Research Article

Comparison between Marketed Formulations of Diclofenac Gel, Emugel and Spray for the Evaluation of Effect of Pressure and Temperature on Their Bioavailability

Mayee R[1*], Rawat S[2], Atre K[1], Mane P[1]

[1]*Dr. Ved Prakash Patil College of Pharmacy, Georai Tanda, Aurangabad*
[2]*Shri Bhagavan College of Pharmacy, Aurangabad*

ABSTRACT
This study involves research to assess the pharmacokinetics of Diclofenac Sodium Marketed formulations as well as the effect of pressure and temperature on its bioavailability by Dermatopharmacokinetic method. This drug is used for the treatment of local pain. This was a single-dose-three arm, open label pharmacokinetic study of marketed formulations of Diclofenac Sodium using 12 healthy Indian male subjects. A marketed Diclofenac Sodium topical formulation was applied on the pre-marked forearms of the subjects as per the dosing schedule. Gel and Emugel formulation was applied on one forearm whereas the spray was applied on the other arm. Subjects received treatment on both arms in normal conditions of temperature and pressure & same treatment with the application of sufficient pressure in the second period & heat in the third period of the study. The study was conducted following open label three way parallel design. A washout period of two days was kept between the three periods of the study. Skin Stratum corneum samples were collected in sterile glass test tubes during each period. The samples were collected pre-dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, & 6.0 hours post-dose application. The Stratum corneum samples were analysed for Diclofenac Sodium concentrations only. Pharmacokinetic parameters of Diclofenac sodium were calculated as C_{max}, t_{max}, AUC (0-t) and AUC (0-∞) Diclofenac Sodium was estimated in Stratum corneum using a validated Spectroscopic method. A total of 12 subjects were enrolled in this study. The bioequivalence values of the test drug are mentioned in the Tables 1-4 and the graphical representation is shown in the Fig. 1-16. This study demonstrated that the bioavailability of the topical formulations increased with the help of pressure and temperature in case of the Gel and Emugel formulations whereas there was negligible effect of temperature and pressure on the Spray formulation of Diclofenac.

Keywords: Stratum corneum, Diclofenac, Dermatopharmacokinetic, Skin stripping.

INTRODUCTION

Bioavailability is typically defined as the rate and extent at which a drug reaches the general circulation from an administered dosage form. In case of topical formulations, the drug has to penetrate through the layers of skin to reach the local site of action which is a complex process only due to the rate limiting barrier of the Stratum corneum. [1]

The penetration of a drug through the skin is a complex process typically rate-limited by the Stratum corneum (SC). This external layer of the skin is composed of terminally differentiated corneocytes embedded in a complex lipid matrix comprising primarily of ceramides, cholesterol, and free fatty acids. Delivery of drug by passive diffusion and the pharmacological effect elicited are dose-related. The better the drug permeates through the skin, the greater is the therapeutic effect.

*Corresponding author: Dr. Rahul V. Mayee,
10, Gurukunj Housing Society, Tilak Nagar, Aurangabad, MS India: 431005; **Tel.:** +91-9850162465;
E-mail: rahul.mayee@rediffmail.com

Therefore, the formulation plays an important role in topical drug delivery as the composition of the vehicle will influence the partitioning and/or the diffusivity of the drug and hence the absolute amount delivered.

The determination of the Bioequivalence of topical products involves the DermatoPharmacoKinetic (DPK) approach. The DPK approach includes measure of drug concentration in the skin, whether directly or indirectly related to the drug's therapeutic action, which can be determined continuously or intermittently for a period of time. This may include the measurement of either drug concentration in Stratum corneum over time and/or drug concentration in serial biopsy samples. The measurement of the change in the Stratum corneum drug concentration as a function of time is the objective of DPK approach and thus is a valid means of comparing a generic and innovator product for their ability to deliver drug to the deeper layers of the skin.

