Evaluation of Pharmacokinetic Studies and Analgesic Activity of Sustained Release Epidural Injection of Analgesic Drug on Wistar Rat

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Abstract: The purpose of this work was to carry out the pharmacokinetic studies and analgesic activity of the developed sustained release in situ gel forming epidural injection of an analgesic drug Diclofenac sodium for the treatment of low back pain. The formulation was prepared using pluronic F 127 as a thermosensitive polymer and HPMC K100 M, HPMC K4 M as release retardant copolymers that gelled at 37°C i.e. body temperature and showed a sustained release of 98.13% over a period of 3 days. In-vivo studies involved the pharmacokinetic and analgesic activity studies of the SR formulation and its comparison with marketed immediate release injection. The Cmax of sustained release and immediate release formulation were found to be 13.21µg/ml and 10.14µg/ml respectively, AUC0-∞ was found to be 933.53µg.hr/ml and 52.47µg.hr/ml respectively. These parameters achieved by the sustained release formulation was found be longer than that of immediate release formulation. The analgesic activity was determined by studying writhing reflex that showed significant decrease in pain as compared with immediate release injection which required frequent administration. Thus, the formulation showed the effectiveness of its use in animals as a sustained drug delivery system that could effectively be employed in clinical studies.

***Index Terms*- Pharmacokinetic Studies, Analgesic Activity, In-Situ Forming Gel, Epidural Injection.**

I. INTRODUCTION

Diclofenac is non-steroidal anti-inflammatory analgesic drug (NSAID) with potent cyclo-oxygenase inhibition activity indicated in the relief of all grades of pain and inflammation associated with a wide range of conditions, including low back pain, arthritic conditions, acute musculo-skeletal disorders and other painful conditions resulting from trauma. The dosage of diclofenac injection is 25 mg per ml, not to exceed 150 mg per day.

Diclofenac Sodium is well-absorbed after oral administration with extensive hepatic metabolism. It exhibits a terminal half life of 1–2 hr. Cmax is reached at approximately 4 hours. Diclofenac is associated with serious dose-dependent gastrointestinal, cardiovascular, and renal adverse effects. The gastrointestinal toxicity of Diclofenac through oral administration can be avoided by injecting drug directly at the target site. The in situ forming gel formulation is developed to provide prolong drug release, increase residence time and bioavailability, reduction in frequent dosing and patient compliance.[1,2,3,4]

The analgesic activity of analgesic or anti-inflammatory agents is evaluated using writhing reflex induced by acetic acid. Writhing reflex is described as stretching, extension of the hind legs and abdominal contractions. Any writhing reflex is regarded as a positive reaction.[5,6]

The present work was focused on pharmacokinetic evaluation and analgesic activity of the developed sustained release insitu gel forming epidural injection of an analgesic drug Diclofenac sodium for the treatment of low back pain over a 3 days period in order to reduce the frequency of administration and to improve patient compliance.

II. MATERIALS AND METHODS

1. Materials:

Diclofenac Sodium was obtained as a gift sample from Emcure Pharmaceuticals Pune. PluronicF127 was provided by Ana lab fine chemical, Mumbai. Hydroxy propyl methyl cellulose K100M, K4M (HPMC K100M, HPMC K4M) was provided by Chemica-biochemic-reagents, Otto chemie,Pvt.Ltd. Methanol, Acetonitrile HPLC grade, Diethyl ether was provided by SD fine chem., Mumbai.

2. Methods:

2.1 Preparation of in situ gel forming formulation

The in situ gel forming formulation was prepared by slow addition and dissolution of thermoresponsive polymer i. e. Pluronic F127, copolymers and tonicity agent with continuous stirring in cold water followed by addition of drug to form a uniform drug solution. The formulation prepared was filled aseptically in 3ml transparent glass ampoules and sterilized by autoclaving at 121C at 15 psi for 20 min and stored at refrigerator condition.[7,8,9]

2.2 In vivo pharmacokinetic studies:

All experimental procedures and protocols used in this research have been evaluated and approved by the Institutional Animal Ethical Committee (IAEC) of AISSMS College of Pharmacy, Pune, established by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (approval no.CPCSEA/IAEC/PT-05/02-2K19. During all the experiments, ethical guidelines were strictly followed. Anaesthetic ether inhalation was the process of killing the animals followed by cervical dislocation.

