ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
July 2018 Vol.:12, Issue:4
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Pharmacokinetic *In Vivo* Evaluation of In-Situ Gel Forming Injectable Drug Delivery System of Analgesic Drug

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An official Publication of Human Journals
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HUMAN

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Submission: 23 June 2018
Accepted: 29 June 2018
Published: 30 July 2018

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Pluronic F127, pharmacokinetics, histopathology, radio imaging, in-situ, intraarticular.

ABSTRACT

The aim of this work was to study the *In vivo* pharmacokinetic analysis of the developed sustained release intraarticular injection of an analgesic drug Tramadol HCl for the treatment of knee pain. The formulation was developed using pluronic F 127 as a thermosensitive gelling agent and HPMC K100M, HPMC K4M as a release retardant polymers in order to achieve a prolonged release over a period of 5 days so as to reduce the frequency of administration and to improve patient compliance and target the dosage form at the knee joint. *In vivo* pharmacokinetic analysis involved administration of sustained release formulation in rats at the knee joint and analysis for various pharmacokinetic parameters of the SR formulation and its comparison with available immediate release injection, *in vivo* gel formation studies, histopathological and radio imaging studies. The *in vitro* drug release was found to be 95.40% in phosphate buffer pH 7.4 at 32°C over a period of 5 days. Pharmacokinetic studies shows the C_{max} of sustained release formulation and immediate release as 491.74µg/ml and 127.98µg/ml respectively. The $AUC_{0-\infty}$of sustained release formulation and immediate release was found to be 12579.10µg.hr/ml and 1699.40µg.hr/ml respectively. The *in vivo* gel formation studies confirms the immediate formation of in situ gel and the histological studies shows the formulation to be biocompatible with no abnormality detected at the site of injection. The X-ray studies confirmed the formation of gel in knee joint and maintaining its stability over a period of 120hrs**.** Thus, the formulation demonstrated the feasibility of its use in animals as a sustained drug delivery system which could be potentially applied in clinical studies.

INTRODUCTION:

Tramadol HCl is a synthetic centrally acting amino cyclohexyl analgesic that acts as an opioid agonist with selectivity for μ receptor have demonstrated that this drug is an effective agent for moderate to severe pain in case of arthritis pain- osteoarthritis pain, rheumatoid arthritis, diabetic neuropathy, postoperative neuralgia and Chemotherapy pain *etc*. Most of the water soluble drug containing formulations release the drug at a faster rate and likely to produce toxic concentrations of the drug on oral administration. Tramadol HCl is a highly water soluble and permeable drug belonging to BCS class I. This drug has good oral bioavailability. Moreover, its elimination half-life is 5 to 7 hrs and the usual oral dosage regimen is 50 to 100 mg every 4 to 6 hours with a maximum dosage of 400 mg/day. The patient non-compliance due to frequent dosing, particularly in long-term use, can be avoided through development of the sustained release system for tramadol hydrochloride. So, in order to retard the drug release, in-situ gel forming gel approach was selected.[1, 2]

The present work was focused on pharmacokinetic evaluation of the in-situ gel forming formulation of Tramadol HCl which contains pluronicas a gel forming polymer releasing the drug over a 5 days period in order to reduce the frequency of administration and to improve patient compliance.

MATERIALS AND METHODS

Materials:

Tramadol HCL was obtained as a gift sample from Emcure Pharmaceuticals Pune. PluronicF127 was provided by Ana lab fine chemical, Mumbai. Hydroxypropyl methyl celluloseK100M, K4M (HPMCK 100M, HPMC K4M) was provided by Chemica-biochemic-reagents, Otto Chemie, Pvt. Ltd. Methanol, Acetonitrile HPLC grade was provided by SD fine chem., Mumbai, carrageenan was provided by Loba Chemie Pvt. Ltd. Mumbai and formalin was provided by New Neeta Chemicals, Pune.