DPK studies offer certain advantages such as: it is painless, the active drug substances (moieties) are protected from

gastric enzymes, it avoids first pass effect, and it is simple to terminate if any adverse or undesired effect is observed. [2-4]

Tape Stripping Technique, consists of the standardized protocol of repeated applications and removal of adhesive tape on the skin surface, whereby consecutive layers of Stratum corneum cells are sampled. Tape stripping is a standard measuring method for the investigation of the dermatopharmacokinetics of topically applied substances using adhesive films. These tape strips are successively applied and removed from the skin after application and penetration of topically applied substances thus; the layers of the corneocytes and certain amount of topically applied substances are removed. The amount of the substances and the amount of Stratum corneum removed with the single tape strip is to be determined for calculation of the penetration profile. The topically applied substances removed from the skin can be thus determined by various analytical methods like HPLC, Mass Spectroscopy and other spectroscopic measurements. [4-5]

Diclofenac is an acetic acid non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Diclofenac is used to treat pain, dysmenorrhoea, ocular inflammation, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and actinic keratosis. Diclofenac has pharmacologic action similar to those of other prototypical NSAIDs. The exact mechanisms have not been clearly established, but many of the actions appear to be associated principally with the inhibition of prostaglandins synthesis. Diclofenac inhibits the synthesis of prostaglandins in body tissues by inhibiting cyclooxgenase; at least 2 isoenzymes, cyclooxygenase-2 (COX-1) and -2 (COX-2) (also referred to as prostaglandin G/H synthase-1[PGHS-1] and [PGHS-2], respectively), which have been identified to catalyze the formation of prostaglandins in the arachidonic acid pathway. The pharmacodynamic effect is thought to reduce prostaglandin E2 (PGE2) synthesis.

SUBJECT AND METHOD
Study Subjects
Sufficient numbers of healthy Indian male human subjects were screened, out of those 09 male subjects were enrolled in the study and 03 male subjects were taken as standby. A total of 12 male subjects were applied with the study medication in the beginning of the study. The screening consent & study consent was taken respectively before drug application. Thereafter, subject's medical records were documented and physical examination was conducted. Inclusion eligibility was also based on successful completion of a clinical health evaluation, which consisted of a personal interview; a complete physical examination (BP, pulse, weight, temperature, and respiratory rate); diagnostic testing that included a 12-lead electrocardiogram and chest radiograph; a laboratory testing that included a complete blood cell count, metabolic and hepatic tests (alanine amino transferase [reference range, 5-55 U/L], aspartate amino transferase [5-34 U/L]), urine analysis, blood chemistry for glucose (70-109 mg/dL), blood urea nitrogen (7-23 mg/dL), and creatinine (0.-1.3 mg/dL), as well as serology tests for hepatitis (B and C), and HIV antibodies. Testing was performed by Central Pathology Laboratory, Shree Pathology Lab CIDCO, Aurangabad. Subjects were excluded if laboratory values were significantly above or below the reference range and/or if all tests had not been performed. In addition, the laboratory

data were reviewed by the investigators of the clinical unit prior to the enrollment of the subjects. Subjects were compensated for participation.

Study Design
This study was carried out as per the ICH (Step 5), 'Guidance for Good Clinical Practices (GCP)' and the principles of Declaration of Helsinki (Scotland, October 2000).The Independent Ethics Committee reviewed the protocol and the informed consent form for this study. A single-dose-three arm, open label, three ways parallel design was used.

Subjects were admitted and housed in the clinical facility at least 2 hour before the application of the dose during each period of the study. Informed consent for the dosing / sampling procedure was obtained from each subject on admission to the clinical facility for the first study period.

These marketed Diclofenac Sodium formulations: 1) [Defenac Gel 15 gm; Lic. No.:JK/01/07-08/131; Mfg. Date: 02/2009; Exp. Date: 01/2012; Mfg. By: Lupin Ltd., Mumbai] 2) [Voveran Emulgel, Lic. No.: KTK/25/460/2001, Batch No.: 8Z099T, Mfg. Date: 12/2008 Exp. Date: 11/2011, Mfg. By: Novartis India Ltd., Bangalore] 3) Diclofenac Duoflam Spray, Lic. No.: AD/248-A, Mfg. Date: 04/08, Exp. Date: 04/2011, Mfg. By: Svizera Health Care, Mumbai.] were applied on the forearm of the study subjects as per the dosing schedule. The dosing procedure was as mentioned below:

- Both the forearms were washed with mild soap and copious amount of water and dried in air.
- One forearm was marked for total of 2 x 8 application sites of 1sq.cm area each for gel and emugel and the other was marked for total of 08 application sites of 1 sq.cm area each for spray respectively
- 5 mm length product was applied on all the sites so that the product completely and smoothly covered the site area.
- The Stratum corneum samples were collected from the sites on the desired pre decided time.

