Journal of Developing Drugs
ISSN: 2329-6631

Research Article Open Access

Comparison of the Topical Analgesic Effects of a Novel Diclofenac Microemulsion to a Marketed Diclofenac Macroemulsion Formulation in Rats Using the Tail Flick Test

Tarique Benbow* **and Jacqueline Campbell**

Department of Pharmacology, University of the West Indies, Kingston, Jamaica

***Corresponding author:** Tarique Benbow, Department of Pharmacology, University of the West Indies, Kingston, Jamaica, Tel: 8764206702; E-mail: tariquebenbow@gmail.com

Rec date: August 08, 2018; **Acc date:** August 29, 2018; **Pub date:** September 05, 2018

Abstract

Current topical preparations do not provide optimal topical drug delivery. Microemulsions have been proposed to increase drug penetration across the skin, leading to increased bioavailability and efficacy of the drug product. This study compares the topical analgesic effects of novel diclofenac diethylamine (DDEA) water-in-oil (w/o) microemulsion to a marketed diclofenac diethylamine (DDEA) oil-in-water (o/w) macroemulsion (Voltaren Emulgel ®) in the treatment of acute thermal pain using the rat tail flick test. Eighteen (N=18) Sprague-Dawley rats were divided into three (3) groups (n=6). The groups were administered the novel microemulsion of DDEA (test group), the marketed macroemulsion of diclofenac (positive control group) and the microemulsion alone (negative control group); the rat tail flick test was performed after topical administration to assess analgesia observed. Mean maximum possible effect (MPE) in percentage was calculated for each group and analyzed using the one-way ANOVA for statistical significance ($p<0.05$) across the groups. The group administered the DDEA o/w microemulsion had the numerical highest MPE (27.459 ± 7.849%). This was statistically significant ($p<0.05$) when compared to negative control group but was not statistically significant ($p>0.05$) when compared to the positive control group. Further studies that specify the mechanism(s) and address limitations are needed to expand these findings to clinical applications.

Keywords: Microemulsion; Macroemulsion; Analgesia; Tail flick test; Diclofenac diethylamine; Pain

Introduction

Pain affects more Americans than diabetes, heart disease, and cancer combined [1]. According to the National Center for Health Statistics, approximately 76.2 million, one in every four Americans, have suffered from pain that lasts longer than 24 hours and millions more suffer from acute pain. Also, estimates suggest that 20% of adults suffer from pain globally and 10% are newly diagnosed with chronic pain each year [2]. Pain, therefore, is a global crisis and significant efforts have gone into the development of drugs that can be used to treat pain. Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness [3]. Although there have been many advancements with analgesics in the treatment of chronic and acute pain; they present with many challenges. Some of these drugs present with severe adverse effects and toxicities, which results in them being contraindicated or unsuitable for treating patients of varying demographics and complications. Others are inadequate in relieving specific types of pain such as localized, and nerve pain. Therefore, the need for safer and more effective analgesics is still a significant concern to the scientific community.

Diclofenac is classified as a non-steroidal anti-inflammatory drug (NSAID) and has anti-inflammatory, analgesic and antipyretic activity. The mechanism of action through which diclofenac exerts its therapeutic action is primarily accepted as through systemic inhibition of the synthesis of prostaglandins by inhibiting cyclooxygenase coenzyme-1 (COX-1) and cyclooxygenase-2 (COX-2) [4]. This enzyme is responsible for the synthesis of prostaglandin PG2 from arachidonic acid in the periphery. It is thought that prostaglandins are involved in the inflammatory and pain pathways, and therefore inhibition of the formation of these chemical mediators inhibits both inflammation and nociception. More specifically, cyclooxygenase-1 is also responsible for the formation of cytoprotective prostaglandins in the stomach, where the gastric juices and hydrochloric acid may persist. Hence inhibition of this enzyme by NSAIDs such as diclofenac results in a decrease in the formation of these cytoprotective prostaglandins, and thus increase the risk of cellular damage in the stomach from the digestive juices. This forms the major side effect experienced when diclofenac is administered orally. One way to negate these challenges is by employing the topical route of administration [5].