Twelve male Wistar rats were used for in vivo examinations; weighing 200-250 g. In accordance with the CPCSEA rules, animals were housed and treated.

2.3 Development of Bio analytical method in plasma:

Bio analytical method was developed and validated for quantitative estimation of Diclofenac sodium in plasma. The Chromatographic parameters selected as follows:

Column: Grace C_{18}column (250*4.6 nm, 5µ) protected with guard column

Mobile phase: Acetonitrile: Deionised Water: Methanol (20:20:60).

Flow-rate: 1ml/ min.

Elution: Isocratic

Injection volume: 20µl

Detector: UV-Visible SPD 20A

Detection wavelength: 254 nm.

2.4 Preparation of calibration standards of spiked plasma:

Stock solution of DS (200 mg/mL) was prepared in methanolic solution. A series of standard solutions at concentrations ranging from 5–25 µg/mL were further diluted by the mobile phase to obtain different working solutions. Spiked plasma was prepared by adding 0.5 mL blank plasma to 50 µL of Diclofenac sodium standard solutions to obtain spiked plasma solutions ranging from 5 to 25 µg/mL. A 200µL of 2 M hydrochloric acid was added, followed by vortexed for 30 sec; subsequently, 1 mL acetonitrile was added for protein precipitation, vortexed for 2 min and centrifuged at 9000 rpm for 10 min, and 20 µL aliquot of the supernatant was injected into the HPLC column for analysis.[3,10]

2.5 Comparison of Plasma concentration profile of Diclofenac Sodium Sustained release formulation and Immediate release formulation in wistar rats:

The pharmacokinetic studies were performed to determine the C_{max}, T_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, K_{el} in order to predict the behaviour of formulation i.e. in situ gel forming injection in the animal model and its a

comparison with the immediate release injection.. The rats were divided in 4 study groups (n=3) and administered with the injections as shown in Table 1 as follows. The blood samples were collected by retro-orbital method at 1, 24, 48 and 72 hrs after injections for sustained release and at 1, 2, 3, 4 and 5 hrs for immediate release in EDTA tubes and stored. The blood plasma was separated using centrifuge and evaluated for Diclofenac sodium content by HPLC. AUC from time zero to the last day of sampling (AUC $_{0\rightarrow t}$) was calculated by the linear trapezoidal rule, maximum blood concentration (C_{max}), time required to obtain the maximum concentration (T_{max}) and the terminal phase elimination rate constant (K_{el}) was estimated from the terminal phase of the plasma concentration-time curve using log linear regression. AUC $_{t\rightarrow\infty}$ was calculated as AUC $_{t\rightarrow\infty}$ = Concentration of the drug obtained at last time interval/ $K_{ele.}$ Plasma drug concentrations in µg/ml were evaluated and graphs were plotted against time in hours. [11,12,13]

Table 1: Animal groups for in vivo studies

Study group	No. of rats in each group	Formulation
Group 1	6	Positive Control (Normal Saline)
Group 2	6	SR Formulation (Test formulation)
Group 3	6	Immediate release Formulation (Marketed)

2.6 Writhing reflex

The writhing reflex induced by acetic acid was adopted for the evaluation of analgesic activity for 3 days. Wistar rats weighing between 200-250 g were used for analgesic activity studies; six wistar rats were kept in each group. The 6 rats were treated with normal saline 10ml / kg as positive control. The six rats were injected single dose of immediate release marketed formulation of diclofenac (20mg / kg) intravenously on first day. Simultaneously, Six rats were injected single dose of sustained release formulation of diclofenac (20mg / kg) via epidural administration on first day. After 30 minutes, all animals were injected with 1% acetic acid (10ml / kg) solution intraperitoneally. This study was continued upto 3days and animals were injected 1% acetic acid (10ml / kg) solution intraperitoneally on each day. The abdominal contractions were counted manually as writhing reflex.[14,15,16]