For the first time the Stratum corneum samples were collected as mentioned in the above procedure. After the pre decided time period the same procedure was repeated, except that after applying the product on all the sites sufficient amount of pressure was applied using pressure belt on all the sites. Then the Stratum corneum samples were collected from the sites on the desired pre decided time. Next apart from applying the pressure, a heat belt was also applied for the sufficient period of time on all the sites where the study product was applied.

Stratum corneum Sampling
Skin Stratum corneum samples were collected in sterile glass test tubes during each period. The samples were collected pre-dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 hours post-dose application. The Stratum corneum samples were analysed for Diclofenac Sodium concentrations only. For each subject the total number of blood draws were 02 (01 for screening and another during post study assessment); the total volume of blood withdrawn (10 ml for the pre-study evaluation and 10 ml for the post study) through the vein puncture did not exceed 20 ml.

Procedure
Study samples were collected as follows. The pre-dose samples were collected within one hour prior to drug application. The post-dose samples were collected within 2 minutes of the scheduled time where the end time of collection to the nearest minute would be recorded.

- Before sampling the drug remaining on the site was removed by mild force using three cotton swabs to ensure the complete removal of residual drug from the site.
- The pre cut (1 sq. cm) adhesion tape was applied on the site and the mild force was applied to ensure the proper adhesion of the tape on the site area. The tape was removed and discarded.
- Eight adhesion tape pieces were applied on the site area in the same manner and each tape was removed from the site before the next one was applied. The removal was done using the forceps and was to be done by one stroke to ensure the complete removal of stratum corneum.
- All 8 samples tapes were collected in a single test tube which were then sealed and stored in the refrigerator at -20°C till analysis.

Analytical Method

A validated UV spectroscopic method was employed by using Chemito-Spectroscan UV 2600, Double Beam UV-Visible Spectrophotometer for the estimation of Diclofenac Sodium in human stratum corneum. This method involved the extraction of the Diclofenac Sodium form sample by using methanol and measuring the absorbance at 285nm. The concentration of Diclofenac Sodium in sample was determined from calibration curve. The standard stock solution of Diclofenac sodium was prepared by weighing 50mg of Diclofenac Sodium powder and shaking it with 60 ml of methanol in a 200-ml volumetric flask, which was then diluted with methanol. From this solution 4ml was diluted up to 100ml with methanol, to get the solution with concentration of 10μg/ml. The test solution was prepared by taking 1, 2, 4, 6, 8, 10 ml from the standard stock solution in six different, labelled (1 μg/ml, 2μg/ml, 4μg/ml, 6μg/ml, 8μg/ml) test tubes and making volume up to 10ml by adding methanol. (Note: no need to add methanol in last μg/ml sample). Methanol was used as blank solution. Calibration Curve was prepared by using various dilutions (1 μg/ml - 10μg/ml) as transferring required quantity of blank solution in to the cuvette and recording the absorbance, then taking the first test tube (1μg/ml), the required quantity of the test solution was transferred into the cuvette and the absorbance was measured recorded at 285 nm. The step 2 and 3 was repeated for remaining dilutions. Finally the graph of concentration versus absorbance (Optical Density *aka* OD) was plotted.

Pharmacokinetic Analysis

Individual plasma concentration– time curves were constructed; Cmax and Tmax were directly obtained from these curves. AUC from time 0 (baseline) to 6 hours (AUC0–6) was calculated using the trapezoidal rule (Chow and Liu, 2000; Chow and Liu, 2007). From the terminal log-decay phase, elimination rate constant (ke) was estimated using linear regression, and t½ was estimated using the following equation: $t\frac{1}{2} = \ln 2 / k_e$ where ln was defined as the natural logarithm. Extrapolation of AUC from baseline to infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC0-6 + (C6/k_e)$ where C6 was defined as concentration at 6 hours.

