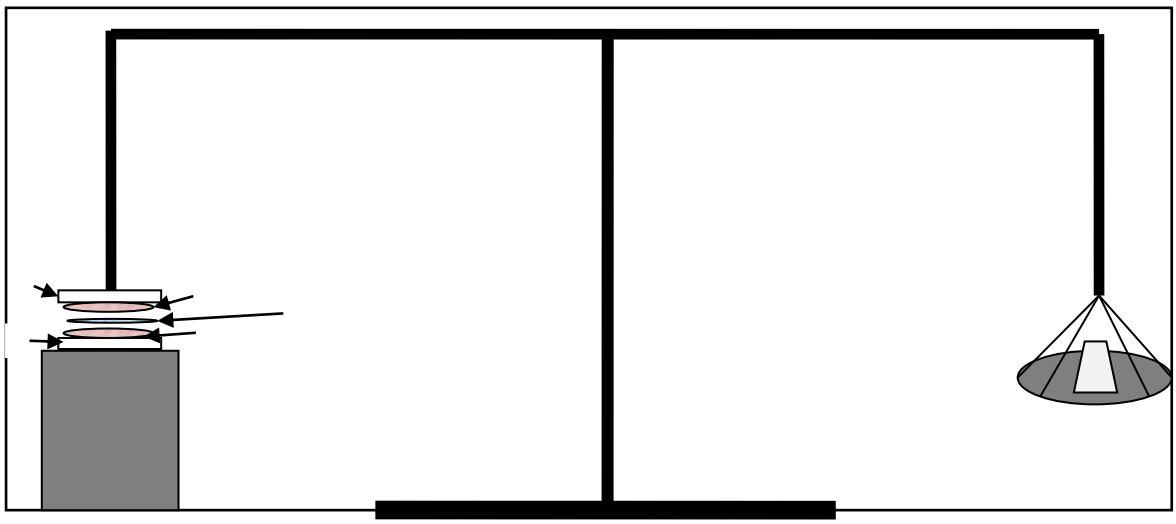

Figure 3. Device used for the measurement of bioadhesive strength. (A) Modified balance; (B) Weights; (C) Platform; (D) Glass plate; (E) Goat skin; (F) IBP gel [32].

