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Research Article

Comparative Analgesic Activity of Achnil in the Rat Formalin Test

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ABSTRACT

Background: NSAIDs are the mainstays for the management of a myriad of painful conditions. Diclofenac has been reported to be efficacious in several conditions. Aceclofenac is a relatively new congener of diclofenac and is reported to have better tolerability. Lornoxicam is another NSAID demonstrated to have good analgesic efficacy. Very few reports are available which have compared the efficacy of different analgesic formulations. This study aimed at evaluating the comparative analgesic potential of parenteral formulations, Voveran, Achnil and Flexilor, reported to contain diclofenac, aceclofenac and lornoxicam respectively.

Procedures: The rat formalin test was utilised to compare the formulations. Composite pain score and no. of licks were evaluated at 15 mins, 6 hr and 12 hr post-dosing.

Findings: Evaluation of these formulations in the rat formalin test at different time-points revealed that achnil was superior to voveran in demonstrating analgesic activity. Observations made for the Achnil treated group revelaed that Achnil was able to reduce the no. of paw licks and composite pain score at all the time points studied. This effect was not only superior to control animals but also than that of the Voveran or Flexilor treated animals. The effects of Voveran and Flexilor reduced to about 52% and 35% repsectively at 6 hrs and results recorded at 12 hrs showed that the effect of Voveran and Flexilor were not significantly different from control.

Conclusions: Duration of action for Achnil injection is better and is more suited for management of conditions involving longer pain durations as compared to Voveran or Flexilor.

Key words: Analgesic activity, NSAID, rat formalin test

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INTRODUCTION:

Inflammation and pain associated with arthritis and spondylitis require immediate attention to control distress. Pharmacological management of these conditions involve the use of NSAIDs administered orally or systemically (1). There is a wide range of drugs available in the category of NSAIDs that may be utilized for this purpose, however, the efficacy of diclofenac sodium is such that it is most commonly prescribed by clinicians to manage pain and inflammation associated with arthritis and ankylosing spondylitis. Lornoxicam, an NSAID of the oxicam class, has been reported have equal efficacy to that of analgesic efficacy as that of diclofenac in management of arthritic conditions (2). Aceclofenac belongs to the same category as of diclofenac and was identified in the early 1990s. It was found to be similar in efficacy but with better GI tolerability in comparison to diclofenac (3). It has also been indicated for the management of lower back pain associated with post-surgical fibrosis apart from rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (4).

Several animal models are available for the evaluation of peripherally acting analagesic agents like the NSAIDs. Of these, the writhing test which involves intraperitoneal injections of chemical irritiants to mimic visceral pain has been frequently used by researchers. Although this test is quite sensitive in determining the analgesic activity, it lacks predictive value. Another commonly used test is the rat formalin test which is known to have the highest predictive value with the determination of analgesic activity (5). The major advantage of this method lies with the fact that the pathophysiology of pain is segregated into the early and late phases of pain response. Injection of dilute formalin induces a characteristic licking, shaking and flinching response in the injected paw which can be scored and a composite pain score can be evaluated. The concentration of formalin used can be a determinant for the time course, behavioural response and the response towards the pharmacological intervention intended. The phases are dependent on a primary afferent drive that inititates and maintains nociceptive sensitization at the spinal level, whereas central modulation is known to participate in the events of the late phase (5, 6). Full-blown inflammation may not be observed but NSAIDs which are known to be active on pain associated with inflammation are active in the late phase. This model offers the advantage of evaluating those categories of drugs whose primary mechanism may be to alleviate pain of nociceptive or neuropathic origin. Evaluations are made in the late phase which last from 15-40 mins of formalin injection. Several peripherally acting analgesics as well as opioids are known to be active in this duration. This phase is reported to correspond with inflammatory pain-related conditions in humans. The objective of the present study was to compare the analgesic effect of injectable marketed pain-relief formulations namely Voveran, Achnil and Flexilor. This study involved evaluation of these formulation for a time-dependent analgesic effect of these formulations.

MATERIALS AND METHODS

Materials:

Achnil, Voveran and Flexilor injections were purchased locally. Other reagents (sodium chloride and formalin) used for the experiment were of analytical 'AR' grade. Solutions were filtered through a 0.22µm nylon syringe filter immediately before injection.