Table 2: Animal groups for Writhing Reflex = 18 Animals

Group	Treatment and Dose of drug	Number of Animals
Positive control	Acetic acid (1%)	06
Immediate release Formulation (Marketed)	Acetic acid (1%) + 20mg/kg	06
Sustained release Formulation	Acetic acid (1%) + 20mg/kg	06

RESULTS AND DISCUSSIONS

1. In vivo pharmacokinetic studies

1.1 Calibration standards of spiked plasma

Table 3: Calibration curve of Diclofenac Sodium in Plasma

Conc (µg/ml)	AUC
5	154124
10	250150
15	474907
20	778152
25	1012302

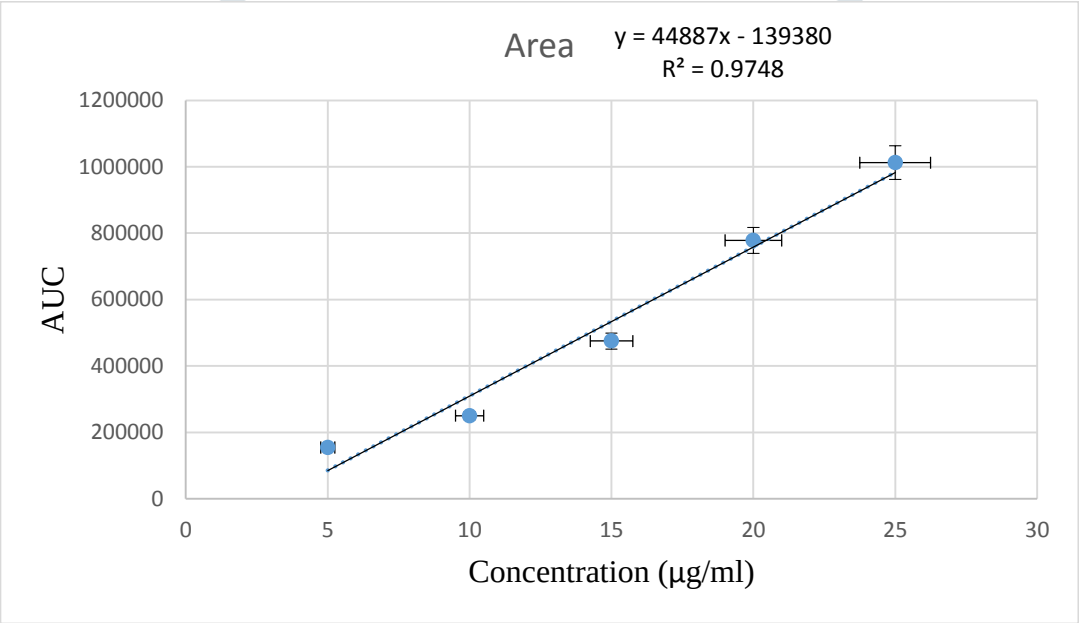


Fig 1.Calibration curve of Diclofenac Sodium in Plasma

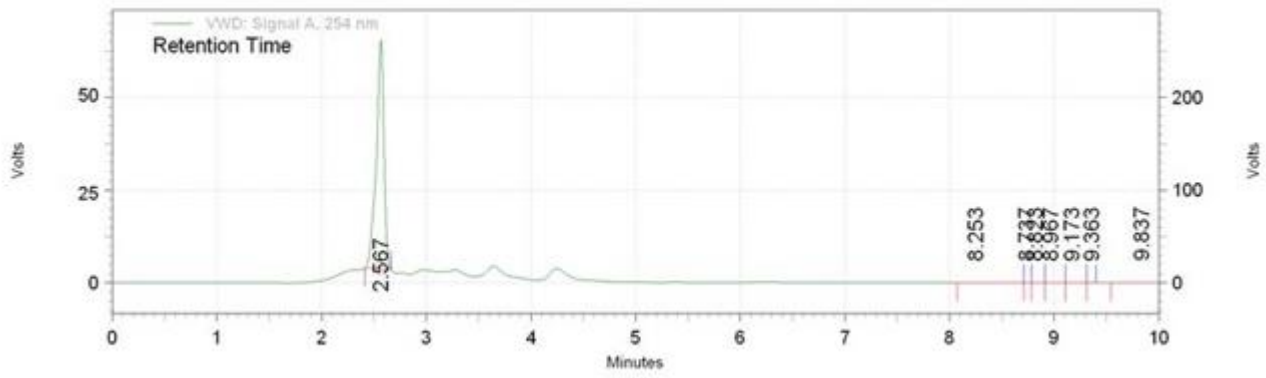


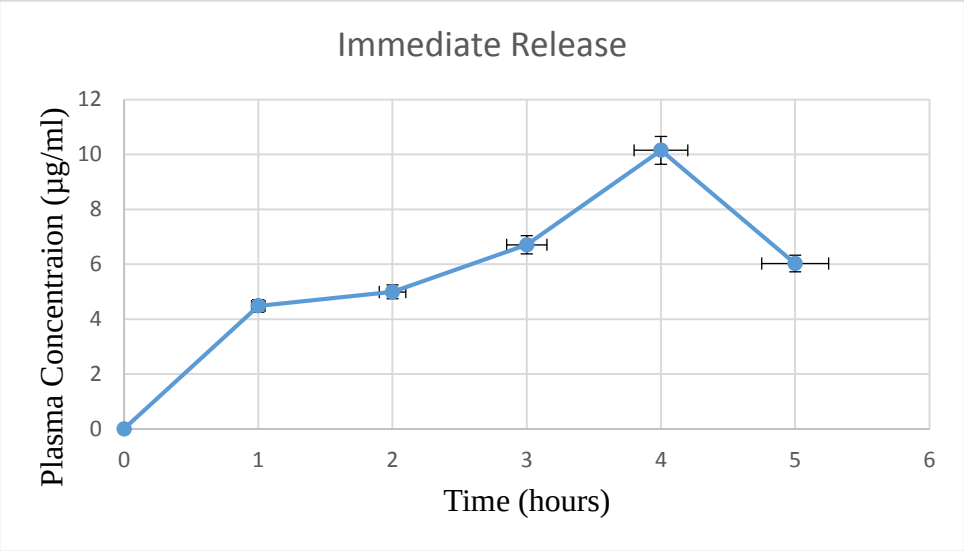