Diclofenac was the first topical NSAID approved in the United States for knee osteoarthritis and is currently the only approved topical NSAID for hand osteoarthritis [2]. Topical administration of diclofenac offers several benefits over alternative routes of administration, including a direct application to a localized area, decreased systemic drug load and therefore, fewer side effects such as the gastrointestinal disturbances [6]. Although beneficial, topical administration of drugs often presents with challenges of its own; the outer layer of the skin is impermeable to most drugs, thereby decreasing bioavailability and ultimately therapeutic response. As such, researchers are often faced with the dilemma of having to balance solubility with bioavailability when considering this route of administration. This, of course, is not an easy task.

Despite the considerable potential of dermal/topical drug delivery, relatively few formulations are commercially available due to the barrier function of stratum corneum, which limits permeation of most exogenous substances [7]. Thus, the problem which needs to be addressed is to overcome the barrier function of stratum corneum and hence enhance drug delivery through the skin. Published approaches include the use of vehicle-chemical enhancers such as ethanol, turpentine, and methanol [8]. These chemical enhancers temporarily diminish the barrier of the skin, to facilitate better absorption of the drug, however in doing so; they increase the risk of entry by pathogens as well as decrease patient acceptability [9]. One of the techniques used to enhance dermal drug delivery is the microemulsion system.

Microemulsions were first introduced by Schulman et al. in 1943. Microemulsions have been studied at great lengths over the last decades by many scientists because of their high potential in food and pharmaceutical applications. Microemulsions are transparent, stable, isotropic mixtures of oil, water, and surfactant, frequently in combination with a co-surfactant [10]. A microemulsion is formed when the interfacial tension at the water/oil interface is brought to a very low level, and the interfacial layer is kept highly flexible and fluid. These systems are achieved by careful and precise selection of components, their respective amounts and through using a co-surfactant which brings flexibility to the water/oil interface [10]. In contrast to ordinary emulsions, microemulsions form upon simple mixing of the components and do not require the high shear conditions generally used in the formation of conventional emulsions [10]. Macroemulsions, require high energy to produce as well as they are not thermodynamically stable and tend to break down or exhibit phase separation (flocculation or coalesce) over time. This result in high production cost, formulation development challenges and decreased shelf-life.

Microemulsions are particularly advantageous because of their low-cost of production and more importantly the promise of improved bioavailability and by extension therapeutic response when used to deliver drugs. The increased absorption of drugs in microemulsion systems is attributed to the enhancement of penetration through the skin by the carrier effect. The dispersed phase in microemulsion systems consists of tiny flexible droplets with a diameter in the range of 100-1000 A°, in contrast, macroemulsion systems consist of droplets 5-140 nm which are much larger than that of microemulsion systems [11]. As a result, the small droplet size in microemulsions leads to a large surface-to-volume ratio in the system. In topical drug applications, this is particularly desirable as it allows for a greater surface area to skin ratio and thereby increases the rate of absorption of the drug when applied topically [11]. These advantages which microemulsion system boasts over macroemulsion system have led many researchers to investigate the superiority of microemulsion as a solution to the challenges faced with topical drug delivery system. An oil-in-water type macroemulsion topical formulation of diclofenac diethylamine (DDEA) is marketed under the trade name Voltaren Emulgel® by Novartis. According to Roberts, this marketed formulation allows only 10% of the dose administered topically to reach the blood unchanged, i.e., 10% bioavailability. Literature reports also indicate that when administered topically in this macroemulsion product, its therapeutic dose falls by a factor of ten (10) and as such it is frequently prescribed with Voltaren tablets to achieve optimal therapeutic effect [12]. This defeats the purpose of administering the drug topically in the first place.

Although there have been many studies investigating the use of microemulsions as drug delivery systems for topical applications, minimal work has gone into comparing the effectiveness of this novel drug delivery system in percutaneous delivery of drugs vs present-day topical delivery systems such as macroemulsions/Emulgel, gels, and creams. This study compares the analgesic effects/efficacy of a novel topical diclofenac diethylamine (DDEA) 1.16% water-in-oil microemulsion formulation to a marketed oil-in-water macroemulsion formulation of diclofenac diethylamine (DDEA) 1.16% in an animal model to acute thermal pain. Analgesia was assessed using the rat tail flick test. The researcher hypothesizes that since the properties of a microemulsion allows for increased permeation rate and total drug quantity transported across the subcutaneous membranes, then this will correspond to an increase in the magnitude of anti-nociception observed (efficacy) when compared to a conventional emulsion (macroemulsion/Emulgel) vehicle at the same dose of diclofenac diethylamine. This should be evident upon assay using a suitable test for analgesia such as the rat tail flick test. In doing so, this research aims to demonstrate the possibility of using a novel drug delivery system to overcome the challenges faced with topical analgesics and how this system can augment or improve current analgesic therapy with diclofenac (a known analgesic) to treat acute pain [13-17].