To evaluate the effect of pressure and temperature on the bioavailability of the formulation tested, Cmax, AUC from baseline to time t (AUC0–t), and $AUC_{0-\infty}$ was carried out for each study. Ratios of Cmax, AUC0–t, and $AUC_{0-\infty}$ for all formulations were calculated, and 90% CIs were obtained. The 90% CIs for the corresponding ratios of Cmax, tmax, AUC0–t, and $AUC_{0-\infty}$ should be within the 80% to 125% range.

RESULT

Twelve subjects were enrolled in this study of Diclofenac (mean age, 25.16 years). The bioequivalence values of the test drug for first time (i.e. when pressure or temperature was not applied) for Emugel, were as follows: Cmax of 24.0 ± 2.398 μg/mL, tmax of 1.78 ± 0.261 h, AUC0-t of 100.5 ± 10.455 h. μg/mL, $AUC_{0-\infty}$ of 178.0± 22.859 h. μg/mL; after applying pressure, Cmax of 31.1 ± 2.742 μg/mL, tmax of 0.79 ± 0.342 h, AUC0-t of 101.3 ± 10.072 h. μg/mL, $AUC_{0-\infty}$ of 179.41 ± 47.627 h. μg/mL; and after using heat belt Cmax of 28.7 ± 2.216 μg/mL, tmax of 1.8 ± 0.261 h, AUC0-t of 100.6 ± 11.15 h. μg/mL, $AUC_{0-\infty}$ of 179.706 ± 21.553 h. μg/mL. and for Gel Cmax of 26.0 ± 2.398 μg/mL, tmax of 1.79 ± 0.261 h, AUC0-t of 99.8 ± 10.455 h. μg/mL, $AUC_{0-\infty}$ of 178.9± 22.859 h. μg/mL; after applying pressure, Cmax of 31.1 ± 2.742 μg/mL, tmax of 1.42 ± 0.342 h, AUC0-t of 102.5 ± 10.072 h. μg/mL, $AUC_{0-\infty}$ of 179.31 ± 47.627 h. μg/mL; and after using heat belt Cmax of 28.2 ± 2.216 μg/mL, tmax of 1.68 ± 0.261 h, AUC0-t of 100.5 ± 11.15 h. μg/mL, $AUC_{0-\infty}$ of 179.7 ± 21.553 h. μg/mL. and for Spray Cmax of 30.0 ± 2.398 μg/mL, tmax of 1.62 ± 0.261 h, AUC0-t of 100 ± 10.455 h. μg/mL, $AUC_{0-\infty}$ of 180.0± 22.859 h. μg/mL; after applying pressure, Cmax of 30.2 ± 2.742 μg/mL, tmax of 1.59 ± 0.342 h, AUC0-t of 100.0 ± 10.072 h. μg/mL, $AUC_{0-\infty}$ of 180.0 ± 47.627 h. μg/mL; and after using heat belt Cmax of 29.4 ± 2.216 μg/mL, tmax of 1.59 ± 0.261 h, AUC0-t of 100.6 ± 11.15 h. μg/mL, $AUC_{0-\infty}$ of 180.0 ± 21.553 h. μg/mL. respectively.

Table 1: DPK values for normal, pressure and temperature for Emugel

Parameters	Emugel DPK Values	DPK Values on application of Pressure	DPK values on application of Temperature
Cmax	24.0±2.398 μg/Ml	31.1± 2.742 μg/mL	28.74± 2.216 μg/mL
Tmax	1.78 ±0.261 h	0.79 ±0.342 h	1.8 ±0.261 h
AUC0-t	100.5± 10.455 h. μg/mL	101.3± 10.072 h. μg/mL	100.6±11.15 h. μg/mL
AUC0-∞	178.0± 22.859 h. μg/mL;	179.421± 47.627 h. μg/mL;	179.0± 21.553 h. μg/mL

**Table 2: DPK values for normal, pressure and temperature for Gel**

Parameters	Gel DPK Values	DPK Values on application of Pressure	DPK values on application of Temperature
Cmax	26.0±2.398 μg/Ml	31.1± 2.742 μg/mL	28.2± 2.216 μg/mL
Tmax	1.79 ±0.261 h	1.42 ±0.342 h	1.68 ±0.261 h
AUC0-t	99.87± 10.455 h. μg/mL	102.5± 10.072 h. μg/mL	100.5±11.15 h. μg/mL
AUC0-∞	178.9± 22.859 h. μg/mL;	179.31± 47.627 h. μg/mL;	179.706± 21.553 h. μg/mL

