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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Anti-Inflammatory and Analgesic Activity of Optimised Lipid-Based Non-Aqueous Nanoemulsion of Naproxen in Experimental Animals

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ABSTRACT
As a nonsteroidal anti-inflammatory medication (NSAID), naproxen exerts analgesic, anti-inflammatory, and antipyretic effects by blocking the production of prostaglandins through the action on cyclooxygenase enzymes. The goal of this investigation was to assess the efficacy of optimised Naproxen NANEs (Non-aqueous Nano Emulsion) in treating pain and inflammation in animal models. Rats were given carrageenan injections into their left hind paws to generate oedema, and the optimised NANEs of Naproxen were tested for their ability to reduce inflammation and pain using the Hot Plate method and the tail-flick test, respectively. Results related to the anti-inflammatory activity revealed that the optimized NANEs of Naproxen produced a maximum percent oedema inhibition (50.92%) as compared to standard naproxen formulation. Similarly, the analgesic effect of the optimized NANEs of Naproxen showed better effect as compared to marketed formulations. Finally, this study concludes that the tested Optimized NANEs of Naproxen exhibited good and acceptable anti-inflammatory and analgesic effect in comparison to the commercial marketed formulation.
Keywords: *NANEs, Naproxen, Prostaglandin, Anti-inflammatory effect, Analgesic effect*

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INTRODUCTION

As an NSAID (Nonsteroidal Anti-inflammatory Drug), naproxen has been shown to have anti-inflammatory, anti-rheumatoid arthritis, analgesic, and antipyretic effects in animal models. Like other NSAIDS, Naproxen may work by preventing the production of prostaglandins. Patients with peptic ulcer disease, gastro esophageal reflux (GERD), irritable bowel syndrome, or other gastrointestinal diseases should not use non-steroidal anti-inflammatory drugs orally [1]. The aforementioned negative effects might be mitigated if the medicine were administered via the skin. Humoral mediators such as kinins, leukotrienes, prostaglandins, and cytokines are produced as part of the intricate process that is inflammation. The levels of corticosteroids in the body tend to rise in cases of persistent inflammation. Reduced inflammation is a result of this compound's ability to suppress leukotrienes [2]. The sensation of pain can emerge after any kind of tissue injury. Pain perception is dynamic and constantly adapts to the body's actual state, which is why there may be no sensation of pain despite the presence of visible and continuous tissue damage. When tissues are damaged, chemical mediators accumulate locally and can powerfully stimulate nociceptors, causing pain. Enzymatic cascades use arachidonic acid as a substrate to produce eicosanoids such prostaglandins, thromboxanes, and leukotrienes in a matter of seconds [3]. All of these substances play a role in pain and inflammation as mediators. Therefore, tissue injury triggers the enzymatic cleavage of circulating high-molecular-weight kininogen to generate bradykinin, another powerful mediator of pain and inflammation [4].

While the drug bioavailability is reduced in conventional emulsions due to their large droplet size, it is increased in nonaqueous nanoemulsions as the particle size decreases. The disadvantages of traditional emulsions, such as phase inversion, phase separation, flocculation, coalescence, creaming, and cracking, are not present in a nonaqueous nanoemulsion. The stability of a formulation can be improved by decreasing the particle size, and vice versa, in NANEs. Lipophilic drugs can be transported by this method. NANEs can be safely administered to the skin and mucous membranes because they are nontoxic and non-irritant. Non-Aqueous Nanoemulsions are a viable delivery method for drugs that are both water-

insoluble and unstable in other forms. Therefore, we formulate naproxen as a nonaqueous nanoemulsion so that it possesses all of the aforementioned qualities. Therefore, the goal of this research was to analyse the analgesic and anti-inflammatory properties of Naproxen in a non-aqueous nano emulsion that had been tailored for lipids [5].

MATERIAL AND METHODS

Chemicals: Naproxen was obtained from the DVVPFs college of Pharmacy's drug laboratory in Ahmednagar (MH), India. Carrageenan was purchased from Dolphin Instruments Pvt Ltd, Mumbai, Ethanol 95% was purchased from Modern Science Apparatus Pvt Ltd, Nashik. The rest of the components employed in this analysis were also of the highest possible quality.

Preparation of lipid based non-aqueous nano emulsion:

Lipid based non-aqueous nano emulsion was prepared in research lab. Naproxen 10% as a medication, 5% Glycerol Monostearate as a surfactant, 5 ml Mineral oil as a continuous phase, and 5 ml of Glycerine as a dispersed phase are utilised to make NANEs of Naproxen, as shown by preliminary experiments employing several methods of preparing NANEs. In first beaker Weighed quantity of Naproxen dissolved in mineral oil then GMS added to second beaker i.e. in glycerin heat it at about 50 to 60 degree centigrade, cool it then it added to second beaker and it homogenize at REMI Ultraturrex high speed homogenizer at 15000-16000 rpm for 3 min [1].