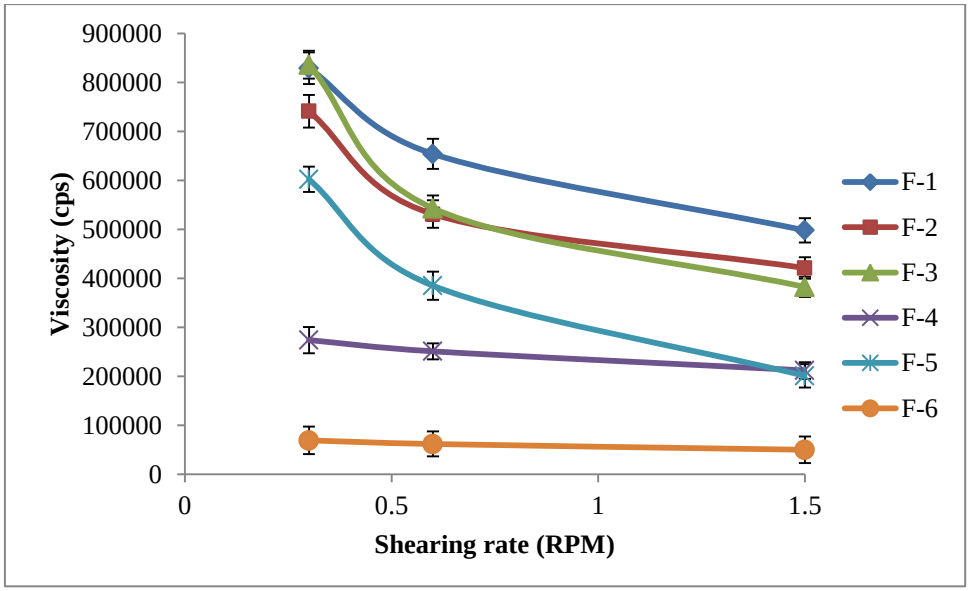

Figure 4. Graph of Viscosity vs Shearing rate in RPM

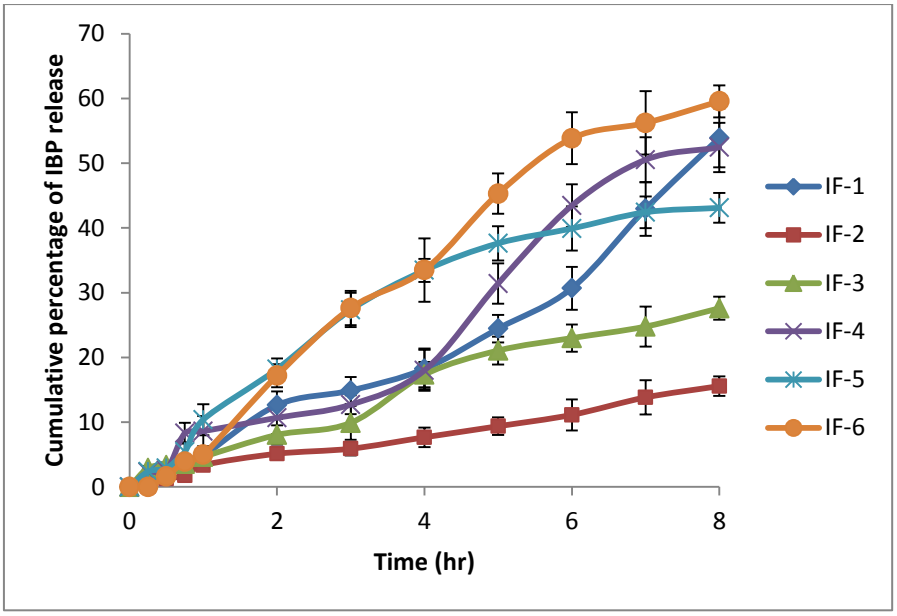

Figure 5. *In vitro* IBP release from different gel formulations.

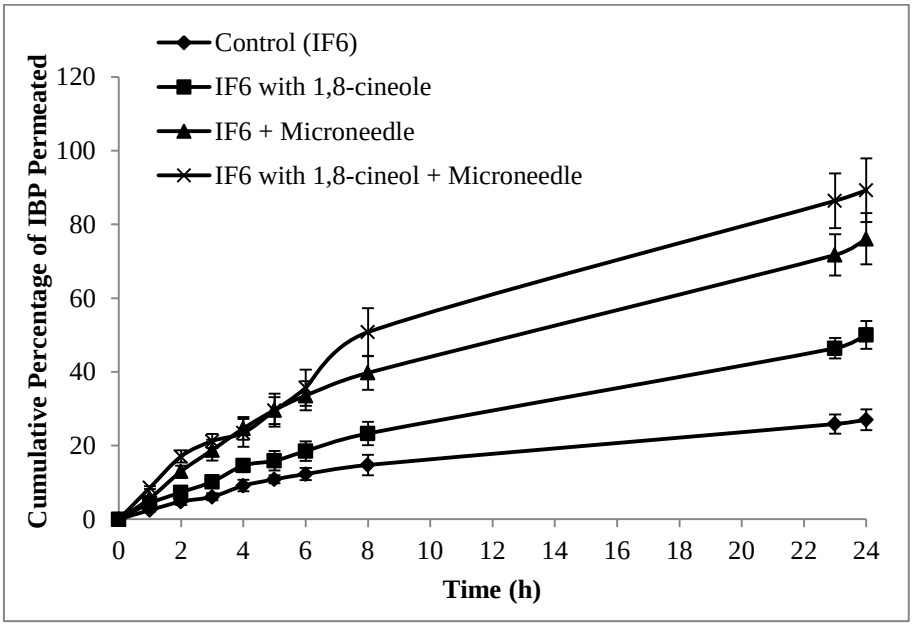

Figure 6. *In-vitro* permeation of IBP from gels across intact and microneedle pressed abdominal skin of rats.

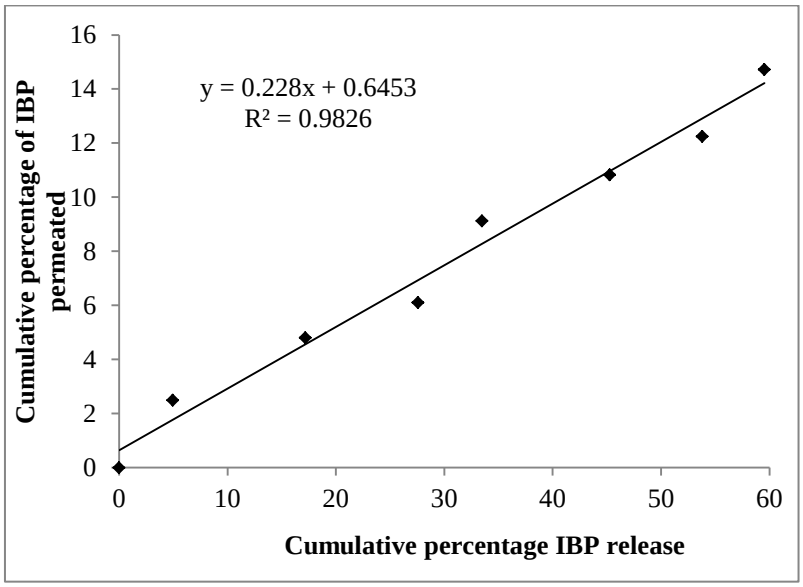

Figure 7. Correlation between cumulative percentage of IBP release and CPP of formulation IF6.