Methods and Materials

The following materials were generously donated by Benjamins PA Manufacturing Company Limited and used as received. These materials include: Diclofenac Diethylamine (DDEA) USP, Isopropyl Alcohol (IPA) USP, Propylene Glycol USP and Glyceryl Monostearate USP.

Voltaren Emulgel (Diclofenac Diethylamine 1.16% w/w) was purchased from a local pharmacy in Kingston Jamaica. Deionized Water was filtered and prepared in-house and conformed to USP Purified water specifications.

Screening of components of microemulsion

In order to prepare an optimized ME system, it was of great importance to select an appropriate oil, surfactant and co-surfactant combination that had a good solubilizing capacity of DDEA. These components were selected on the basis of a solubility study reported by Thakkar et al., who developed w/o MEs using five different co-surfactants, not including glycerol monostearate as surfactant, combined with isopropyl alcohol as co-surfactant. The oil phase for the ME prepared was propylene glycol monolaurate and deionized water was used as the aqueous phase in the ME formulation. All components of the ME conformed to USP 2015 specifications.

Preparation of microemulsions

ME formulations were formed spontaneously by mixing heated Glycerol Monostearate (60°C) with Isopropyl Alcohol at 2:1 ratio to form a surfactant/cosurfactant mixture. In a separate container the DDEA was added to the desired quantity of water and mixed gently at room temperature until homogenous. The aqueous solution of DDEA was then added to the surfactant co-surfactant mixture and with propylene glycol and mixed at 650 rpm for 5 minsuntil homogenous. The end product was a microemulsion system with Diclofenac Diethylamine 1.16% w/w incorporated.

ME without DDEA formulations were also prepared to be used as a control in the study. The preparation steps of these formulations were

similarly to that of the ME with DDEA; with the exception that no DDEA was incorporated in these formulations.

Characterization of microemulsion

pH measurement: The apparent pH of the tested ME formulation was determined by a digital pH meter (Accumet XL20, pH meter). All measurements were performed in triplicate at 25°C.

Conductivity measurement: The conductivity of a microemulsion can be used to determine the type of microemulsion system that exists. That is; water-in-oil will yield a low conductivity reading conversely, oil-in-water will yield a high conductivity reading. For this experiment we used the ThermoScientific Orion Star °A222 Conductivity portable meter. All measurements were taken in triplicates at 25°C.

Viscosity measurement: The viscosity of the microemulsion was determined by three repetitive readings using a Brookfield °DVE viscometer at 25°C.

Droplet size measurement: The droplet size of microemulsion formulations was determined using Delsa Nano Particle Size Analyzer (Beckman coulter Inc., Brea, CA). This was determined at 25°C and at 90° angle in triplicate.

Methods

Study design: The study was an experimental study conducted at the University of the West Indies Mona Campus in the Department of Pharmacology over three (3) days, April 26-27th, 2018.

Sample size and population: The sample consisted of 18 Sprague Dawley rats (N=18), 9 males and 9 females. The rats used in the experiment were screened and selected through the inclusion and exclusion criteria described in Table 1. The rats were randomly divided into three groups with each group consisting of 6 rats total (Male=3, Female=3).

Group one (Test Group) was administered the novel DDEA 1.16% w/o microemulsion (Product A). Group two (Positive Control group) was administered the marketed DDEA 1.16% o/w macroemulsion gel (Product B). Group three (Negative Control group) was administered the base microemulsion without any drug (Product C). The rats were maintained at 12 hours light/dark cycle with food and water ad libitum.

Inclusion Criteria	Exclusion Criteria
Species=Sprague Dawley Rats	Used in Prior Pain Studies/Experiments
Weight=150-350 g	Signs/Symptoms of Illness as per Table 2
Age=3 weeks old	

Table 1: Inclusion and exclusion criteria for rats selected for the study.