Animals:

The Albino Wistar rats (150-200 gm) both male and female were procured from animal house (Animal House No:1670/PO/ReBiBt/S/12/CPCSEA) of DVVPFs college of Pharmacy, Ahmednagar (MH). The animals were housed at laboratory for 12 hrs day and night conditions for acclimatization up to one week. The rats were given pellets of rat food (obtained from Prashant Enterprises in Pune) and running water from the tap whenever they wanted it. Institutional Animal Ethics Committee approval was acquired before to conducting the experiment. (Coph/IAEC/ 2021/02).

1. Anti-inflammatory activity (Carrageenan induced paw oedema):

Albino Wistar rats (both sexes) weighing between 150 and 200 gm were chosen for the study and split into four groups of six rats each (n=6) and marked with picric acid and was treated with vehicle,0.1 mL 1% Carrageenan into the sub plantar tissue to Group II, standard drug Naproxen (10% naproxen gel containing 100 mg naproxen/gm topically) and optimized formulation of NANEs, Sub plantar tissue of left hind paw was treated with (10% Naproxen Nonaqueous nanoemulsion containing 100 mg naproxen /ml topically) by applying it 50 times with a gentle rubbing motion using the index finger. All rats in groups II, III, and IV (with the exception of group I) were injected with 0.1 ml of 1% Carrageenan into the sub plantar tissue of the left hind paw an hour after receiving the above treatments. Injections of carrageenan into the foot were followed by measurements of swelling with a Digital Plethysmometer taken at 0, 1, 2, 3, and 4 hours (Laboratory enterprises). To the right hind paw, we applied 0.1 ml of vehicle (NANEs without Naproxen topically)[6-9].

Group I - Normal (NANEs without Naproxen topically)
Group II - Inflammatory Control (received 0.1 mL 1% Carrageenan into the sub plantar tissue)
Group III - Inflammation treated with standard drug (topically 10% naproxen gel containing 100mg naproxen /gm 60 min before 0.1 mL 1% Carrageenan)
Group IV - Inflammation treated with optimized formulation, (topically 10% naproxen NANEs containing 100 mg naproxen/ml 60 min before 0.1 mL 1% Carrageenan)

The percent inhibition of paw oedema induced by carrageenan was calculated for each group after 4 hrs using following formula

$$\text{Inhibition of oedema (\%)} = \frac{\text{V control} - \text{V treated}}{\text{V control}} \times 100$$

Evaluation of Analgesic activity:

Analgesic activity (tail-flick test):

The tail-flick test was used to determine the analgesic effect of the substance. Animals were randomly assigned to one of four groups of six (n=6) albino wistar rats, each weighing between 150 and 200 gm, each and marked with picric acid and were treated with vehicle, standard drug Diclofenac gel 1%, Naproxen (10% naproxen gel containing 100 mg naproxen/gm) and optimized formulation NANEs, (10% naproxen Nonaqueous nanoemulsion containing 100 mg naproxen/ml) topically to 3-5 cm portion of rat tail. After 30 minutes, the gel and NANEs remaining on the skin's surface were washed away with a piece of cotton. The distal 2 - 3 cm part of the rat tail was submerged in 55°C ± 0.5°C water. As reaction time, the time it took the rat to remove its tail from hot water was recorded. The reaction time was observed at

0, 30, 60, 90, and 120 minutes after the following treatments were applied. 15 seconds was chosen as the cutoff time to prevent tissue harm [7-10].

Group I - Normal (NANEs without Naproxen topically to tail portion)
Group II - Standard 1 (Diclofenac 1% w/w gel topically)
Group III - Standard 2 (10% naproxen gel containing 100mg naproxen/gm topically)
Group IV - Treated with optimized formulation NANEs (10% Naproxen Nonaqueous Nano emulsion containing 100 mg naproxen/ml topically)

The percentage protection against tail-flick response was used to assess the % analgesia and was calculated using following formula.

$$\text{Percentage protection (\%)} = \frac{\text{Test latency} - \text{Control latency}}{\text{Cut off time - Control latency}} \times 100$$

Analgesic activity (Hot Plate):

Using the Hot Plate technique, analgesic activity was assessed. For the study, albino wistar rats of either sexes weighing between 150 and 200 gm were chosen and divided into four groups of six animals (n=6) each and marked with picric acid and were treated with vehicle, standard drug Diclofenac gel 1%, Naproxen (10% naproxen gel containing 100 mg naproxen/gm) and optimized formulation NANEs (10% naproxen Nonaqueous nanoemulsion containing 100 mg naproxen/ml) topically to all paws of rat. The residual gel and NANEs on the paw's surface were wiped away with a piece of cotton 30 minutes after drug application [7-11].

