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Evaluation of anti-inflammatory and analgesic activity of optimized lipid based non-aqueous nanoemulsion of naproxen in experimental animals

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***Abstract*---** Naproxen is a nonsteroidal anti-inflammatory drug, it has analgesic, anti-inflammatory and antipyretic effect by inhibiting prostaglandin synthesis via inhibition of cyclooxygenase enzymes. This study was designed to evaluate analgesic and Anti-inflammatory effect of optimized NANEs (Non-aqueous Nano Emulsion) of Naproxen in experimental animals. The anti-inflammatory activity of the optimized NANEs of Naproxen was evaluated using right hind paw oedema size of rats induced by carrageenan injection and analgesic effect was evaluated using Hot Plate method and Tail-flick method applied on rats. Results related to the anti-inflammatory activity revealed that the optimized NANEs of Naproxen produced a maximum percent oedema inhibition as compared to standard naproxen formulation. Similarly, the analgesic effect of the optimized NANEs of Naproxen shows 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.

Introduction

Naproxen is an NSAID (Nonsteroidal Anti-inflammatory Drug) that shows anti-inflammatory, anti- rheumatoid arthritis, analgesic and antipyretic activities in animal models. The mechanism of action of Naproxen like that of other NSAIDS, may be related to inhibition of prostaglandin synthesis. Non-steroidal anti-inflammatory drug`s oral administration contraindicated in patients with peptic ulcer disease, gastro esophageal reflux (GERD), irritable bowel syndrome, or other gastrointestinal disorders. Administration to drug through skin could reduce the above-mentioned side effects.

Inflammation plays important roles in combating the pathogen and saving the integrity of the organism. Inflammation activates cells as macrophages to destroy the pathogen and produce the specific immune response. Then it provides a physical barrier to prevent the spread of the pathogen. Finally, it initiates the repair of the injured tissue. Inflammation could be produced by the presence of antigen, or by tissue damage. Inflammation is a complex process that leads to production of various humoral mediators like kinins, leukotrienes, prostaglandins and cytokines. In many cases of inflammation, membrane phospholipids are converted to arachidonic acid by phospholipase A2. Cyclooxygenase acts to convert arachidonic acid to prostaglandins and thromboxanes, whereas lipoxygenase acts to convert arachidonic acid to leukotrienes. In chronic inflammation corticosteroid concentration get increased. It inhibits the leukotrienes and decreasing inflammation. After any tissue injury pain can be developed, but without tissue damage, strong stimuli could be also painful. Pain sensitivity is dynamically changing with the actual physiological status: pain is sometimes absent when tissue damage is obvious and ongoing. Tissue injury is the main inducer of pain, leads to local accumulation of chemical mediators that can strongly activate nociceptors. Cell membrane distress by injurious forces or agents leads to activation of membrane bound enzymes particularly phospholipase A2, which produce arachidonic acid from membrane phospholipids. Arachidonic acid is the substrate for enzymatic cascades that generate within seconds prostaglandins, thromboxanes and leukotriens. These all products are mediators of pain and inflammation. Thus, tissue injury induces enzymatic cleavage of circulating high-molecular-weight kininogen to produce bradykinin, another potent mediator of pain and inflammation. Further, mast cells in damaged tissue degranulate, releasing histamine and chemotactic agents that promote infiltration of injured tissue with neutrophils and eosinophils. Many tissues injury-induced chemical mediators like histamine, bradykinin and prostaglandins that leads to induce dilatation and increase permeability of tissue capillaries. As a result of this localized edema develop. Thus, this work was aimed to study the anti-inflammatory and analgesic effects of optimized lipid based non-aqueous nano emulsion of Naproxen.[1, 2,3,4,5]

Materials and Methods

Chemicals

Naproxen as a drug was obtained from drug laboratory of DVVPFs college of Pharmacy, Ahmednagar (MH), India. Carrageenan was purchased from Dolphin

Instruments Pvt Ltd, Mumbai, Ethanol 95% was purchase from Modern Science Apparatus Pvt Ltd, Nashik. All other material used in this study are of analytical grade.

Preparation of lipid based non-aqueous nano emulsion

Lipid based non-aqueous nano emulsion was prepared in college lab at Department of Pharmaceutics of Dr Vithalrao Vikhe Patil Foundations College of Pharmacy, Ahmednagar (Maharashtra). From the preliminary studies by using different method of preparation of NANEs, it is clear that 10 % Naproxen as a drug, 5% Glycerol Monostearate as a surfactant, 5 ml Mineral oil as a continuous phase and 5 ml of Glycerine as a dispersed phase are used to prepared NANEs of Naproxen. 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 remis 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 animals had free access to rat food pellet (purchased from Prashant Enterprises, Pune) and tap water *ad libitum*. Before performing the experiment, the ethical clearance was obtained from the Institutional Animal Ethics Committee (Coph/IAEC/ 2021/02).[6,7,8]