Animals:

Female Sprague-Dawley rats (10-12 weeks old) were used for the study. They were provided with pelleted chow and purified drinking water *ad libitum*. They were maintained on a 12hr light/dark cycle. All the procedures to be performed were approved by the Institutional Animal Ethics Committee and were followed in accordance with the CPCSEA Guidelines, Ministry of Environment and Forests, Govt. Of India. Animals were acclimatized in standard animal house environmental conditions for 5 days before the start of experiment. On last day of acclimatization, animals were randomized on the basis of body weights and were allocated to 4 groups for each time point (i.e. 15 min, 6 hr and 12 hr). The grouping and treatment allocation is shown in Table 1.

Group*	No. of Animals‡	Treatment	Dose (mg/kg)°	Route of Administration
G-1†	6	Control	----	Intramuscular
G-2	6	Achnil	15.43	
G-3	6	Voveran	7.75	
G-4	6	Flexilor	0.82	

Table 1: Grouping and treatment allocation of the test system

Such sets were allocated for each time-point (i.e. 15 min, 6hr and 12 hr), but treatment was administered at once. Total animals used for the study were 72.

† G-1 was treated with equivalent volume of saline.

‡ For each time point indicated.

°Equivalent human single administration dose, and the volume of administation was less than 0.5 ml/kg.

Study Design:

The animals were treated with normal saline or test formulations (Table 1) simultaneously so that a time-dependent analgesic effect may be evaluated. After treatment, 50 µl of sterile 5% formalin was injected subcutaneously into the dorsal surface of the left hind paw of the animal with the use of a 1 mL syringe with a 26-gauge needle (Formalin injections for each time point

$$Composite\ Pain\ Score = \frac{T_1 + 2T_2 + 3T_3}{time\ (secs)}$$

corresponded with the durations mentioned above). Accordingly, injections of formalin were administered to the entire set of animals at 15 min, 6 hr and 12 hr post-treatment. At each time point, a fresh set of animals (previously treated with the formulations/control) was utilised. Immediately after formalin injection, each animal was placed in a glass chamber for pain-scoring and the stop clock was started simultaneously. The total time (sec) spent in each scoring profile

given below was measured as an indicator of pain. Observation period was for 20-30 min after formalin injection to correspond with the late phase of pain. Pain scoring and rating was done as per method described in Table 2 (Abbott et al., 1995; Watson et al., 1997):

where T_1, T_2, and T_3 are the durations (in sec) spent in categories 1, 2 or 3 respectively during the early and late phases. Animals were observed for late phase (20 min post formalin injection) and the observations continued upto 30 mins. Total no. of paw licks in each phase were calculated by an individual blinded to the treatments.

Score	Evaluation
0	Both forepaws are placed on the floor, and weight is evenly distributed. During locomotion, there is no discernible favoring of the injected paw.
1	The injected paw rests lightly on the floor or on another part of the animal's body, and little or no weight is placed upon it. During locomotion, there is an obvious limp. This rating is also given to normal grooming, during which both paws are elevated and "washed", and to rearing where neither forepaw is in contact with the floor, although either may be in contact with the wall. Attempts to sleep by curling up with both paws off the floor are also given this rating.
2	The injected paw is elevated, and not in contact with any surface. The uninjected paw is placed firmly on the floor. Attempts to sleep by curling up with only the injected paw off the floor, even when it is tucked under the body, are given this rating.
3	The injected paw is licked, bitten, or shaken, while the uninjected paw is not. This behavior is quite distinct from normal grooming (rating l), although transitions between the two are common. Figure below illustrates the major pain rating categories for rats.

*Compostie pain score is a cumulative grading of all scores observed in each phase

Statistical analysis:

Data is presented as mean±SEM of 6 animals. All the available data was subjected to one-way analysis of variance followed by Bonferroni's *post-hoc* test. *, ** and *** indicate P<0.05, P<0.01 and P<0.001 respectively.

RESULTS

This study involved the estimation of analgesic activity of Achnil and its comparison with other marketed formulations. Effect of Achnil for analgesic activity was evaluated intermittently for a period of 12 hrs including different time points (15 min, 6 hr and 12 hr).

Effect on composite pain score:

Data related to time-dependent effect of the formulations on Composite Pain Score are given in Figures 1a - 1c.