Table 3: DPK values for normal, pressure and temperature for Spray

Parameters	Spray DPK Values	DPK Values on application of Pressure	DPK values on application of Temperature
Cmax	30.0±2.398 μg/Ml	30.2± 2.742 μg/mL	29.4± 2.216 μg/mL
Tmax	1.62 ±0.261 h	1.58 ±0.342 h	1.59 ±0.261 h
AUC0-t	100.0± 10.455 h. μg/mL	100.0± 10.072 h. μg/mL	100±11.15 h. μg/mL
AUC0-∞	180.0± 22.859 h. μg/mL;	180.0± 47.627 h. μg/mL;	180.0± 21.553 h. μg/mL

Table 4: Comparison between the normal DPK values and effect of pressure and temperature on DPK of Diclofenac Emugel, Gel and Spray formulations

Parameters	Test Drug DPK (Normal Values)			Effect of Pressure			Effect of Temperature		
	Emugel	Gel	Spray	Emugel	Gel	Spray	Emugel	Gel	Spray
Cmax in µg/Ml	24	26	30	31.1	31.1	30.2	28.7	28.2	29.4
Tmax In Hrs	1.78	1.79	1.62	0.79	1.42	1.58	1.8	1.68	1.59
AUC(0-t) In h. µg/mL	100.5	99.8	100	101.3	102.5	100	100.6	100.5	100
AUC(0-∞) In h. µg/mL	178	178.9	180	179.4	179.3	180	179	179.7	180