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

The albino wistar rats of either sex weighing between 150-200gm was selected for study and divided into four groups of 6 animals (n=6) each and marked with picric acid and was treated with vehicle,0.1 mL 1% Carrageenan in to the sub plantar tissue to Group II, standard drug Naproxen (10% naproxen gel containing 100 mg naproxen/gm topically) and optimized formulation of NANEs, F1 (10% Naproxen Nonaqueous nanoemulsion containing 100 mg naproxen /ml topically) to the sub plantar tissue of left hind paw by gently rubbing 50 times with the index finger. 1 hrs after receiving the above-mentioned treatments all the rats in groups II, III and IV except group I, injected 0.1 ml of 1% Carrageenan into the sub plantar tissue of left hind paw. Swelling of carrageenan injected foot was measured at 0, 1, 2, 3, 4 hrs using Digital Plethysmometer (Laboratory enterprises). The right hind paw was applied with 0.1 ml of vehicle (NANEs without Naproxen topically).[6,7,8,9]

Group I - Normal (NANEs without Naproxen topically)
Group II - Inflammatory Control (received 0.1 mL 1% Carrageenan in to the sub plantar tissue)
Group III - Inflammation treated with standard drug (topically 10% naproxen gel containing

100 mg naproxen /gm 60 min before 0.1 mL 1% Carrageenan)

Group IV - Inflammation treated with optimized formulation, F1 (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:

a. Analgesic activity using the tail-flick test in rats

Analgesic activity was evaluated by the tail-flick method. The albino wistar rats of either sex weighing between 150-200gm were selected for study and divided into four groups of 6 animals (n=6) each and marked with picric acid and were treated with vehicle, standard drug Diclofenac (Voveran Emulgel 1%) from Novartis India Ltd, Naproxen (10% naproxen gel containing 100 mg naproxen/gm) and optimized formulation NANEs, F1 (10% naproxen Nonaqueous nanoemulsion containing 100 mg naproxen/ml) topically to 3-5 cm portion of rat tail. 30 minutes after the drug application, the gel, NANEs remaining on the surface of the skin was wiped off with piece of cotton. The distal 2 - 3 cm portion of rat tail was immersed in hot water maintained at 55 ± 0.5ºC. The time taken by the rat to withdraw the tail from hot water was noted as reaction time. The reaction time was recorded at 0, 30, 60, 90 and 120 min after the application of the below treatments. The cut off time was considered as 15 second to prevent tissue injury.[7,10]

Group I - Normal (NANEs without Naproxen topically to tail portion)

Group II - Standard 1 (Diclofenac 1% w/w emulgel 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.

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

b. Analgesic activity using the Hot Plate test in rats:

Analgesic activity was evaluated by the Hot Plate method. The albino wistar rats of either sex weighing between 150-200gm were selected for study and divided into four groups of 6 animals (n=6) each and marked with picric acid and were treated with vehicle, standard drug Diclofenac (Voveran Emulgel 1%) from Novartis India Ltd, 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. 30 minutes after the drug application, the gel, NANEs remaining on the surface of the paw was wiped off with piece of cotton.

Animals were placed on a hot plate maintained at a temperature of 55 ± 0.5°C. The latency to lick the paw or jump from the hot plate was noted as the reaction time. The reaction time was noted at 0, 30, 60, 90 and 120 min after the application of the below treatments. The cut off time was considered as 15 second to prevent tissue injury.[7,11]

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 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.

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

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test using Graph Pad Instat version 5. A difference in the mean P value <0.05 was considered as statistically significant.[7,11]

Results

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

Topical treatment of the rats with optimized NANEs of Naproxen significantly ($P < 0.01$) inhibited carrageenan induced rat paw edema as compared to control group. Maximum inhibition of paw edema was observed with NANEs at 4 hrs when 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
			0	1	2	3	4	
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,	0.69±0.007	1.14±0.01	1.93±0.01	2.15±0.01	2.16±0.009	-

		sub plantar injection						
3	Inflammation treated with Standard Drug	10% Naproxen gel containing 100mg naproxen/gm topically 60 min before 0.1 mL 1% Carrageenan	0.71±0.004^{ns}	1.03±0.03*	1.14±0.008*	1.24±0.009**	1.11±0.005**	48.61
4	Inflammation treated with Optimized formulation F1	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 Values are Mean ± SEM, n=6, when compared with inflammatory control by using one way ANOVA followed by Dunnette's multiple comparison test.