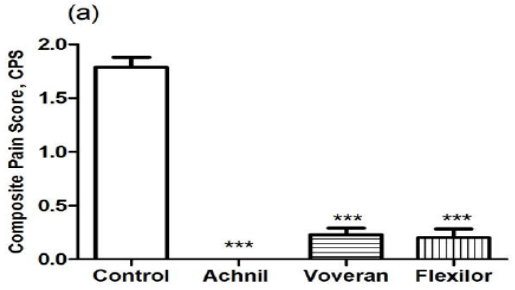

Figure 1a: Composite Pain Score (CPS) recorded in late phase of the rat formalin test (15 MIN). Data is presented as Mean ± SEM(n = 6). '***' indicates P<0.001 as compared to control group

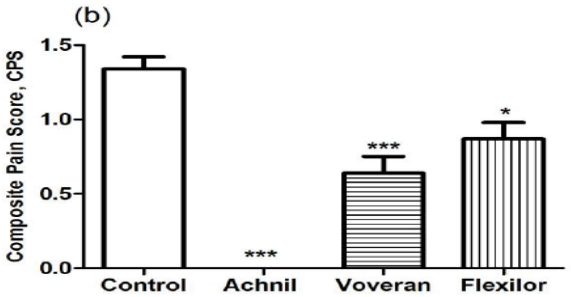

Figure 1b: Composite Pain Score (CPS) recorded in late phase of the rat formalin test (6 HR). Data is presented as Mean ± SEM (n = 6).'*' and '***' indicate P<0.05 and P<0.001 respectively as compared to control group

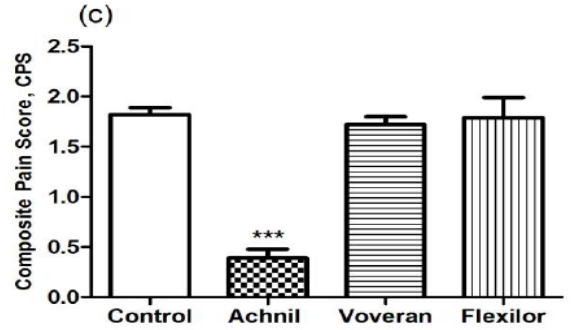

Figure 1c: Composite Pain Score (CPS) recorded in late phase of the rat formalin test (12 HR). Data is presented as Mean ± SEM (n = 6). '***' indicates P<0.001 as compared to control group

Observations made at 15 mins showed that composite pain score (CPS) was significantly lower in all the treated groups (Figure 1a). Achnil showed nearly 100% inhibition (P<0.001) of pain responses, Voveran showed comparable inhibition at 87.18% (P<0.001) and Flexilor showed nearly 89% inhibition (P<0.001) . At 6hrs, the analgesic effect of Achnil was comparable to the earlier time point suggesting a continued effect (P<0.001). However, the analgesic effect of Voveran decreased to about 52.10%, though it was significantly higher (P<0.001) as compared to control (Figure 1b). The results for Flexilor suggested that this formulation showed nearly 35% inhibition of

pain response (P<0.05), an effect found to be significantly better as compared to control. Recordings made at 12 hrs showed 78.44% analgesic activity for Achnil (P<0.001 as compared to control) but that of Voveran and Flexilor were not found to be significantly different from control readings (Figure 1c). This indicated a substantial decrease in the analgesic activity of Voveran and Flexilor, which can be accounted for by their respective elimination half-lives. This suggested that the analgesic activity of single-dose Achnil is retained upto 12 hrs. In case of Voveran and Flexilor, the analgesic activity at 12 hrs may be considered negligible and were not found to be significantly different as compared to control.

Effect on number of paw licks/bites:

Data for no. of paw licks are shown in Figures 2a - 2c.

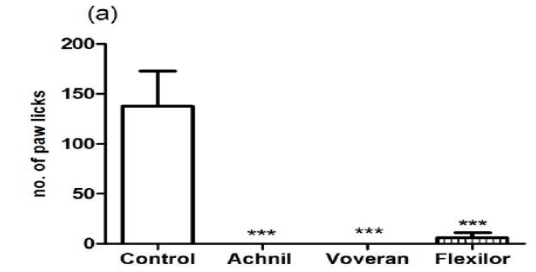

Figure 2a: No. of paw licks in the late phase of the rat formalin test (15 MIN). Data is presented as Mean ± SEM (n = 6). '***' indicates P<0.001 as compared to control group

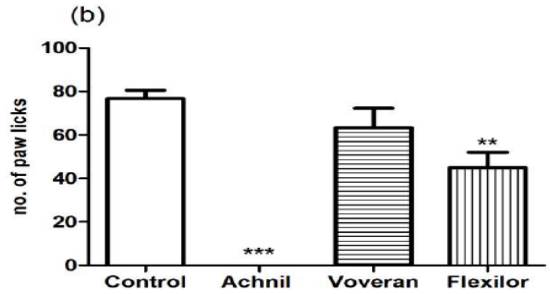

Figure 2b: No. of paw licks in the late phase of the rat formalin test (6 HR). Data is presented as Mean ± SEM (n = 6). '***' indicates P<0.001 as compared to control group