Parameter	Observation
General Appearance	Dehydration, decreased body weight, missing anatomy, abnormal posture, hypothermia, fractured appendage, swelling, tissue masses, prolapse, paraphimosis
Skin and fur	Discoloration, urine stain, pallor, redness, cyanosis, icterus, wound, sore, abscess, ulcer, alopecia, ruffled fur
Eyes	Exophthalmos, microphthalmia, ptosis, reddened eye, lacrimation, discharge, opacity
Nose, Mouth, and Head	Head tilted, nasal discharge, malocclusion, salivation
Respiration	Sneezing, dyspnoea, tachypnoea, rales
Urine	Discolouration, blood in urine, polyuria, anuria
Faeces	Discolouration, blood in the faeces, softness/ diarrhoea
Locomotor	Hyperactivity, hyperactivity, coma, ataxia, circling, muscle, tremors,

Table 2: Signs and symptoms used to determine if each rat was ill and therefore ineligible for participation in the study.

Rat tail flick assay: A modified version of the rat tail flick test was employed in this study to assess the level of analgesia observed from each product used. Three repetitive measurements evaluated the baseline latency for each rat (i.e., before any test substance is applied) and the average of the readings calculated and used as the baseline latency for each rat. A 0.5 g of each test substance (i.e., Product A, B and C respectively) was then applied and massaged into the distal portion of the tail (last 3 mm of the tail) for one (1) minute respectively. Any excess test substance remaining on the tail was removed using a clean cloth. Following topical administration, the exposed or tested area of the rat's tail was exposed to the radiant heat source (infrared radiation). An intensity of 50% was maintained throughout the experiment. The latency time was recorded and noted. A maximum tail flick latency of 10 seconds was used to minimize tissue damage to the tail. Each rat was tested twice at 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes and 180 minutes post topical administration. The average Post Drug Latency at each test point was recorded and tabulated for data analysis.

Blinding of study: To ensure that the study was blinded, the division of the rats into the three (30) groups and the application of the test substances was conducted by a laboratory technician, who was not involved in the tail flick assay. Following which the researcher conducted the tail flick exercise on the rats as described above.

Data analysis: The Percentage Maximum Possible Effect (MPE%) for each group at each test point was calculated from the post drug latency using equation one (1) (Table 3).

$$Equation\ 1: \quad \%MPE = \frac{PostDrugLatency - BaselineLatency}{10sec - BaselineLatency} \times 100$$

The data was analyzed for statistical difference using the one-way ANOVA and Games-Howell post-hoc tests using SPSS (version 25, IBM, Armonk, NY, USA). A p-value of <0.05 was considered statistically significant.

Ethical considerations: Ethical approval for this study was obtained from the University of the West Indies/Faculty of Medical Sciences Animal Ethics Committee.

Results and Discussion

Product	pH=25°C	Conductivity =25°C S/m	Viscosity (cps)	Avg. Droplet Size (nm)
Product A	4.03 ± 0.02	2	22 ± 1.56	2 nm
Product B	5.0 ± 0.03	14.2	21.20 ± 0.43	100 nm
Product C	4.30 ± 0.02	2.7	21 ± 1.49	1.3 nm

Table 3: Physical Characteristics of products used in the experiment. Notes: Product A-water-in-oil Microemulsion with Diclofenac Diethylamine; Product B-oil-in-water Macroemulsion with Diclofenac Diethylamine; Product C-water-in-oil Microemulsion without Diclofenac Diethylamine.