Methods:

The formulation was prepared by using cold method. Drug was dissolved in water followed by addition of thermosensitive polymer Pluronic F127, stirred and kept in refrigerator at 4°c for 24 hrs to dissolve completely and form a clear solution. The other excipients like

copolymer HPMC K100M, HPMC K4M and tonicity adjusting agent NaCl were added to this solution and stirred continuously to dissolve to give complete clear viscous solution which was filled aseptically in transparent glass ampoules 2ml and sterilized by autoclaving at 121°C at 15 psi for 20 min and stored at refrigerator condition.[3, 4, 5]

***In vivo* pharmacokinetic studies:**

In vivo examinations were accomplished using 12 male Wistar rats; weighing 200-250 g. Animals were housed and handled according to the CPCSEA guidelines.

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of AISSMS College of Pharmacy, Pune, constituted under 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-18/01-2K17. Ethical guidelines were strictly followed during all the experiments. The process of killing the animals was anesthetic ether inhalation followed by cervical dislocation.

Development of Bioanalytical method in plasma:

A HPLC method for quantitative estimation of Tramadol Hydrochloride in plasma was developed and validated as:

Chromatographic parameters selected:

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

Mobile phase: Acetonitrile: Methanol (50:50) with addition of 0.1% Triethylamine.

Flow-rate: 1ml/ min.

Elution: Isocratic

Injection volume: 20µl

Detector: UV-Visible SPD 20A

Detection wavelength: 271 nm.

Preparation of calibration standards of spiked plasma:

Spiked plasma was prepared by taking 0.25ml plasma, 0.25ml stock solution Tramadol Hydrochloride and 0.50ml of Acetonitrile. Appropriate quantities of this stock solution were added into EDTA tube to obtain spiked plasma solutions in the range of 40-200µg/ml. The contents of the tubes were vortexed for 5 min and then it was centrifuged for 15 minutes at 3000 rpm. After centrifugation, the supernatant was separated and evaporated to dryness. The dried residue was diluted to 10ml with mobile phase. 20µl of this solution was injected into the column for analysis.[6, 7]

Comparison of Plasma concentration profile of Tramadol HCl Sustained release formulation and Immediate release formulation in rats:

The pharmacokinetic studies were conducted 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 Gelatin matrix powder in the animal model. *In vivo* examinations were accomplished using 12 male Wistar rats. Animals were housed and handled according to the CPCSEA guidelines. The process of killing the animals was performed using anaesthetic overdose method. *In vivo* experimental protocol was approved by the IAEC. A HPLC method for quantitative estimation of drug in plasma was developed and validated.

12 male rats, weighing 200–250 g, were divided in 4 study groups (n=3) and administered with the injection as shown in Table 1 as follows. The blood samples were collected from tail vein at 1, 24, 48, 72, 96 and 120 hr after injections for sustained release and at 15, 30, 45, 60, 90, 120 mins for immediate release in EDTA tubes and stored. The blood plasma was separated and assayed for Tramadol HCl 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/ $K_{el.}$ Plasma drug concentration in µg/ml were evaluated and graphs were plotted against time in hours.[6, 7,12]

Table 1: Animal groups for *in vivo* studies

Animal Groups	Administration
Group A (Control)	0.05 ml of SWFI given intra-articularly.
Group B (positive control)	0.05 ml of placebo formulation given intra-articularly.
Group C (Standard)	0.05 ml of IR formulation given intra-articularly at a dose of 0.5mg / 250gm of rat
Group D (Test)	0.05 ml of sustain release Tramadol Hcl formulation given intra-articularly at a dose of 2.5 mg/ 250gm of rat

In situ gel formation:

Group B animals (Test) was given 0.05 ml of sustained release Tramadol Hcl formulation intra-articularly in the knee joint region. The formation of stiffer gel was evaluated immediately after giving injection by carefully cutting the skin surrounding the site of injection to confirm the in situ gel formation of the Tramadol HCl formulation.[8,]

Histopathological evaluation of knee joint tissue:

Formulation treated, placebo treated and control rats were killed at a scheduled time and the knee joint area was cut carefully at the injection site, together with the surrounding tissue. To evaluate the biocompatibility of the in situ gel forming on the injection site, the surrounding tissue was harvested for histopathological analysis. The harvested tissues were preserved in 10% formalin. Tissue processing was done to dehydrate in ascending grades of alcohol, clearing in xylene and embedded in paraffin wax. Sections were cut to 3µ thickness with the Rotary microtome and stained with standard Hematoxylin and Eosin (H & E) solution. The stained sections were examined under microscope for tissue necrosis, inflammation (inflammation was induced in the left knee joint by intra-articular injections of 0.02 mL of 1% carrageenan in the B, C, and D group) or any changes in cellular arrangement. Further, it was sent to Chaitanya laboratory Pune. Where, following procedure and evaluation parameters was studied.[8, 9, 10.11]

These tissues were trimmed and processed routinely. Prior to processing bones were decalcified using Gooding and Stewart solution. Tissue processing was done to dehydrate in ascending grades of alcohol, clearing in xylene and embedded in paraffin wax. Paraffin wax

embedded tissue blocks were sections at 4-5 µm thickness with the Rotary Microtome. Slides of bones were stained with Hematoxylin& Eosin (H & E) stain. The prepared slides were examined under microscope by Pathologist to note histopathological lesions if any.

Following parameters were evaluated:

- Synovial lining cell layer
- Synovial hyperplasia
- Synovial vascularity
- Infiltration of inflammatory cells
- Pannus formation
- Cartilage erosion
- Bone erosion

Radiographic imaging Studies:

Radiographic imaging study were carried out to confirm the *in vivo* gel formation at the site of injection and its stability over 120hrs. The evaluation of dosage form in animal model renders support the *in vitro* studies. Wistar rats were selected as animal model and formulation injection was administered intra-articularly at the knee joint region of rats. The behaviour of Tramadol HCl formulation in rats was observed using a radiographic imaging technique and compared with the control rat. At different time intervals of 24hr, 72 hr, 96 hr and 120 hr X-ray images were taken under the supervision of a radiologist, to follow the nature, movement and location of in situ gel formed.[13, 14, 15]

RESULTS AND DISCUSSION

In vivo bioavailability studies:

Table 2: Calibration curve of Tramadol HCl in Plasma

Conc (µg/ml)	AUC
40	8226899
80	10579853
120	14263983
160	16415868
200	18927642

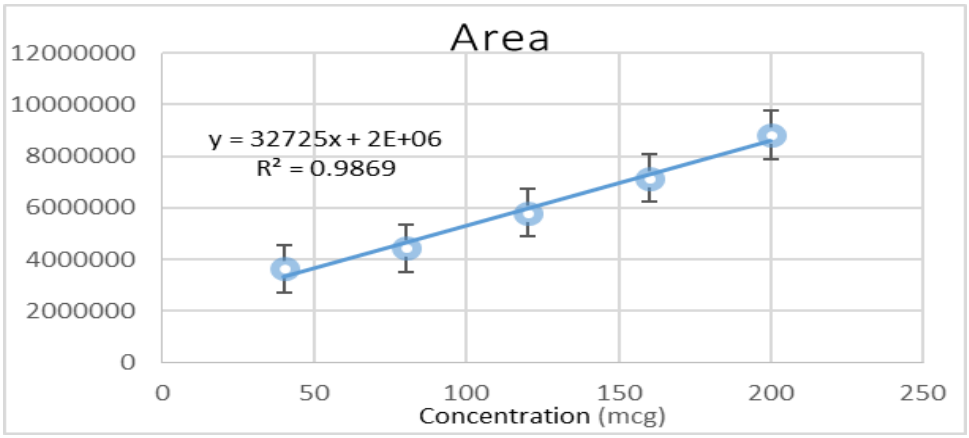


Fig 1. Calibration curve of Tramadol HCl in Plasma

Above fig1. shows calibration curve and R^2 value found to be 0.9869.