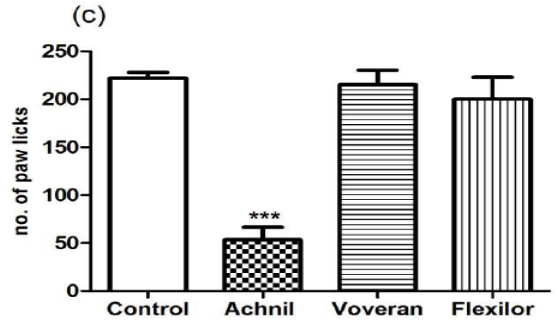

Figure 2c: No. of paw licks in the late phase of the rat formalin test (12 HR). Data is presented as Mean ± SEM (n = 6). '***' indicates P<0.001 as compared to control group

The licking response was similar in all the treatment groups suggesting that the formulations are equally efficacious in mediating the analgesic activity when measured at 15 min post-dosing (Figure 2a). After 6 hrs, the effect of Voveran (17.39%) on licking response was not significantly different from control, but that of Achnil showed 100% reduction in the no. of licks. Flexilor was found to be significantly better as compared to control with nearly 39.2% reduction in the no. of licks, however, the activity was not found to be at par with Achnil (Figure 2b). The effect of Achnil was found to be statistically significant (*P*<0.001) until this stage. Data recorded at 12 hrs (Figure 2c) showed that Achnil showed a 75.94% reduction in the no. of licks (*P*<0.001) but that of Voveran and Flexilor were not significantly different from control. While Achnil showed a reduction of the paw licking responses (*P*<0.001), the licking responses for Voveran and Flexilor were found to be significant only in the initial duration of the study. The data revealed that while the effect of a single dose of Achnil is retained for upto 12 hrs, injection of Voveran and Flexilor for analgesic activity is effective only for the initial duration and the activity reduces to a significant extent at 12hrs.

DISCUSSION

Pharmacotherapy of pain related to inflammatory conditions like rheumatoid arthritis and osteoarthritis, ankylosing spondylitis, other conditions like dental surgery related pain or post-surgical fibrosis frequently involves the use of NSAIDs. Phenylacetic acid derivatives (like diclofenac) form the staple class of peripheral COX-inhibitors and are known to have wide applications in therapy (1). Apart from non-selective COX-inhibition these agents are also reported to reduce the synthesis of leukotrienes. Aceclofenac, as compared to diclofenac, has shown equivalent efficacy and better tolerability in several experimental and clinical studies (3). Several studies claim that lornoxicam (Flexilor injection) is another NSAID which is reported to be equal in efficacy to diclofenac sodium (2). Further, aceclofenac has a rapid onset and longer duration of action in comparison to diclofenac (9). Clinically, aceclofenac has been indicated for post-operative dental pain, management of moderate to severe pain conditions like ear pain, gynecological pain, ankylosing spondylitis and arthritis (4, 10). The present study was undertaken to evaluate the comparative analgesic efficacy of Achnil (a novel formulation of aceclofenac), Voveran (marketed formulation of

diclofenac sodium) and Flexilor injection (Lornoxicam). Several experimental and clinical studies have tried to dissect the efficacy of aceclofenac. McCormack and Urquhart (1995) reported the efficacy of aceclofenac and other NSAIDs in the rat formalin test (11). On similar lines, Kajal et al (2014) reported that phenylacetic acid derivatives (belonging to the same chemical class of diclofenac and aceclofenac), have positive activity in the rat formalin test (12). This provides credence to the observations that the formalin test is an useful model for evaluating novel formulations of aceclofenac, like Achnil, to evaluate its analgesic potential. The rat formalin test has been identified as a model to study the different class of analgesics. It offers a reliable model of nociception and also helps in identifying preliminary mechanisms related to pain (5, 6, 13). Different groups have reported descriptive scoring methods that may be utilized with this model for a subjective evaluation of different analgesic agents (7, 8, 14). This type of scoring paradigm offers a good semi-quantitative estimate of analgesic activity. The experimental set-ups for evaluation of analgesic agents offers a myriad of tests that may be used for different class of potential analgesic agents, but these models often require complex surgical procedures and the pain produced as a result of such induction is often erratic. Furthermore, restraint of animals required for the evaluation leads to false positives during the subjective evaluation. The formalin test is a pain assessment procedure which eliminates some of these difficulties. Additionally, it offers the advantage that dose related assessment of analgesic activity of the test item is possible and it is unlikely that variation in results might arise out of motor abnormality (15).