Pharmacokinetic parameters

C_{max}a, t_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ for each study are shown in Table 1 - 4 and depicted in Fig. 1 to 16.
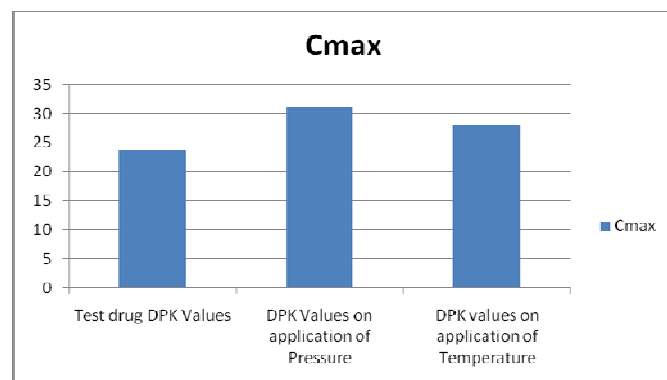
Fig. 1: Graphical Representation for the values of Cmax for Emugel
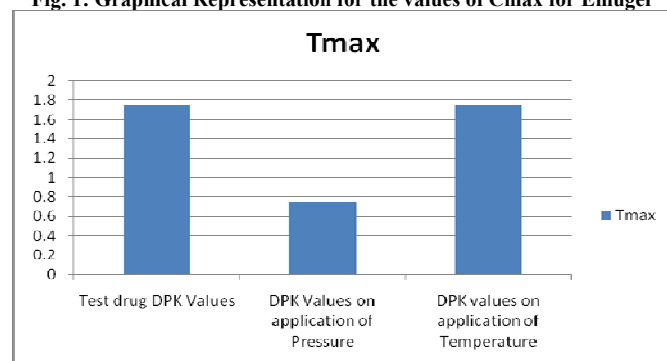
Fig. 2: Graphical Representation for the values of Tmax for Emugel
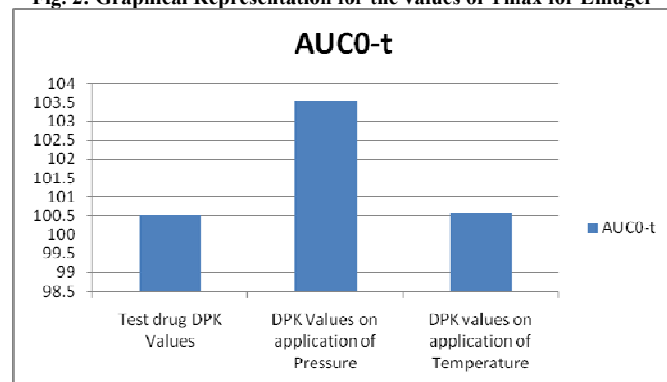
Fig. 3: Graphical Representation for the values of AUC(0-t) for Emugel
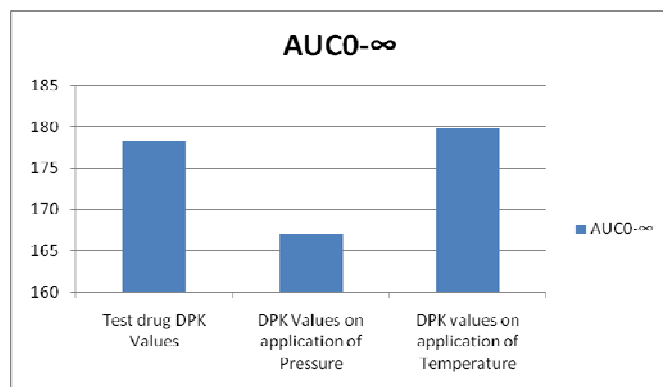
Fig. 4: Graphical Representation for the values of AUC(0-∞) for Emugel
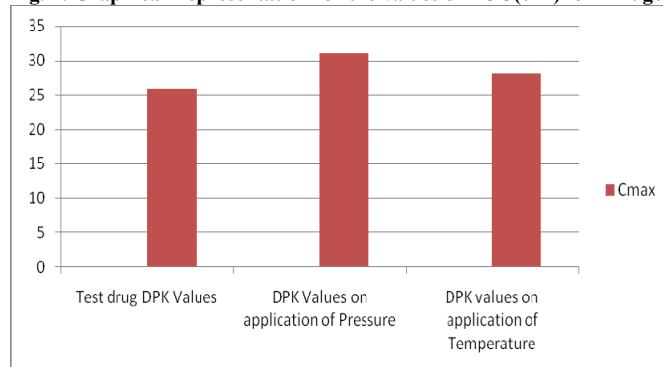
Fig. 5: Graphical Representation for the values of Cmax for Gel
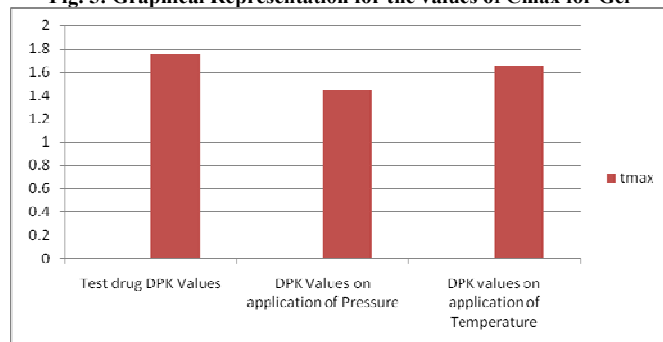
Fig. 6: Graphical Representation for the values of Tmax for Gel
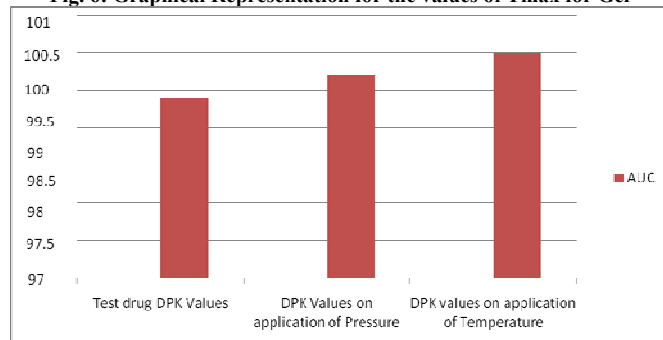
Fig. 7: Graphical Representation for the values of AUC(0-t) for Gel
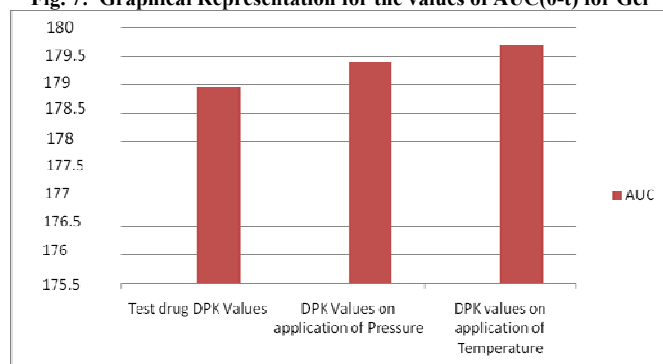
Fig. 8: Graphical Representation for the values of AUC (0 to ∞) for Gel
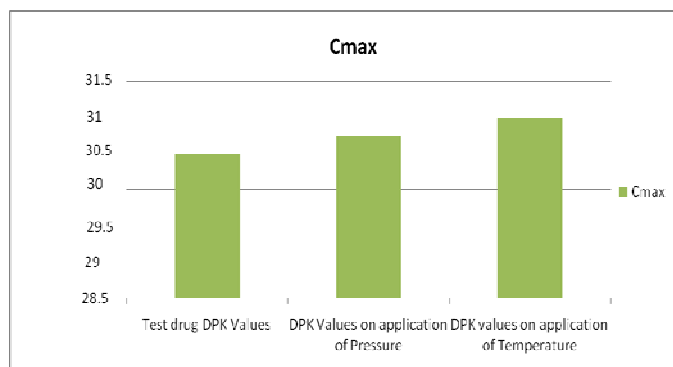
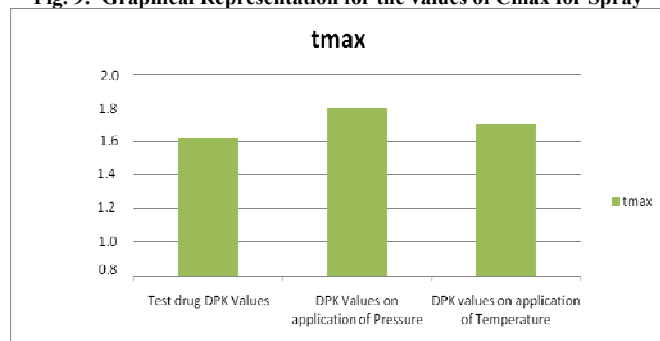**Fig. 9: Graphical Representation for the values of Cmax for Spray**

