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A SIMPLE NON-INVASIVE COMPARATIVE LOG LINEAR PK-PD MODEL OF DEXIBUPROFEN TABLETS IN INDIAN HEALTHY HUMAN MALE VOLUNTEERS

Punnamchand Loya[1]* & Madhusudan N. Saraf [2]

[1] Associate Professor, RBVRR Women's College of Pharmacy, Osmania University 3-4-343, Barkatpura, Hyderabad -27, INDIA.

[2] Principal & Professor of Pharmacology, The Bombay College of Pharmacy, Kalina, Santacruz (E), Mumbai-400 098.

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***Correspondence for Author**
Dr. Punnamchand Loya
Associate Professor, RBVRR Women's College of Pharmacy, Osmania University 3-4-343, Barkatpura, Hyderabad -27, INDIA.

ABSTRACT

The aim of the present work was to develop a simple PK-PD model for NSAIDs taking dexibuprofen as a study drug. In this study, the pharmacokinetics and pharmacodynamics of dexibuprofen were investigated in Indian healthy volunteers during a fasting state for over 10 h. The pharmacodynamic study was carried by checking the inhibition of methyl nicotinate (MN) induced erythema after a single oral administration of 400 mg tablets of dexibuprofen using Laser Doppler Flowmeter (LDF) for a period of 12 h. In addition, the concentration of dexibuprofen in blood samples was determined by HPLC. The time courses of the plasma concentration of dexibuprofen and the % inhibition of methyl nicotinate (MN) induced erythema were analyzed**.** It was found that there exists a direct relation between concentration-effect of dexibuprofen. Hence a simple log linear PK-PD was applied and the applied PK-PD model helps in predicting the clinical performance of new NSAID formulations.

KEYWORDS: Dexibuprofen, HPLC-UV, LDF, Human Volunteers & Log-Linear PK-PD.

INTRODUCTION

Dexibuprofen (Fig. 1) is the single pharmacologically effective enantiomers of rac-ibuprofen and acts by inhibiting prostaglandins synthesis and formation of thromboxane via blockade of

cyclooxygenase (both COX-1 and COX-2) enzymes. It is used similarly like ibuprofen in the management of mild to moderate pain and inflammatory conditions such as dysmenorrhoea, headache, postoperative pain, dental pain, sprains, and soft-tissue rheumatism. Dexibuprofen also inhibits leucotriene production and the nitric oxide synthesis inhibition by iNOS induction. Dexibuprofen was found to be safer when compared with other NSAIDs in the risk of gastrointestinal bleeding or ulcer perforation as it has been observed that a dose of 1:0.75 (rac-ibuprofen Vs dexibuprofen) would be needed to obtain comparable pharmacodynamic effects. [1-5]

Pharmacokinetic-Pharmacodynamic models search revealed that very few models were published for the anti-inflammatory drugs after administering the drug parenterally, orally and applied topically. [6-9] Data on the relationships between plasma concentration and anti-inflammatory effects of NSAIDs are limited because most inflammation models do not permit pharmacodynamic (PD) modeling in human volunteers. [9] Based on this literature, it was felt necessary to standardize and validate a simple non-invasive PD model in human volunteers to perform the effect-time study on healthy human male volunteers after a single oral administration of 400 mg tablets of dexibuprofen, which will also help in predicting the clinical performance of new formulations of NSAIDs.

The study was carried to examine the relationship between dexibuprofen plasma concentration and inhibition methyl nicotinate induced erythema by dexibuprofen after oral administration to healthy volunteers. This should enable a prediction of the time-course of the therapeutic and side effect profiles of dexibuprofen for oral dosing strategies. Therefore, the objective of this study was to assess the applicability of pharmacokinetic-pharmacodynamic (PK-PD) modeling in the description of this relationship.