The present study evaluated two critical parameters associated with the rat formalin test: composite pain score and no. of paw licks. A reduction in these parameters is associated with analgesic activity of the test formulations. With composite pain score, a trend towards a gradual decrease in analgesic activity of all the formulations as time progressed was found. It has been reported that diclofenac is prone to biotransformation and excretion in the urine within 6 hrs of administration (16, 17) which may be responsible for a gradual reduction in activity. Similarly lornoxicam has a reported elimination half-life of 3-4 hours, which might be responsible for reduction in activity observed at 6hrs and no activity at 12 hrs (18). Achnil was able to show a sustained efficacy for upto 12 hrs, whereby the activity remained nearly constant, as indicated by the composite pain score. The gradual lowering of activity of Achnil was found to be superior to diclofenac and lornoxicam with regard to analgesic effect such that even at the end of 12 hrs the analgesic effect was only reduced by around 21-24% and was superior than control group in exhibiting analgesia. On the contrary, analgesic effect of Voveran and Flexilor fell about 35-50% at about 6 hrs and continued to decrease further as time progressed. This was evident from the composite pain score recorded in the present study. This is in agreement with the reports of Davies and Anderson (1997) and Prasad et al (2009) suggesting a similar effect (17, 18). Kirchheiner et al (2003) also reported that concentrations of diclofenac fall below detection levels within 12 hrs of administration indicating towards the lower analgesic activity observed in the present study (19). These data are suggestive for the lower efficacy of Voveran observed in the later phases of the present study. Flexilor injection showed reduced activity at 6 hrs and further at 12 hrs there was complete absence of analgesic activity. However, data from the no. of paw licks showed that all the formulations showed comparable efficacy at 15 mins but the activity of Voveran and Flexilor were significantly reduced at 6 hrs post-dosing. Activity of Achnil remained nearly constant for about 12 hrs. Effect of Achnil showed significantly higher efficacy as compared to control but data recorded at 12 hrs showed somewhat lower inhibition of pain. This may be attributed to the metabolism and excretion of Achnil. Overall, it was observed that for both the parameters evaluated in the study, effect of Achnil remained nearly constant upto 12 hrs, reducing modestly, still showing good analgesic effect whereas data for Voveran and Flexilor revealed a significant reduction in activity at 6 hrs indicating that multiple injections of both formulations may be required for a sustained analgesic effect over the day. These readings were found to be in concert with previous reports (9, 20). The increased duration of action of Achnil may be attributed to the sustained biotransformation of aceclofenac to diclofenac (21, 22), which also has analgesic activity. It is imperative to note that diclofenac (active ingredient of Voveran) has a plasma half-life of 2 hours whereas that of lornoxicam and aceclofenac (active ingredients of Flexilor and Achnil) are 3-5 hours. It can be thus be expected that these products would be effective for only

shorter duration (< 6 hrs) and frequency of dosing will need to be increased to achieve analgesic effect over an extended period of time. However, the novel formulation of aceclofenac, Achnil, provides sustained effect as indicated by the results of the present study. This type of effect for prolonged duration is advantageous in management of post-operative conditions like knee/hip-replacements or other conditions of chronic pain. Such an effect was not evident with either of the comparators and thus multiple injections may be needed if analgesic efficacy is required for 6-12 hours. It is therefore suggested that Achnil has better efficacy as compared to Voveran and Flexilor, eliminating the need for multiple injections.

It was evident from the results that Achnil has a longer duration of action and better efficacy as compared to Voveran and Flexilor. While, single dose of Voveran and Flexilor were not able to sustain its analgesic effect after the initial recordings, it may be extrapolated that Voveran and Flexilor would require multiple injections to maintain analgesic effect for a longer duration. A single injection of Achnil was not only superior in efficacy but also showed effect for a prolonged duration in comparison to Voveran and Flexilor. Hence, Achnil injection is superior and better suited for management of chronic pain as compared to Voveran and Flexilor injections.