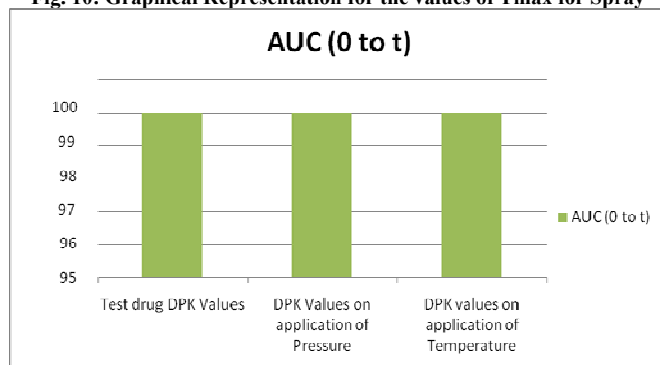**Fig. 10: Graphical Representation for the values of Tmax for Spray**

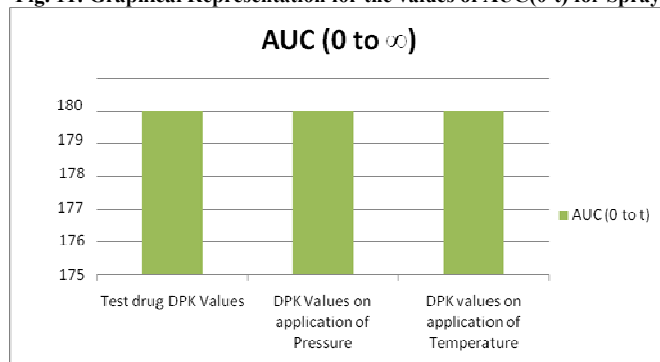**Fig. 11: Graphical Representation for the values of AUC(0-t) for Spray**