MATERIALS & METHODS

Chemicals and Reagents

Dexibuprofen was obtained from Medley Pharmaceuticals Ltd, Mumbai. Acetonitrile and methanol (HPLC grade) were purchased from Qualigens Fine Chemicals, Mumbai. Potassium dihydrogen phosphate (AR grade) was purchased from S.D. Fine Chem. Ltd., Mumbai. Freshly prepared double distilled water was used throughout the study. Fresh frozen human plasma used in the method development was obtained from the National Plasma Fractionation Center, K.E.M. Hospital, Mumbai, and was stored at –20° C until used.

Chromatographic Conditions

Pure sample of dexibuprofen was dissolved in methanol and the UV scan was then taken to determine the $\lambda_{max.}$ The chromatographic conditions for the determination of dexibuprofen in plasma was developed using UV detection. Separation was carried out on C_{18} column using a mixture of acetonitrile: methanol: 0.05 M potassium dihydrogen phosphate as a mobile phase. A single step sample preparation method using acetonitrile as precipitating solvent was developed for the extraction of dexibuprofen from plasma samples. The analytical method developed for estimation of dexibuprofen from plasma was validated for its selectivity, limit of detection and quantitation, precision, accuracy, linearity, recovery and stability in plasma. The Preparation of standard & test Solutions, Chromatographic Conditions, Sample Preparation & Method validation were described in detailed our earlier manuscript. [10]

Pharmacokinetic Study

The study was conducted as per the protocol approved by local Independent Ethics Committee and as per CDSCO guidelines. [10] The study was conducted on 12 healthy human male volunteers in the age group 20-30 years as a 12 X 2 single dose, comparative, open label, randomized and complete crossover design with an at least 7-day wash-out interval. Written Informed Consent was obtained from each volunteer prior to the study. After overnight fasting of 10 h, all subjects received one reference tablet of IBUSOFT® 400 (each tablet containing 400 mg dexibuprofen, manufactured by Emcure Pharmaceuticals Ltd, Pune, India) or test formulations (Medley Pharmaceuticals Ltd, Mumbai, India) per oral with 240 ml water at an ambient temperature. A Venous blood samples (5 ml) were collected before dosing (0 h) and then at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 8.0, 10.0 and 12.00 h post drug administration. The blood samples collected at various time intervals were immediately centrifuged at 3000 g for 15 min at 4 °C, plasma was separated and stored in vials at -20 °C until analysed. The plasma concentration profile thus obtained was fed into S-inverse (S-INV), a computer software on BASICA® Version 1.12, to determine the pharmacokinetic parameters. The detailed procedure followed for conducting the study and statistical analysis applied [11, 12] were discussed in earlier manuscript. [13]

Pharmacodynamic Study - Methyl Nicotinate Induced Erythema

Methyl nicotinate (MN) induced skin erythema was studied using the method described by Poelman. [14] A total of 12 volunteers were selected for this study. Volunteers were acclimatized to the environment (RT 23±1° C, RH 45±5 %) in supine position for at least 15

min, before the actual blood flow measurements. A site on the flexor aspect of forearm was selected with the precaution that no major vein would face the Laser Doppler probe during the measurement. Resting Cutaneous blood flow (CBF) was measured for 10 min using Laser Doppler Flowmeter (LDF) (Periflux PF3, Perimed, Sweden.) equipped with standard probe (Probe 408). Cutaneous blood flow was recorded for 10 min, before dosing (0 h) and then at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 8.0 and 10.0 h following drug administration immediately after methyl nicotinate treatment. For each time point a new site was selected and the resting CBF (baseline reading) for the respective site was taken prior to MN application and also CBF after MN application was recorded of the same site at respective time point. The signals from the LDF were recorded as PU and processed using perisoft software program. The Mean Perfusion unit (PU) was measured for baseline (untreated) and as well as after applying MN (treated) for volunteers after a single dose of 400 mg standard and test dexibuprofen formulation. Significance at various time points was checked with respect to the basal value using t-test. The detailed procedure followed for blood flow measurement and statistical analysis applied were discussed in our earlier article. [15]

Results were expressed as percentage inhibition (effect) for the different time points of the erythematous reaction induced by MN, calculated using the formula:

$$\text{Percentage inhibition} = \frac{PU(b) - PU(t)}{PU(b)} \times 100$$

PU (b): Mean PU after MN application minus resting CBF before dosing (0 h) of the site.
PU (t): Mean PU at various time points after MN application minus resting CBF at various time points for the respective site.