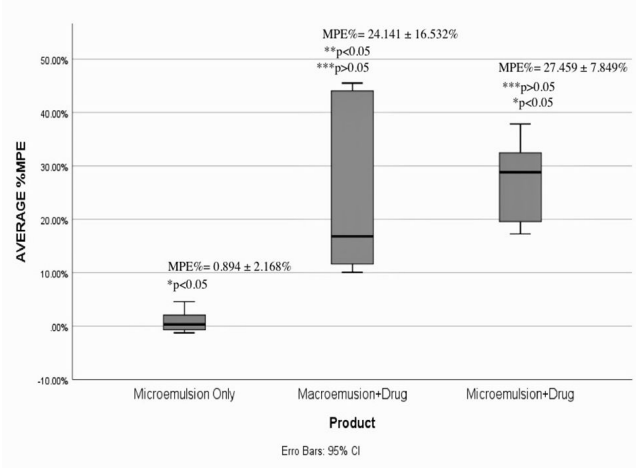

Figure 1: Mean Percentage Maximum Possible Effect (MPE%) for Groups (Microemulsion only, Macroemulsion with DDEA 1.16% w/w and water-in-oil Microemulsion with DDEA 1.16% w/w) used in the experiment. Note: *p<0.05 for microemulsion+Drug group compared with microemulsion only group; **p<0.05 for macroemulsion+Drug group compared with microemulsion only; ***p>0.05 for microemulsion+Drug group compared with macroemulsion+DDEA.

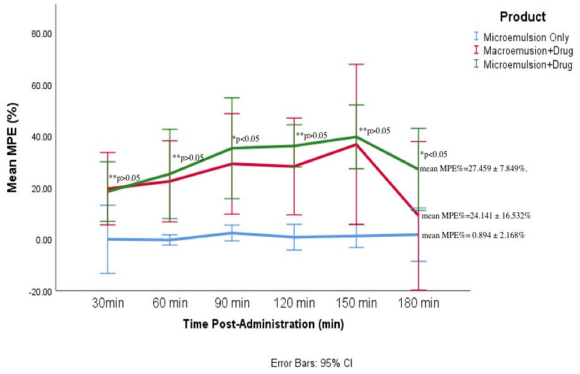

Figure 2: Mean Percentage Maximum Possible Effect (MPE%) for each group vs the time post-administration of each drug product used in the experiment. Notes: The maximum %MPE experienced at any test point for both the group administered the w/o microemulsion with diclofenac diethylamine 1.16% and the o/w macroemulsion with diclofenac diethylamine 1.16% was observed at the 150 min interval. Where the mean %MPE for the w/o microemulsion with diclofenac diethylamine 1.16% at 150 minutes post administration, was numerically higher than that noted from the group administered o/w macroemulsion with diclofenac diethylamine 1.16% at 150 minutes post administration. However, the difference between the two means was not statistically significant as per one-way ANOVA-Games-Howell analysis in Table 1. 6 [F (2, 7.47), p=898]. *p<0.05 for comparison of group administered the microemulsion+DDEA and Macroemulsion +DDEA; **p>0.05 for comparison of group administered the microemulsion+DDEA and Macroemulsion+DDEA.

The results of the physical characterization of the emulsion systems formed confirmed the existence of a microemulsion system for Products A and C. Droplet size determination showed that average droplets size was less than 5 nm for the microemulsion formulations developed. Meanwhile the droplet size for the marketed product; Product B yielder larger values, averaging at 100 nm. The small droplet size implies that the surface tension between the oil and water phases were brought very low allowing for a flexible micelle formation in the microemulsion systems. Also, further characterization of the formulations was conducted to determine the type of microemulsion systems formed i.e., water-in-oil or oil-in-water Permeation enhancer [18]. A low conductivity indicated a water-in-oil microemulsion system; in an emulsion system the continuous phase operates as the main conductor and since in a water-in-oil emulsion system, the oil phase is the continuous then it is expected that such a system will display very low conductivity readings. This was shown in Products A and Product C (Conductivity=2.0 and 2.7 S/m @20°C respectively). However, the marketed Product B (marketed product) had a high conductivity reading (Conductivity=14.2 S/m @20°C), which confirmed the existence of an oil-in-water type emulsion system (Table 2).

In the tail flick model assay, the animals in the test group (w/o microemulsion with DDEA), showed an increase in the mean percentage maximum possible effect (MPE%) from baseline through

the duration of the experiment when compared to the negative control group (microemulsion only) (Figure 1). This difference between the mean MPE% for animals in the negative control group (w/o microemulsion only) and the test group (w/o microemulsion with diclofenac diethylamine 1.16%) was statistically significant (26.565%*; p=0.001, CI=95%). This provides evidences that the novel microemulsion with DDEA was able to deliver the drug, effectively across the layers of the skin and thus to its site of action to effect anti-nociception or analgesia. This finding corresponds with that of other researchers.