The animals were placed on a plate kept at 55°C ± 0.5°C. The reaction time was measured by how long it took the animal to either lick its paw or hop off the hot plate. The following treatments were applied, and the reaction time was recorded at 0, 30, 60, 90, and 120 minutes. For this reason, 15 seconds was chosen as the cutoff time to avoid tissue damage.

Group I - Normal (NANEs without Naproxen topically to paw of rats)
Group II - Standard 1 (Diclofenac 1% w/w gel topically)
Group III - Standard 2 (10% naproxen gel containing 100mg naproxen/gm topically)
Group IV - Treated with optimized formulation NANEs (10% naproxen Nonaqueous nanoemulsion containing 100 mg naproxen /ml topically)

The percentage protection against paw licking response was used to assess the % analgesia and was calculated using following formula.

$$\text{Percentage protection (\%)} = \frac{\text{Test latency} - \text{Control latency}}{\text{Cut off time - Control latency}} \times 100$$

Statistical analysis:

Statistical significance was shown by the mean standard error of the mean. Graph Pad Instat version 5 was used to do one-way analysis of variance (ANOVA) and Dunnette's multiple comparison test to determine whether or not statistical significance existed. A statistically significant difference in the means was defined as a P value less than 0.05.

RESULTS

Anti-inflammatory effect in rat paw edema:

Rat paw edoema generated by carrageenan was considerably ($P < 0.01$) reduced when rats were treated topically with optimal NANEs of Naproxen. At 4 hours, NANEs showed the greatest effect in reducing paw edema compared to the control group.

Table 1: Anti-inflammatory activity using Carrageenan induced paw oedema in rats

Sr. no.	Group (n=6)	Treatment	Paw oedema (volume in ml) at different hrs					% Inhibition after 4 hrs
			0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	
1	Normal	NANEs without Naproxen	0.70±0.008	0.71±0.009	0.71±0.01	0.71±0.01	0.70±0.01	-
2	Inflammatory Control	0.1 mL 1% Carrageenan, sub plantar injection	0.69±0.007	1.14±0.01	1.93±0.01	2.15±0.01	2.16±0.009	-
3	Inflammation treated with Standard Drug	10% Naproxen gel containing 100mg naproxen/gm topically 60 min before 0.1	0.71±0.004^{ns}	1.03±0.03*	1.14±0.008*	1.24±0.009**	1.11±0.005**	48.61

		mL 1% Carrageenan						
4	Inflammation treated with Optimized formulation	10% Naproxen NANEs containing 100 mg naproxen/ml topically 60 min before 0.1 mL 1% Carrageenan	0.72±0.003^{ns}	0.96±0.006*	1.17±0.03*	1.14±0.005**	1.06±0.008**	50.92

ns- Nonsignificant, P> 0.05, * P< 0.05, **P<0.01 One-way analysis of variance followed by Dunnette's multiple comparison test was used to compare experimental and control groups for inflammation; experimental values are presented as Mean ± SEM (n=6).

Evaluation of Analgesic activity:

Analgesic activity using the tail-flick test in rats:

Compared to normal animals, topical treatment of rats with optimal NANEs of Naproxen significantly (P < 0.01) increased latency to flip tail. At 120 minutes, the Optimized formulation showed the greatest nociception inhibition. At 120 minutes, the maximal nociception inhibition by Diclofenac was observed.

Table 2: Analgesic activity using the tail-flick test in rats

Sr. no.	Group (n=6)	Treatment	Tail Flick Latency (Seconds)					% Analgesia after 120 min
			0 min	30 min	60 min	90 min	120 min	
1	Normal	NANEs without Naproxen	3.25±0.008	4.08±0.10	3.63±0.08	4.38±0.06	3.80±0.05	-
2	Standard 1	Diclofenac 1% w/w gel topically	3.51±0.09^{ns}	4.81±0.09*	6.45±0.07**	8.56±0.10**	9.50±0.06**	50.89
3	Standard 2	10% naproxen gel containing 100 mg naproxen/gm topically	3.15±0.04^{ns}	4.50±0.05*	6.01±0.06*	8.05±0.07*	9.00±0.05*	46.42
4	Optimized formulation	10 % Naproxen NANEs containing 100 mg aproxen/ml topically	3.68±0.05^{ns}	4 .38±0.08^{ns}	6.20±0.05*	8.08±0.08*	9.25±0.04**	48.66

ns –non significant, P> 0.05, * P< 0.05, **P<0.01 When compared with normal, values are presented as the mean ± standard error of the mean (n=6), and analysis of variance (ANOVA) is followed by Dunnette's multiple comparison test.

Analgesic activity (Hot Plate test) in rats:

When compared to normal animals, the paw-licking reaction of the rats that were given a topical therapy with optimal NANEs of naproxen had a significant (P < 0.01) decrease. At a time point of 120 minutes, the Optimized formulation showed the greatest degree of nociception inhibition. At a duration of 120 minutes, diclofenac demonstrated the greatest degree of nociception inhibition.