Pharmacokinetic-Pharmacodynamic Modeling

To evaluate possible relation between the pharmacodynamic effect (inhibition of inflammation induced by methyl nicotinate) and dexibuprofen plasma concentrations, the effect was plotted against the concentrations were analyzed by linear regression analysis using SPSS software, V 17.0.

RESULTS

Under the chromatographic conditions employed the mean retention time (Rt) of the dexibuprofen was found to be 4.720±0.3 min and no endogenous peak from plasma was

found to interfere near the retention time of dexibuprofen. The calibration curve of dexibuprofen was linear over the working concentration range of 1-40 µg/ml in human plasma with seven-point (including zero point) with coefficient of correlation (r^2) was found > 0.99 for dexibuprofen from plasma. The LOD & LOD of dexibuprofen in plasma was verified as 0.5 µg/ml & 1.0 µg/ml, respectively. The % CV for *intra-day* and *inter-day* precision of the method was found to be in the range of 1.021-10.46. The *intra-day* and *inter-day* accuracy of the method was found to be in the range of 100.35-106.0 %. The mean recoveries of QC standards of dexibuprofen were found to be above 90 %. Three freeze-thaw cycles of the QC standards appeared to have no effect on stability of the analyte. QC samples stored in a freezer at -20 °C remained stable for at least one month. These studies suggested that human plasma samples containing dexibuprofen can be handled under normal laboratory conditions without significant loss of compound.

The observed C_{max} of dexibuprofen was found to be 30.00±3.20 µg/ml and 28.95±4.35 µg/ml; with the corresponding T_{max} was found to be 1.83±0.65 h and 1.83±0.44 h following a single oral dose of 400 mg dexibuprofen test and reference formulation, respectively. The AUC_{0-t} of dexibuprofen was found to be 125.63±20.07 µg.h/ml and 115.36±27.78 µg.h/ml; while the AUC_{0-inf} was found to be 139.90±25.79 µg.h/ml and 130.36±25.27 µg.h/ml following a single oral dose of 400 mg dexibuprofen test and reference formulation, respectively. The observed pharmacokinetic parameters, ratio of test/reference (T/R), 90 % confidence intervals (90 CIs) and their statistical values for dexibuprofen after an oral administration of 400 mg dexibuprofen tablets were within the range of 80-125 % in accordance with the Food and Drug Administration Bioequivalence Guideline. [16, 17] The detailed results obtained were discussed in our earlier publication. [13]

The observed mean maximum inhibition (max effect, E_{max}) was 93.64±4.57 % and 95.22±3.61 %; and the corresponding mean time to maximum effect was found to be 2.08±0.36 h and 2.17±0.33 h for the test and reference formulations, respectively. The mean normalized individual perfusion units (PU) following a single oral dose of test and reference formulation of dexibuprofen at various time points were found to significantly inhibit the MN induced inflammation for 8 h compared with respect to the resting CBF (UN treated basal value) at $p<0.05$. The detailed obtained results were discussed in our earlier publication. [15]

The time Vs concentration profile of dexibuprofen (Test and Reference formulations) in human plasma paralleled the inhibition of inflammation induced by MN (Fig 2 & 3). The PK

and PD values at various time points of dexibuprofen obtained after a single oral dose of test and reference formulations were tabulated in Table 1. A linear plot of the mean percent inhibition (effect) Vs mean concentration was developed and the correlation coefficient (r^2) were found to be 0.874 for test formulation and 0.803 for reference formulation, respectively (Fig. 4 & 5) and while logarithmic linear plot of the percent mean inhibition (effect) Vs mean concentration the correlation coefficient (r^2) were found to be 0.903 and 0.888 for test and reference formulation, respectively (Fig. 6 & 7)