Thakkar in his study demonstrated that the microemulsion of diclofenac diethylamine was able to facilitate Microemulsions absorption drug across the rat skin in his *in-vitro* model using the Franz-diffusion cell. Studies conducted to explain the mechanism of topical drug delivery through microemulsion systems explains that in these systems the drug is contained in both the oil phase and the aqueous phase of the emulsion system. This is advantageous as it allows for transdermal delivery to be accomplished through both passive diffusion for the drug in the oil phase (unionized form) and the transappendgeal route for the drug in the aqueous phase (ionized form). Since there was no statistically significant change in the mean MPE% for the rats in the negative control group (mean %MPE=0.894 ± 2.168%) from baseline, we can deduce that the microemulsion vehicle alone did not possess any therapeutic activity [19-22]. This was important to note; because we can then attest the analgesic activity seen in the groups to the action of DDEA only.

The mean MPE% for the animals in the test group (w/o microemulsion with DDEA) was numerically greater than the mean MPE% for the animals in the positive control group (o/w macroemulsion with DDEA); (3.318%**; $p<0.05$) [23]. However, the results of the one-way ANOVA, showed that this difference was not statistically significant [$F(2, 7.345)=11.105$, $p=0.006$] (Figure 2). This reveals that the transdermal delivery of drug using the novel microemulsion formulation developed did not yield statistically significant analgesic activity when compare to the marketed macroemulsion product. However, what is apparent is that both drug delivery systems displayed similar therapeutic response (analgesia). Inherent limitations for the microemulsion system developed could justify the insignificant statistical difference in the mean percentage maximum possible effect observed between the positive control and the test groups in the study. The components of the microemulsion system may not have been the most optimal. To combat this, further studies can be conducted to compare the analgesic effects of multiple microemulsion systems using different chemical for the oil and aqueous phases to determine the most optimal or effective formulation. Additionally, further studies can be conducted to investigate the effect of increased concentration of the drug in these system vs therapeutic response to elucidate the extent of drug absorption from this formulation or other microemulsion formulations. The microemulsion may possess some potential advantages which may be of interest to the pharmaceutical drug industry. For example, microemulsions require very limited amount of energy to produce as oppose to conventional macroemulsion. This has positive implications on reducing the cost to products of drug products which will ultimately lead to increased affordability for many patients.

Limitations

In this study, we assumed that an increase in the penetration of diclofenac would give rise to higher serum concentration of diclofenac and thus bioavailability leading to greater therapeutic efficacy of the drug. Since only the outcome or the therapeutic benefit was measured, the data in the study could not undoubtedly prove that there was a quantitatively higher concentration of diclofenac across the skin when compared to the macroemulsion dosage form used. Subsequent experiments could investigate the effect of varying concentrations and doses of diclofenac diethylamine in microemulsion systems and the level of antinociception experienced from the dose used and comparison of macroemulsions at the same doses of drug incorporated. This could be employed through either animal or human *in-vitro* studies using the Franz-diffusion cell model to measure the amount of drug that crosses the skin at varying doses [24,25].

Another limitation of this study was the sample size used in the study. As aforementioned this was determined through the crude method, and as such was not as robust and suitable to ensure the most appropriate effect size was ascertained. This could have been combatted by first conducting a pilot study, which would result in a more suitable effect size and ultimately the more appropriate sample size selection.

Conclusion

The novel diclofenac diethylamine 1.16% w/o microemulsion induced analgesia to acute thermal pain. However, when compared with the marketed diclofenac diethylamine 1.16% w/o macroemulsion, there is was no statistically significant difference in the magnitude of analgesia observed to acute thermal pain ($p>0.05$). However, the use of further studies that specify the mechanism(s) and address limitations confounded to this study are needed to expand the findings to clinical applications [26,27].