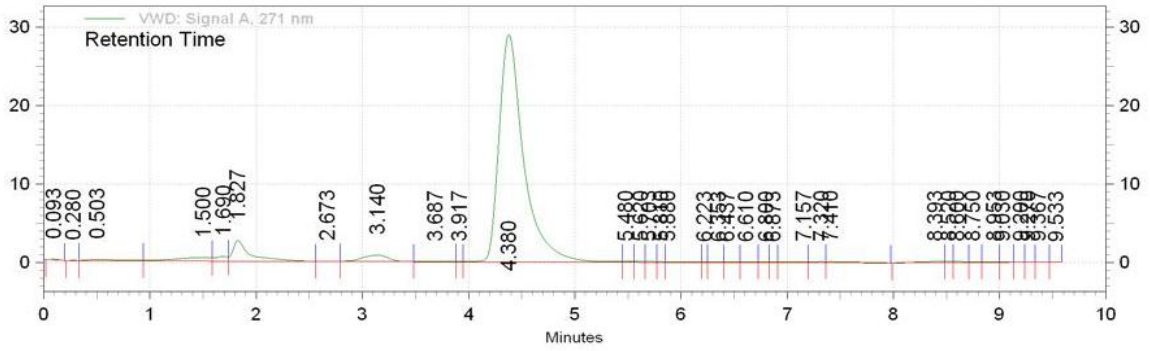


Fig 2. Chromatograph of Tramadol HCl in Plasma

In above chromatograph Fig 2. shows retention time 4.38 min with flow rate 1ml/min and run time 10 min at 271 nm. When the concentrations of Tramadol HCl and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship [r^2=0.993] was observed between the concentration of Tramadol HCl 40-200µg/ml. The regression equation of Tramadol HCl concentration over its peak area was found to be Y= 68094x + 6E+06.

Comparison of Plasma concentration profile of Tramadol HCl Sustained release formulation and immediate release formulation in rats:

The mean pharmacokinetic parameters for the sustain release test formulation and immediate release injection were studied and compared. The Tramadol Hcl plasma concentration Vs time curve after administration of sustained release formulation and immediate release is shown in fig 3.

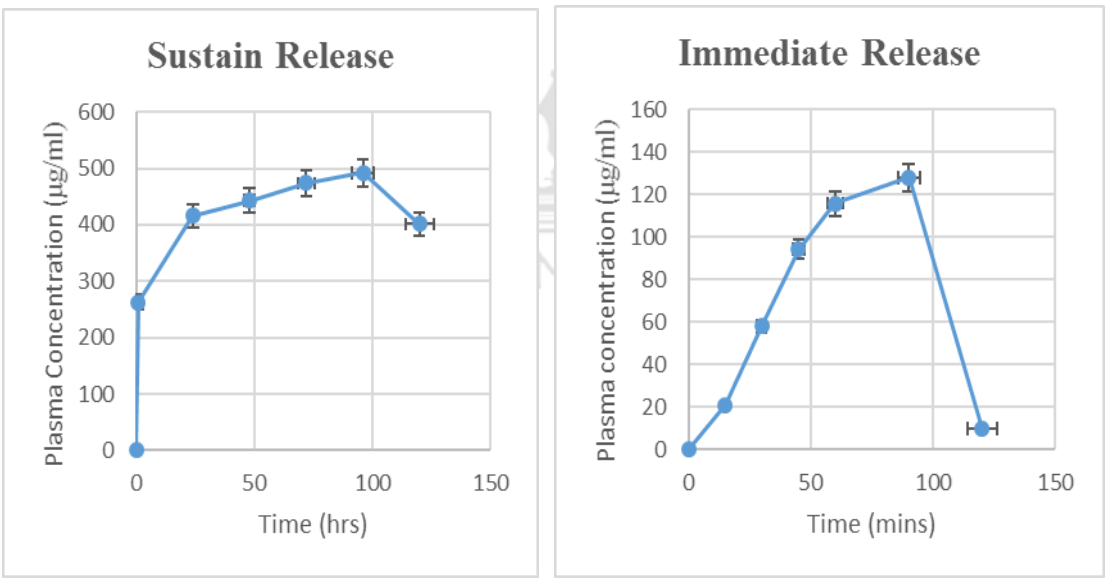


Fig 3. Plasma drug concentration Vs time profile in rats. Mean ± SD (n=3)