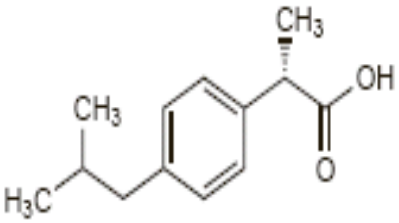

Fig 1: Chemical Structure of Dexibuprofen

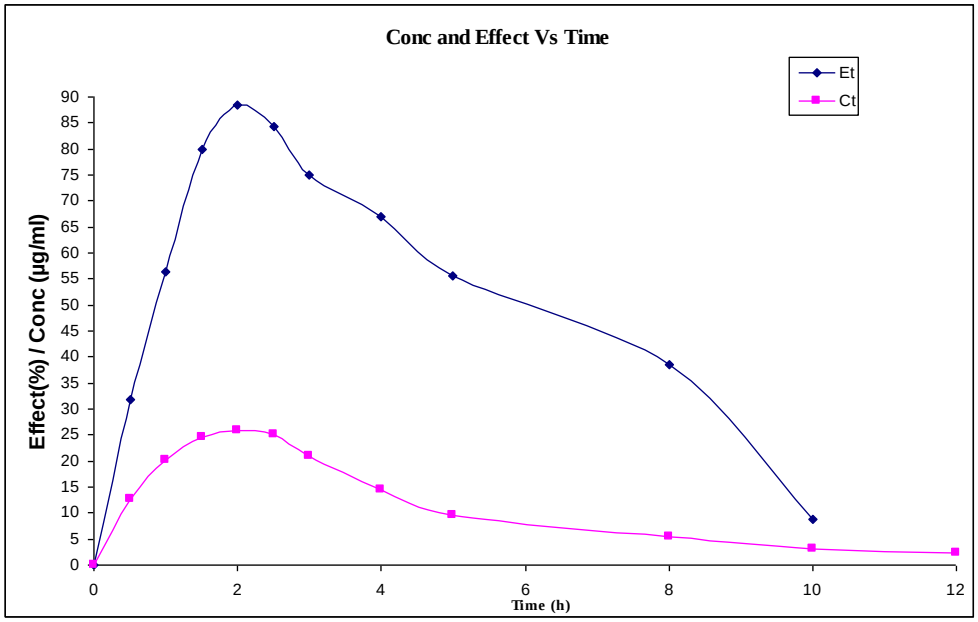

Fig 2: Effect (Inhibition) and Concentration Vs Time of Dexibuprofen Test Formulation in 12 Healthy Male Volunteers

For the above graphs: Et – Effect Vs Time Graph of Dexibuprofen (Pharmacodynamic); Ct – Concentration Vs Time Graph of Dexibuprofen (Pharmacokinetic)