References

1. Department of Health and Human Services (2017) USA.
2. Baraf HS, Gold MS, Petruschke RA, Wieman MS (2012) Tolerability of topical diclofenac sodium 1% gel for osteoarthritis in seniors and patients with comorbidities. The American Journal of Geriatric Pharmacotherapy 10: 47-60.
3. Fan SH, Ali NA, Basri DF (2014) Evaluation of analgesic activity of the methanol extract from the galls of Quercus infectoria (Olivier) in rats. Evidence-Based Complementary and Alternative Medicine.
4. Graham DY, Opekun AR, Willingham FF, Qureshi WA (2005) Visible small-intestinal mucosal injury in chronic NSAID users. Clinical Gastroenterology and Hepatology 3: 55-59.
5. Lacy CF, Armstrong LL, Goldman MP, Lance L (2004-2005) Drug Information Handbook: Pocket, Lexi-Comp.
6. Stoicea N, Fiorda-Diaz J, Joseph N, Shabsigh M, Arias-Morales C, et al. (2017) Advanced analgesic drug delivery and nanobiotechnology. Drugs 77: 1069-1076.
7. Vadlamudi CH, Narendran H, Nagaswaram T, Yaga G, Thanniru J (2014) Microemulsions based transdermal drug delivery systems. Current Drug Discovery Technologies 11: 169-180.
8. Chaudhary H, Kohli K, Amin S, Rathee P, Kumar V (2011) Optimization and formulation design of gels of Diclofenac and Curcumin for transdermal drug delivery by Box-Behnken statistical design. Journal of Pharmaceutical Sciences 100: 580-593.
9. Shah SN (2012) Developing an efficacious diclofenac diethylamine transdermal formulation. Journal of Food and Drug Analysis 20: 464-470.
10. Kogan A, Garti N (2006) Microemulsions as transdermal drug delivery vehicles. Advances in Colloid and Interface Science 123: 369-385.
11. Sharma MK (1985) Introduction to Macro- and Microemulsions. ACS Symposium Series.

12. Thakkar PJ, Madan P, Lin S (2014) Transdermal delivery of diclofenac using water-in-oil microemulsion. Pharmaceutical Development and Technology 19: 373-384.
13. Grampurohit N, Ravikumar P, Mallya R (2011) Microemulsions for topical use- a review. Ind J Pharm Edu Res 45: 100-107.
14. Derle DV, Sagar BS, Pimpale S (2006) Microemulsion as a vehicle for transdermal permeation of nimesulide. Indian Journal of Pharmaceutical Sciences.
15. Lopes LB (2014) Overcoming the cutaneous barrier with microemulsions. Pharmaceutics 6: 52-77.
16. Kreilgaard M (2002) Influence of microemulsionss on cutaneous. Bulletin Technique GatteFosse, pp: 79-100.
17. Hathout RM, Mansour S, Mortada ND, Geneidi AS, Guy RH (2010) Uptake of microemulsion components into the stratum corneum and their molecular effects on skin barrier function. Molecular Pharmaceutics 7: 1266-1273.
18. Marwah H, Garg T, Goyal AK, Rath G (2014) Permeation enhancer strategies in transdermal drug delivery. Drug Delivery 23: 564-578.
19. Schoellhammer CM, Blankschtein D, Langer R (2014) Skin permeabilization for transdermal drug delivery: recent advances and future prospects. Expert Opinion on Drug Delivery 11: 393-407.
20. Neubert RH (2011) Potentials of new nanocarriers for dermal and transdermal drug delivery. European Journal of Pharmaceutics and Biopharmaceutics 77: 1-2.
21. Alkrad JA, Talhouni AA (2018) In vivo and in vitro study of transdermal application of diclofenac sodium using nonionic microemulsions as colloidal drug delivery systems. Journal of Drug Delivery Science and Technology 43: 27-33.
22. Prasanth IPL (2012) Vesicles – mechanism of transdermal permeation: a review. Asian Journal of Pharmaceutical and Clinical Research.
23. Charan J, Kantharia ND (2013) How to calculate sample size in animal studies? Journal of Pharmacology and Pharmacotherapeutics 4: 303.
24. Marie E (1997) Human Anatomy and Physiology (4th edn.).
25. Apparatus PH (2018) Tail flick test. Kingston, Jamaica.
26. Gan TJ (2010) Diclofenac: an update on its mechanism of action and safety profile. Current Medical Research and Opinion 26: 1715-1731.
27. Singh P, Roberts MS (1994) Skin permeability and local tissue concentrations of non-steroidal anti-inflammatory drugs after topical. Journal of Pharmacology Experimental Therapeutics 268: 144-151.