Table 3: Mean Pharmacokinetic parameters

Parameters	Sustained release (SR) Formulation	Immediate Release (IR) Formulation
C_{max}	491.74± 8.35 µg/ml	127.98± 3.18 µg/ml
C_{min}	262.27± 4.86 µg/ml	20.92 ± 2.64 µg/ml
T_{max}	96± 0.8 hrs	90± 0.5 mins
K_{ele}	4.55± 0.28	1.61± 0.05
$AUC_{0\text{-}120hr}$ (SR) $AUC_{0\text{-}120min}$ (IR)	12455.09 ±14.8 µg.hr/ml	1620.11± 11.6 µg.hr/ml
$AUC_{0\text{-}\infty}$	12579.10 ±15.2 µg.hr/ml	1699.40 ±13.4 µg.hr/ml

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

It was seen from the above figures and table that the blood plasma concentration profile of the drug after administration of formulation showed sustained drug release in the systemic circulation for 120 hours. The increase in peak plasma concentration (Cmax) for Tramadol HCl sustain release formulation in plasma after intra-articular administration, time (Tmax) to reach Cmax and AUC as compared to immediate release injection may be attributed to the slow diffusion of the drug from the polymeric matrix.

***In vivo* gel Formation:**

A stiffer gel clot was observed immediately after injection.

Fig 4: *In vivo* gel formation in rat

In vivo formation of gel immediately after giving the injection as shown in fig 4 occurred due to the optimized concentration of thermosensitive polymer (pluronic F127) which formed the gel at the physiological conditions within the knee joint at 32°c.

Histopathological analysis:

Histopathological reports revealed the following outcomes.