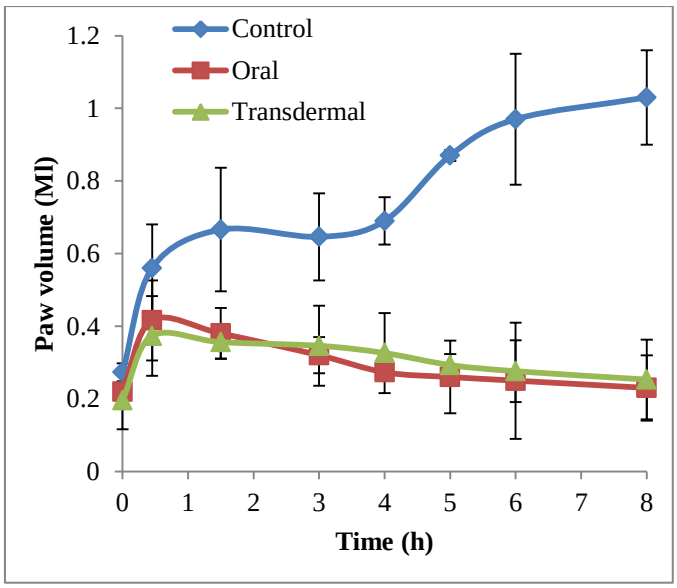

Figure 8. Comparison of paw volume of transdermally treated IBP gel with control and orally administered IBP suspension in carrageenan induced paw oedema in rats.

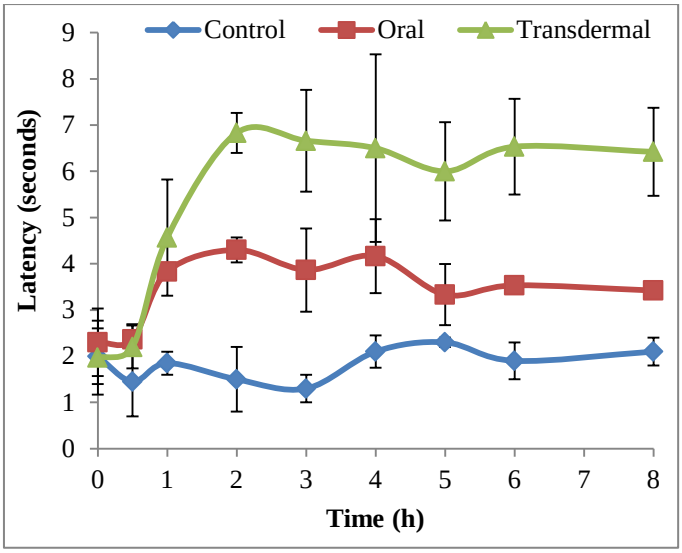

Figure 9a. Comparison of analgesic latency of transdermal IBP gel with control and oral IBP suspension by tail flick method in rats.

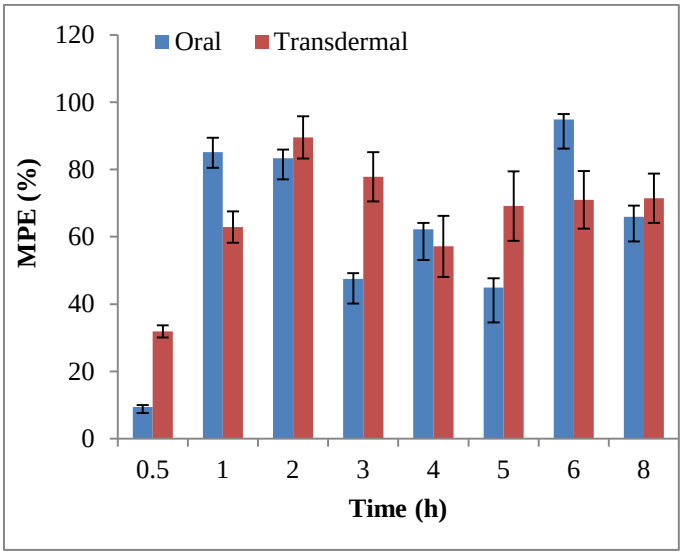

Figure 9b. Comparison of MPE (%) of transdermal IBP gel with oral IBP suspension by tail flick method in rats.