Fig 2. Chromatograph of Diclofenac Sodium in Plasma

Above Fig 1. shows calibration curve and regression coefficient R2 was found to be 0.974. In the chromatograph above, Fig 2.shows retention time 2.56 min at flow rate 1ml / min and run time 5 min at 254

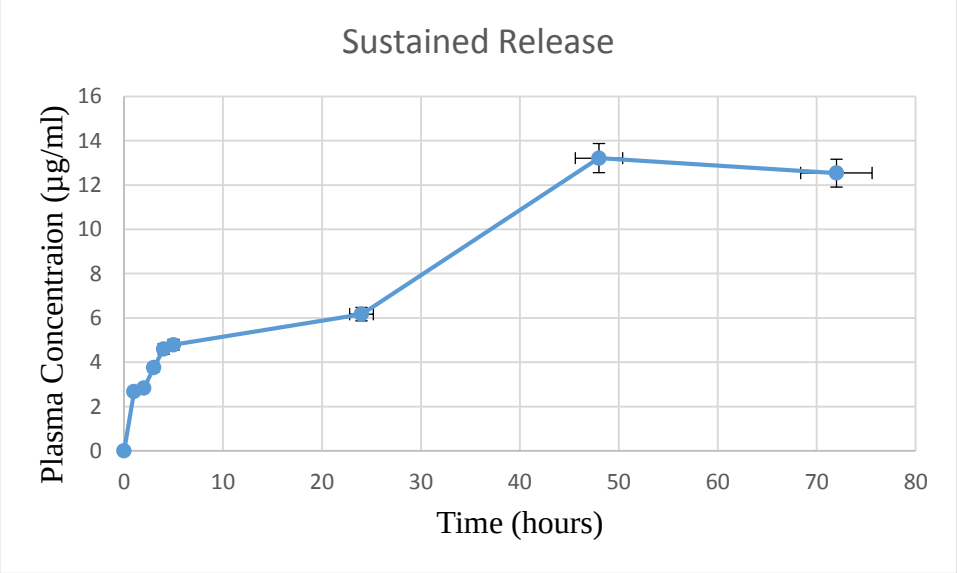
nm. A good linear relationship between the concentration of Diclofenac Sodium 5-25µg / ml was observed when the concentrations of Diclofenac Sodium and its respective peak areas were subjected to regression analysis using the least square method. The Diclofenac Sodium concentration regression equation above its peak area was found to be y= 44887x-13938.