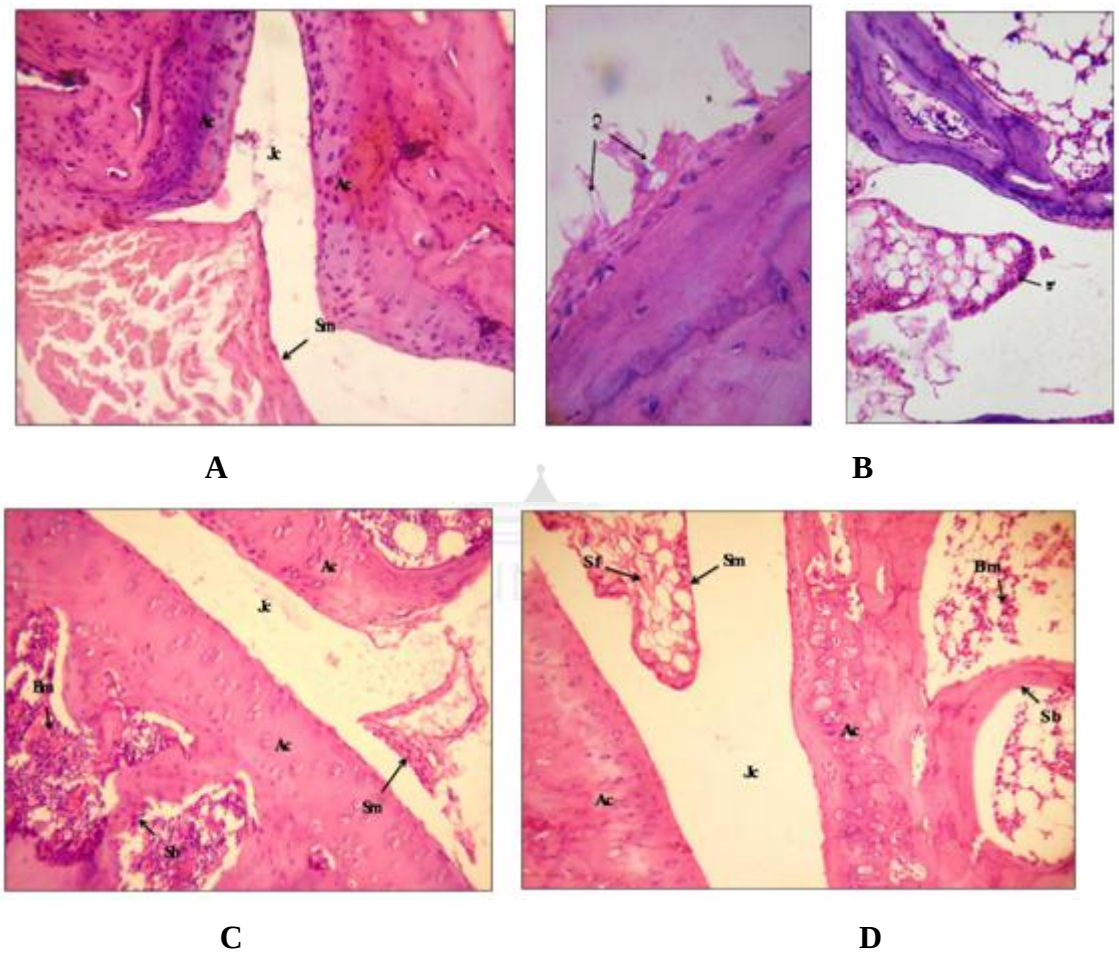


Fig 5: Optical micrographs of intra-articular tissue A) Control B) positive control C) Standard D) Test

Rats of control group did not reveal any lesion of pathological significance at knee joint. Treatment of Carrageenan induces multifocal mild inflammation at synovial area and focal mild cartilage erosion at knee joint suggestive of arthritic lesions. Treatment of Standard and test drug in Carrageenan induced inflammation reduces the adverse effect at knee joint. Severity of the observed lesions were recorded as NAD =No Abnormality Detected, Minimal changes (+1), Mild changes (+2), Moderate changes (+3), Severe changes (+4) and distribution was recorded as focal, multifocal and diffuse.

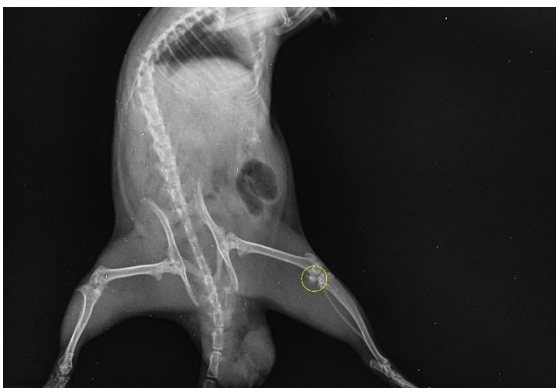
Table 4: Observations for histopathology studies

Sample	Observations
Control	Showing normal histology, Articular cartilage(Ac), Synovial membrane (Sm), Spongy Bone(Sb), Joint cavity (Jc) {H& E, 100X}
Positive control	Showing infiltration of inflammatory cells in synovial area(In){H& E, 100X} and cartilage erosions (Ce) {H& E, 400X}
Standard	Showing normal histology, Articular cartilage(Ac), Synovial membrane (Sm), Spongy Bone(Sb), Bone Marrow cells(Bm), Joint cavity (Jc){H& E, 100X}
Test	Showing normal histology, Articular cartilage (Ac), Synovial membrane (Sm), Synovial folds(Sm), Spongy Bone (Sb), Bone Marrow cells (Bm), Joint cavity (Jc){H& E, 100X} i.e no inflammation, necrosis and found normal when compared to control tissues. The biocompatibility could be attributed to the use of biocompatible polymers other excipients within their approved limits.