Table 3: Analgesic activity using the Hot plate test in rats

Sr. no.	Group (n=6)	Treatment	Paw licking response (Seconds)					% Analgesia after 120 min
			0 min	30 min	60 min	90 min	120 min	
1	Normal	NANEs without Naproxen	3.25±0.07	4.16±0.04	3.55±0.07	4.45±0.07	5.05±0.07	-
2	Standard 1	Diclofenac 1% w/w gel topically	3.50±0.03^{ns}	5.05±0.07*	6.48±0.06**	9.05±0.07**	10.45±0.08**	54.27
3	Standard 2	10% naproxen gel containing 100 mg naproxen/gm topically	3.45±0.04^{ns}	4.65±0.04*	6.05±0.07*	8.05±0.08*	8.98±0.11*	39.49
4	Optimized formulation	10 % Naproxen NANEs containing 100 mg naproxen/ml topically	3.60±0.05^{ns}	4.88±0.06*	6.38±0.06**	8.48±0.07**	9.18±0.14**	41.50

ns –non significant, P> 0.05, * P< 0.05, **P<0.01 Normality testing was performed using one-way ANOVA and Dunnette's multiple comparison test, and all values were expressed as Mean ± SEM for a sample size of 6.

DISCUSSION

Naproxen, when applied topically in the form of optimised NANEs, considerably reduced the extent of edema generated by carrageenan injection into the subplantar area of each rat's left hind paw, as seen in the current investigation. NANEs of Naproxen applied topically to rats significantly ($P < 0.01$) increased latency to flick tail and decreased paw licking response in the tail-flick and hot plate tests, indicating analgesic efficacy. Experiments evaluating the analgesic and anti-inflammatory effects of improved NANEs of Naproxen show that all the examined compounds had a respectable impact on pain and inflammation.

It demonstrated the efficacy of Naproxen's improved NANEs, allowing for the pursuit of additional clinical studies.

REFERENCES

1. Bhagat, B. V., & Rachh, P. R. (2021). Formulation and development of lipid based non-aqueous nano emulsion for selected NSAID. *Journal of Advanced Scientific Research*, *12*(04 Suppl 1), 79-89.
2. Okur, N. Ü., Apaydın, Ş., Yavaşoğlu, N. Ü. K., Yavaşoğlu, A., & Karasulu, H. Y. (2011). Evaluation of skin permeation and anti-inflammatory and analgesic effects of new naproxen microemulsion formulations. *International journal of pharmaceutics*, *416*(1), 136-144.
3. Alhakamy, N. A., Kotta, S., Ali, J., Alam, M. S., Hosny, K. M., Shaik, R. A., ... & Md, S. (2021). Formulation Development, Statistical Optimization, In Vitro and In Vivo Evaluation of Etoricoxib-Loaded Eucalyptus Oil-Based Nanoemulgel for Topical Delivery. *Applied Sciences*, *11*(16), 7294.
4. Gupta, S. K., Bansal, P., Bhardwaj, R. K., Jaiswal, J., & Velpandian, T. (2002). Comparison of analgesic and anti-inflammatory activity of meloxicam gel with diclofenac and piroxicam gels in animal models: pharmacokinetic parameters after topical application. *Skin Pharmacology and Physiology*, *15*(2), 105-111.
5. Abdallah, F. I., Dawaba, H. M., Mansour, A., & Samy, A. M. (2011). Evaluation of the anti-inflammatory and analgesic effects of piroxicamloaded microemulsion in topical formulations. *Int J Pharm Pharm Sci*, *3*(2), 66-70.
6. Vogel, H.G. (2002). Drug Discovery and Evaluation: Pharmacological Assays, *Springer-Verlag Berlin Heidelberg*, 2, 725-771.
7. Tunner R.A. (1971). Screening Methods in Pharmacology, *Academic Press*, 227-233.
8. Kandadi, P., Syed, M. A., Goparaboina, S., & Veerabrahma, K. (2012). Albumin coupled lipid nanoemulsions of diclofenac for targeted delivery to inflammation. *Nanomedicine: Nanotechnology, Biology and Medicine*, *8*(7), 1162-1171.
9. Winter, C. A., Risley, E. A., & Nuss, G. W. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proceedings of the society for experimental biology and medicine*, *111*(3), 544-547.
10. Niemegeers, C. J. E., Verbruggen, F. J., & Janssen, P. A. J. (1964). Effect of various drugs on carrageenin-induced oedema in the rat hind paw. *Journal of Pharmacy and Pharmacology*, *16*(12), 810-816.
11. Randall, L. O. (1957). A method for measurement of analgesic activity of inflamed tissue. *Arch Int Pharmacodyn Ther*, *111*, 409-411.

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