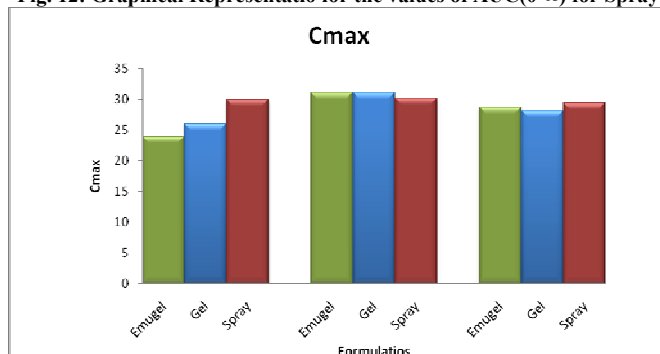**Fig. 12: Graphical Representatio for the values of AUC(0-∞) for Spray**

Fig. 13: Comparison between the Values of Cmax of Emugel, Gel and Spray
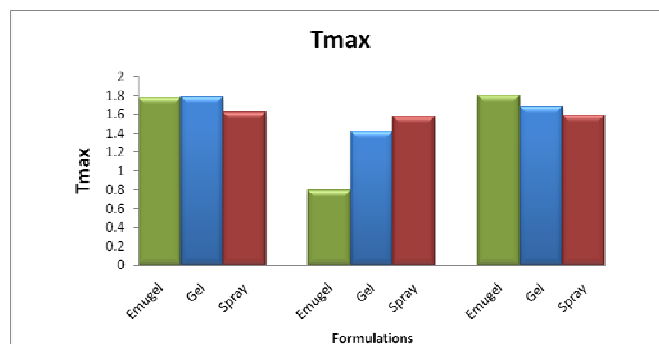

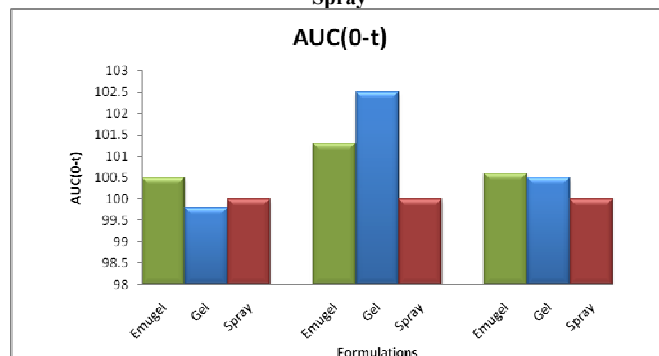**Fig. 14: Comparison between the Values of Tmax of Emugel, Gel and Spray**

Fig. 15: Comparison between the Values of AUC(0-t) of Emugel, Gel and Spray
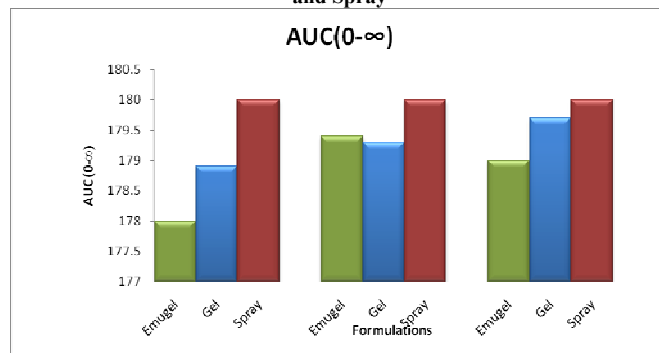

Fig. 16: Comparison between the Values of AUC(0-∞) of Emugel, Gel and Spray

DISCUSSION

The results of our study showed that the bioavailability of the topical formulations increased with the help of pressure and temperature in case of the Gel and Emugel whereas there was negligible effect of temperature and pressure on the Spray formulation of diclofenac.

No moderate or serious Adverse Events (AEs) were reported by the investigators. Potential recall bias of AEs in this study was not likely because only one dose of each formulation was administered during each treatment period, subjects were under medical surveillance in the clinical unit, and the duration of the washout period was only 2 days.

CONCLUSION

This study has demonstrated that for the topical formulations, the physical parameters like pressure or temperature acts as an aid not only to achieve higher tissue concentration but also to give the rapid onset of action. Emugel and Gel formulations shows marked elevation in Cmax and AUC values and Tmax is achieved faster, whereas Spray formulation shows negligible effect on DPK parameters after application of Pressure and Temperature.

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