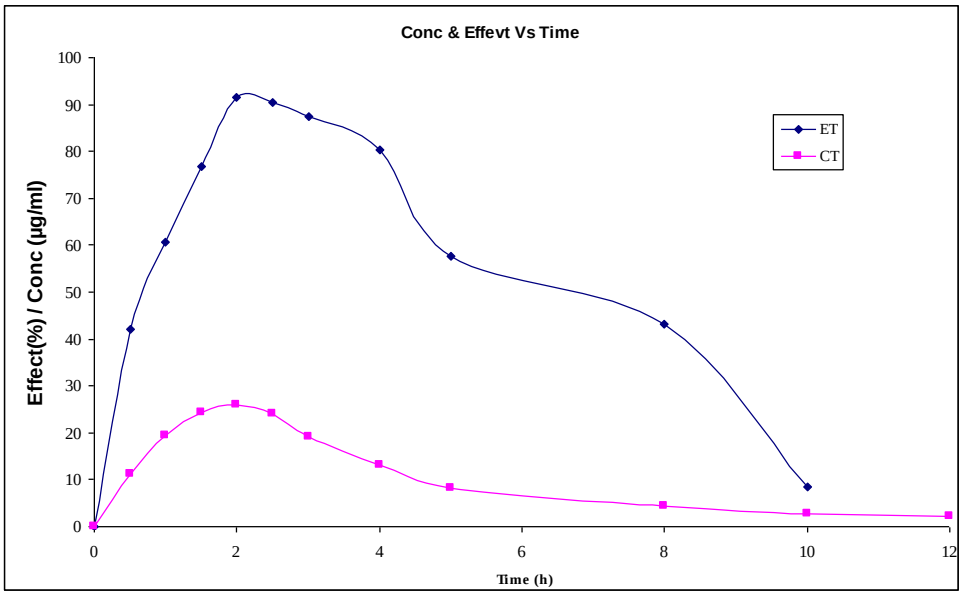

Fig 3: Effect and Concentration Vs Time of Dexibuprofen Reference Formulation in 12 Healthy Male Volunteers

For the above graph: Et – Effect Vs Time Graph of Dexibuprofen (Pharmacodynamic); Ct – Concentration Vs Time Graph of Dexibuprofen (Pharmacokinetic)

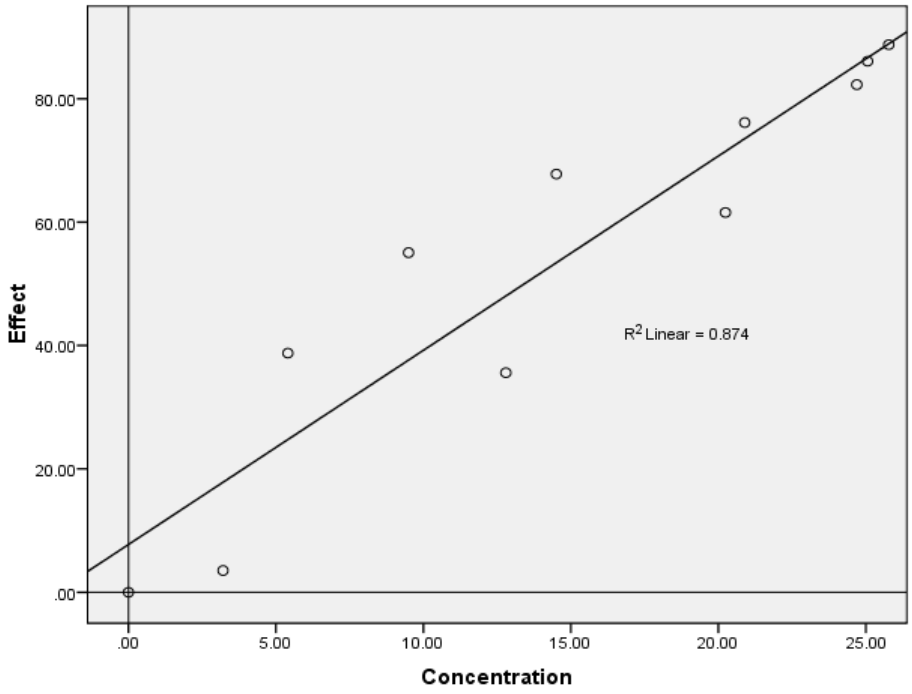

Fig 4: Percent Inhibition (Effect) Vs Concentration of Dexibuprofen Test Formulation in 12 Healthy Male Volunteers (Linear)