1.2 Comparison of Plasma concentration profile of Diclofenac Sodium Sustained release formulation and Immediate release formulation in rats:

The pharmacokinetic parameters were studied and compared for the formulation of sustained release and immediate release injection. After administration of sustained release formulation and immediate release, the Diclofenac Sodium in plasma concentration Vs time profile is shown in fig 3.(a)and (b).



a) Immediate Release



b) Sustained Release

Fig 3.(a)and (b): Plasma drug concentration Vs time profile in rats.

Table 4: Mean Pharmacokinetic parameters

Parameters	Sustained release (SR) Formulation	Immediate Release (IR) Formulation
C_{max}	13.21 µg/ml	10.14 µg/ml
T_{max}	48 hrs	4 hrs
K_{ele}	0.01377	0.18
$t_{1/2}$	21.86 hrs	1.67 hrs
$AUC_{0\text{-}72hr}$ (SR) $AUC_{0\text{-}72min}$ (IR)	22.77 µg.hr/ml	19 µg.hr/ml
$AUC_{0\text{-}\infty}$	933.53 µg.hr/ml	52.47 µg.hr/ml

*All values are expressed as mean ± SD, n = 3

the above figures and table shows that the blood plasma concentration profile was achieved by sustained drug release in 72 hours. The peak plasma concentration (Cmax), Tmax and AUC of sustained release formulation is increased after epidural administration which may be attributed to the slow diffusion of drug from the polymeric matrix.

1.3 Writhing reflex

The writhing reflex was performed to show the analgesic effect of Diclofenac sodium upon epidural administration. The number of writhing reflex i.e. abdominal contractions is given in table no.5. The positive control group showed significantly higher number of writhing reflex values. The number of writhing reflex of sustained release formulation were lower than marketed immediate release formulation showing the analgesic effect i.e. pain reduction up to 3 days. The writhing reflex of immediate release formulation was lowered on first day but increased on 2nd day. The immediate release formulation requires repeated administration of injection which can be overcome by sustained release epidural injection. From this test results, we can also predict that the tolerance to Diclofenac sodium did not develop via epidural administration.

Table 5: Effects of Writhing reflex

Group	Writhing reflex			
	Day1	Day2	Day3	SD
Positive control	24	23	24	0.57
Immediate release Formulation (Marketed)	9	21	22	7.23
Sustained release Formulation	11	9	7	2

*All values are expressed as mean ± SD, n = 3

CONCLUSION:

The pharmacokinetic studies and analgesic activity of the developed sustained release formulation shows satisfactory and significant results when compared with immediate release formulation. We can consider that in situ gel forming sustained release injectable formulation is a promising new alternative for increasing the epidural availability of Diclofenac sodium after administration at the target site.

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DECLARATION OF INTEREST

We have no personal relationships with other people or organizations that could inappropriately influence the presented work or any affiliation with any organization with a financial interest, direct or indirect, in the subject matter or materials discussed in the manuscript.

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