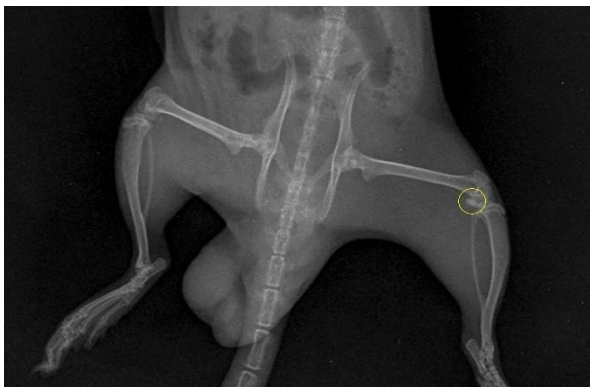
Radioimaging studies:

To strengthen the *in vitro* release study findings, *in vivo* radiographic imaging study was carried out. It is shown from the X-ray studies that the gel formation occurred in knee joint region at 24hr Fig 6a, it starts expanding and releasing the drug in sustained manner after 48 hrs,72hrs (Fig 6b, 6c) and at 120hrs (fig 6d) it becomes very tiny in size which indicates that the drug release is sustained for 5 days.

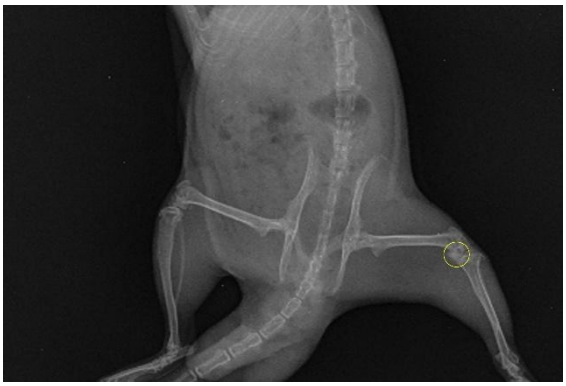
Figure 6) X Ray images of rats taken at various time intervals:



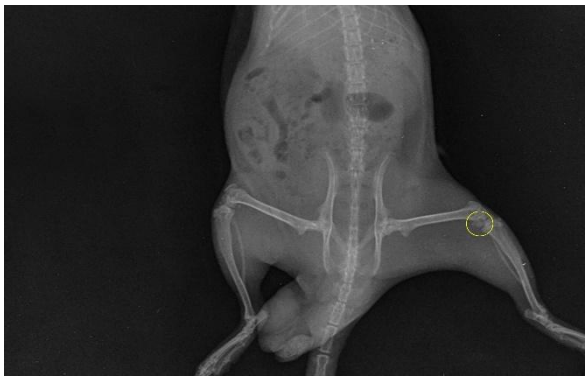
6a) Gel depot in knee joint region at 24hr



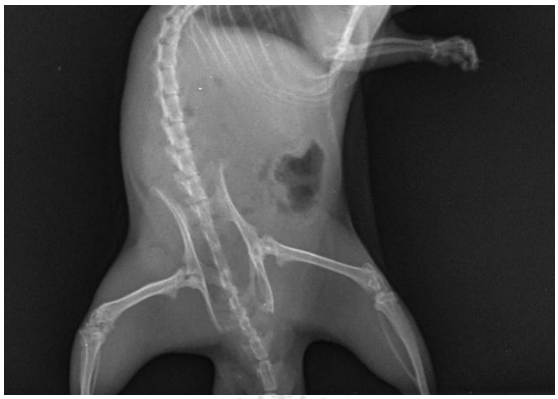
6b) Gel depot in knee joint region at 72hr



6c) Gel depot in knee joint region at 96hr



6d) Gel depot in knee joint region at 120hr



6e) control

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CONCLUSION:

In vivo pharmacokinetic studies exhibited satisfactory results in rats with a steady release profile over a period of 5 days. The X-ray studies confirmed the *in-vivo* gel formation and is stability for 120 hrs. Histopathology study proved that the formulation was non-toxic and biocompatible. Therefore; it can be concluded that in-situ gel forming formulation of Tramadol Hydrochloride could be rationally proceeded to clinical studies by virtue of their promising outcomes in animals.

ACKNOWLEDGMENT:

The authors are thankful to AISSMS College of Pharmacy for providing the facilities to carry out the study.

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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