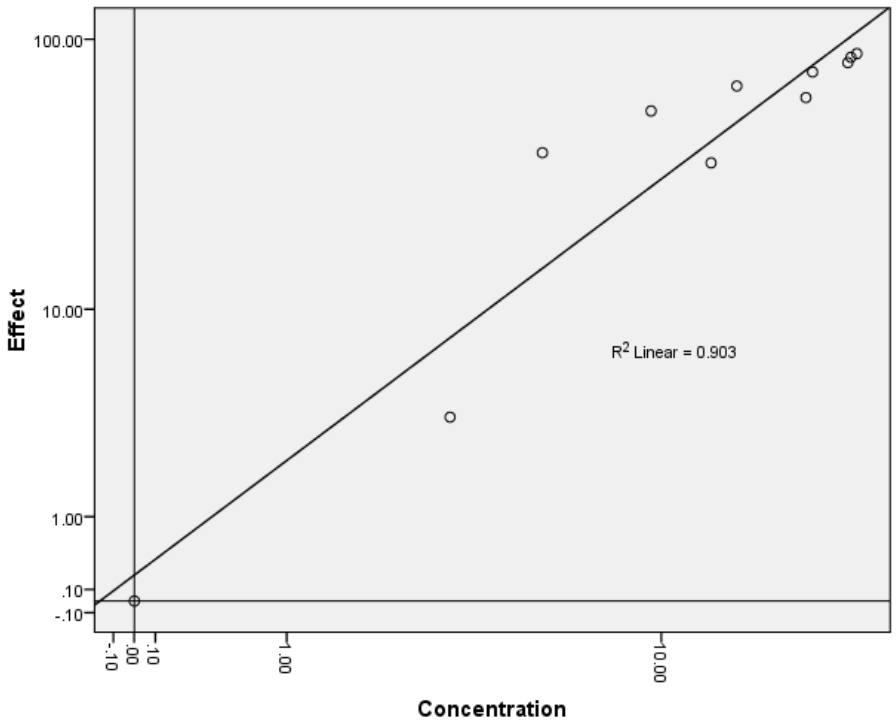

Fig 5: Percent Inhibition (Effect) Vs Concentration of Dexibuprofen Test Formulation in 12 Healthy Male Volunteers (Log Linear)

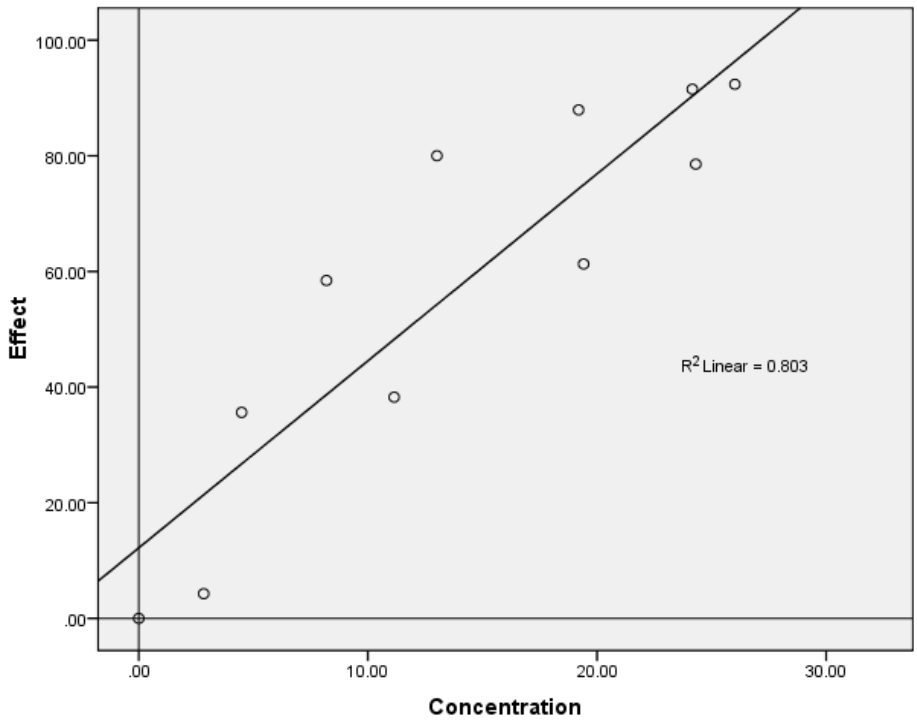

Fig 6: Percent Inhibition (Effect) Vs Concentration of Dexibuprofen Reference Formulation in 12 Healthy Male Volunteers (Linear)