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REFERENCES

1. Patil P, Jaida J, Palani A, Rao K, Anandam B. A comparative study of efficacy and safety of diclofenac and aceclofenac in the treatment of osteoarthritis. J Drug Delivery Therap. 2012; 2: 139-43.
2. Kidd B, Frenzel W. A multicenter, randomized, double blind study comparing lornoxicam with diclofenac in osteoarthritis. J Rheumatol. 1996; 23:1605-11.
3. Grau M, Montero J, Guasch J, Felipe A, Carrasco E, Julia S. The pharmacological profile of aceclofenac, a new nonsteroidal antiinflammatory and analgesic drug. Agents and Action. 1991; 32:125-9.
4. Legrand E. Aceclofenac in the management of inflammatory pain. Expert Opin Pharmacother. 2004; 5:1347-67.
5. Munro G. Pharmacological assessment of the rat formalin test utilizing the clinically used analgesic drugs gabapentin, lamotrigine, morphine, duloxetine, tramadol and ibuprofen: influence of low and high formalin concentrations. Eur J Pharmacol. 2009; 605:95-102.
6. Hunskaar S, Fasmer O, Hole K. Formalin test in mice, a useful technique for evaluating mild analgesics. J Neurosci Meth. 1985; 14:69-76.
7. Abbott F, Franklin K, Westbrook R. The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain. 1995; 60:91-102.
8. Watson G, Sufka K, Coderre T. Optimal scoring strategies and weights for the formalin test in rats. Pain. 1997; 70:53-8.
9. Chunduri N, Kollu T, Goteki V, Mallela K, Madasu K. Efficacy of aceclofenac and diclofenac sodium for relief of postoperative pain after third molar surgery: A randomised open label comparative study. J Pharmacol Pharmacother. 2013; 4:144-5.
10. Seymour R, Frame J, Negus T, Hawkesford J, Marsden J, Matthew I. The comparative efficacy of aceclofenac and ibuprofen in postoperative pain after third molar surgery. Br J Oral Maxillofac Surg. 1998; 36:375-9.
11. McCormack K, Urquhart E. Correlation between Nonsteroidal Anti-Inflammatory Drug Efficacy in a Clinical Pain Model and the Dissociation of their Anti-Inflammatory and Analgesic Properties in Animal Models. Clin Drug Invest. 1995; 9:88-97.
12. Kajal A, Bala S, Sharma N, Kamboj S, Saini V. Therapeutic Potential of Hydrazones as Anti-Inflammatory Agents. Int J Med Chem. 2014;761030.
13. Dubuisson D, Dennis S. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain. 1997; 4:161-74.
14. Wheeler-Aceto H, Cowan A. Standardization of the rat paw formalin test for the evaluation of analgesics. Psychopharmacology. 1991; 104:35-44.
15. Quintans-Junior L, Oliveira M, Santana M, Santana M, Guimaraes A, Siqueira J, De Sousa D, Almeida R. α-Terpineol reduces nociceptive behavior in mice. Pharm Biol. 2011; 49:583-6.
16. Shakeel F, Mohammed S, Shafiq S. Comparative Pharmacokinetic Profile of Aceclofenac from Oral and Transdermal Application. J Bioequiv Bioavailab. 2009; 1:13-7.
17. Davies N, Anderson K. Clinical pharmacokinetics of diclofenac. Therapeutic insights and pitfalls. Clin Pharmacokinet. 1997; 33:184-213.
18. Prasad Byrav DS, Medhi B, Prakash A, Patyar S, Wadhwa S. Lornoxicam: a newer NSAID. Ind J Pharm Med Res. 2009; 20:27-31.
19. Kirchheiner J, Meineke I, Steinbach N, Meisel C, Roots I, Brockmoller J. Pharmacokinetics of diclofenac and inhibition of cyclooxygenases 1 and 2: no relationship to the CYP2C9 genetic polymorphism in humans. Br J Clin Pharmacol. 2003; 55:51–61.
20. Jyothsna K, Deshpande N, Vijayalakshmi G. Efficacy and safety of diclofenac sodium and aceclofenac in controlling post extraction dental pain: A randomised open label comparative study. J Pharmacol Toxicol. 2011; 11:41–7.
21. Hinz B, Rau T, Auge D. Aceclofenac spares cyclooxygenase-1 as a result of limited but sustained biotransformation to diclofenac. Clin Pharmacol Ther. 2003; 74:222–35.
22. Musmade P, Subramaniam G, Srinivasan K. High-performance liquid chromatography and pharmacokinetics of aceclofenac in rats. Anal Chim Acta. 2007; 585:103-9.