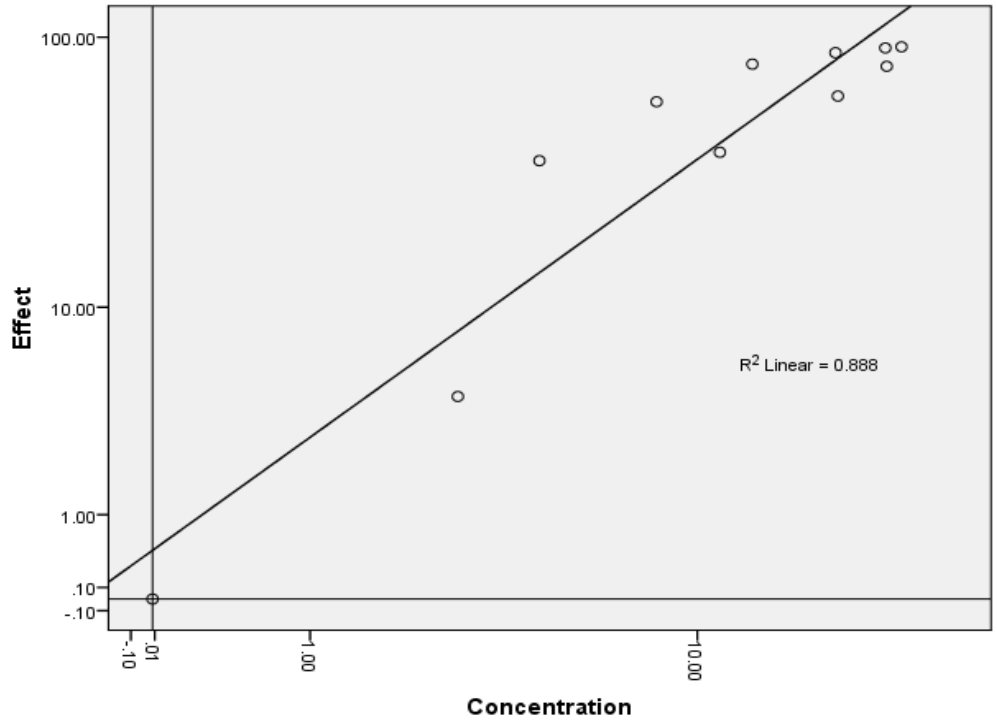

Fig 7: Percent Inhibition (Effect) Vs Concentration of Dexibuprofen Reference Formulation in 12 Healthy Male Volunteers (Log Linear)

Table 1: Concentration and Effect of Dexibuprofen Test and Reference Formulations

Time (h)	Test Formulation		Reference Formulation	
	Concentration (µg/ml)	Effect (% inhibition)	Concentration (µg/ml)	Effect (% inhibition)
0.0	0	0	0	0
0.5	12.794	35.59	11.147	38.25
1.0	20.239	61.56	19.415	61.29
1.5	24.692	82.29	24.31	78.55
2.0	25.776	88.76	26.016	92.36
2.5	25.058	86.08	24.16	91.52
3.0	20.893	76.14	19.197	87.94
4.0	14.507	67.80	13.012	80.01
6.0	9.495	55.05	8.191	58.44
8.0	5.402	38.76	4.488	35.63
10.0	3.205	3.53	2.832	4.28
12.0	2.267	-	2.16	-

DISCUSSION

A non-invasive methyl nicotinate induced erythema pharmacodynamic model for dexibuprofen using LDF was standardized and was successfully applied to perform the effect-time study in healthy human male volunteer after a single oral administration of 400 mg of dexibuprofen test and reference formulations. The topical application of MN rapidly

produces erythema or local inflammation by the liberation of prostaglandins results in significant change in microcirculation due to the vasodilation and the duration of which varied from subject to subject and was concentration dependent i.e. MN induced changes in microcirculation was found to be reproducible with a variation of ± 20 % in a same volunteer when measured at the same time on different days under the same experimental conditions. [1, 14, 18] Therefore the topical application of aqueous MN solution induced erythema was standardised at concentrations of 1, 5 and 10 % (w/v) [18, 19] and found that the topical application of MN, produced a rise in CBF by 10 to 15 fold with respect to the basal CBF; it remained constant for 10 minutes after which it declined gradually. Hence a 10 % (w/v) MN solution was used to induce erythema or local inflammation for effect-time study of dexibuprofen in healthy human male volunteers.

The developed MN model was used to evaluate the effect-time of dexibuprofen 400 mg test and reference formulations by checking the inhibition of MN induced inflammation by dexibuprofen. The inhibition of MN induced inflammation by dexibuprofen was checked for 10 h and found that dexibuprofen significantly inhibited the inflammation induced by MN for 8 h comparing when compared to the resting CBF (UN treated basal value) at $p<0.05$ using T-test. The effect of dexibuprofen was observed for 8 h where the concentration of was above 4 µg/ml for both test and reference formulation. It indicates that for dexibuprofen to show its activity of inhibiting the inflammation induced by MN the concentration in the blood has to be more than the 4 µg/ml. Hence, the inhibition at 10 h was not significant compared to the basal value for both test and reference dexibuprofen tablets as the concentration were less.

The effect of dexibuprofen was calculated from the percent inhibition of MN induced inflammation by dexibuprofen, after subtracting the basal CBF value at each site from the MN treated value for each time point. It was observed that dexibuprofen inhibited the inflammation by almost 35 % in the 0.5 h of dosing of dexibuprofen 400 mg test and reference formulations. The maximal inhibition of inflammation induced by methyl nicotinate was found to be 90 % and it was observed at 2.5 h after dosing of dexibuprofen 400 mg test and reference formulations. The maximal effect calculated from the effect-time study of dexibuprofen test and reference formulation was evaluated statistically using ANOVA and found that there was no statistical significance observed in the maximal effect for test and reference formulations.

The PK profile was compared with PD profile and found that the time to reach the maximal effect for the test and reference formulation from effect-time overlaps with that of time to achieve maximum plasma concentration from concentration-time study. The plasma concentration Vs time profile of dexibuprofen (test and reference formulations) in human paralleled the time Vs inhibition of MN induced local inflammation by dexibuprofen (Fig 2 & 3). When the pharmacological effects are seen immediately and directly related to the bioactive compound concentration, a PD model such as a linear or a sigmoid E_{max} model can be applied to characterize the relationship between bioactive compound concentration and effect. [20] A plot of percent inhibition of MN induced inflammation by dexibuprofen Vs the concentration of dexibuprofen was developed using simple linear and logarithmic linear regression (Fig 4-7). It was found that the plot of logarithmic linear correlation gave better regression co-efficient compare to the simple linear correlation. Thus a simple log linear PK-PD model for dexibuprofen was developed.

CONCLUSION

A non-invasive method on LDF has been successfully applied for the pharmacodynamic study of dexibuprofen in healthy human volunteers. The PK and PD profiles of dexibuprofen were compared. It was found that there exists a simple log linear PK-PD model was applied for the dexibuprofen. This model can be used as a predictive tool for PK studies and also in predicting the time-course of therapeutic effects of dexibuprofen and related compounds for oral dosing strategies under a new set of experimental conditions like multiple dosing, increasing or decreasing doses of dexibuprofen and related compounds.

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