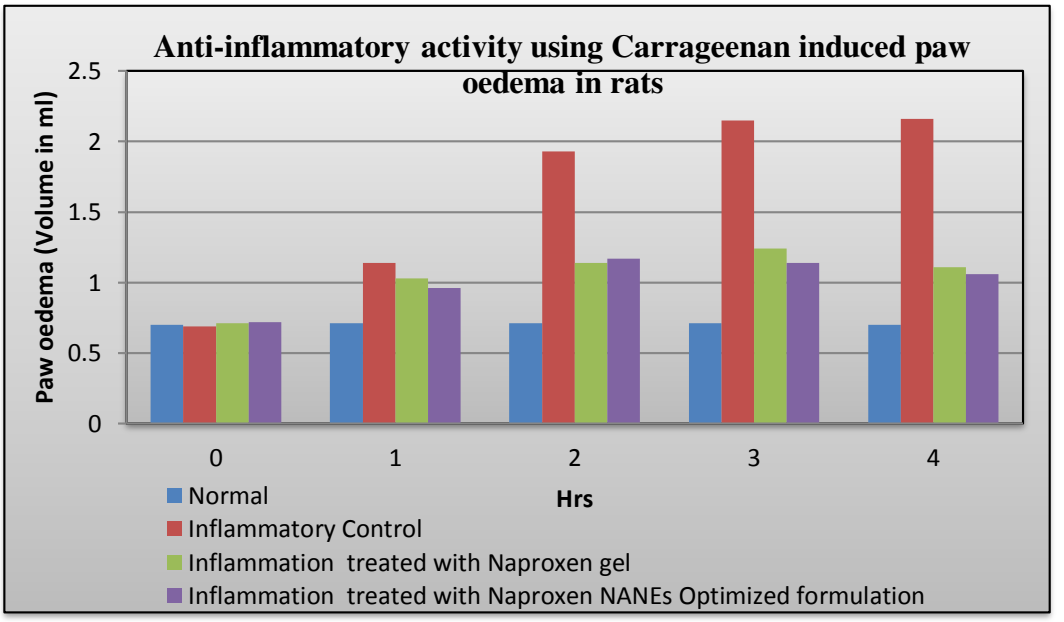

Figure 1: Anti-inflammatory activity using Carrageenan induced paw oedema in rats

Evaluation of Analgesic activity:
a. Analgesic activity using the tail-flick test in rats:

Topical treatment of the rats with optimized NANEs of Naproxen significantly ($P < 0.01$) increased latency to flick tail as compared to normal animals. The highest nociception inhibition was exhibited by Optimized formulation at 120 min. The maximum nociception inhibition by Diclofenac was observed at 120 min.

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

Sr. no.	Group (n=6)	Treatment	Tail Flick Latency (Second) at min					% Analgesia
			0	30	60	90	120	
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 naproxen/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 –nonsignificant, P> 0.05, * P< 0.05, **P<0.01 Values are Mean ± SEM, n=6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test.

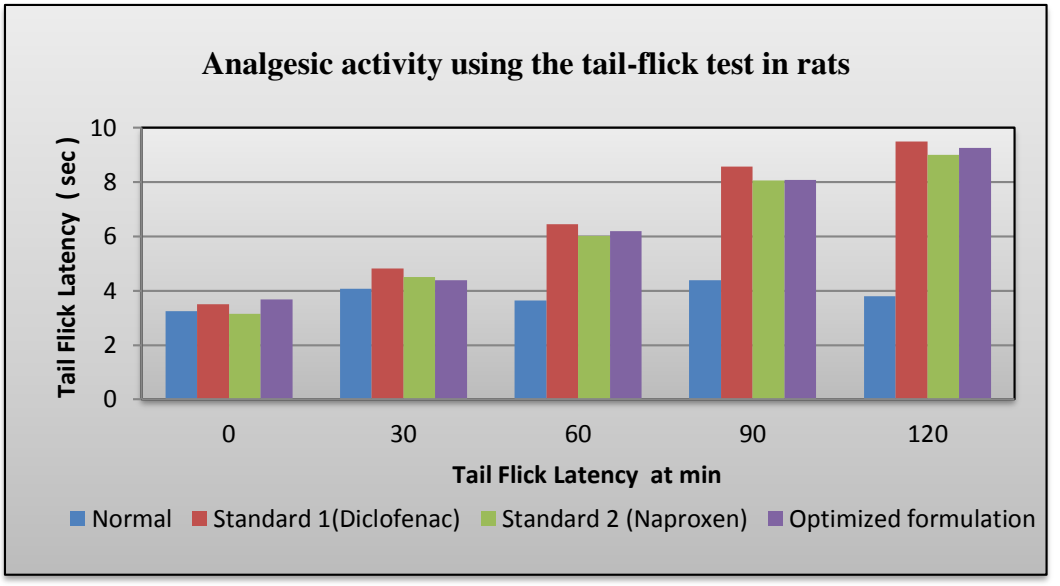

Figure 2: Analgesic activity using the tail-flick test in rats

b. Analgesic activity using the Hot Plate test in rats:
Topical treatment of the rats with optimized NANEs of Naproxen significantly ($P < 0.01$) reduced paw licking response as compared to normal animals. The highest nociception inhibition was exhibited by Optimized formulation at 120 min. The maximum nociception inhibition by Diclofenac was observed at 120 min.

Table 3
Analgesic activity using the Hot plate test in rats

Sr. no.	Group (n=6)	Treatment	Paw licking response (second) at min					% Analgesia
			0	30	60	90	120	
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 –nonsignificant, P> 0.05, * P< 0.05, **P<0.01 Values are Mean ± SEM, n=6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test.

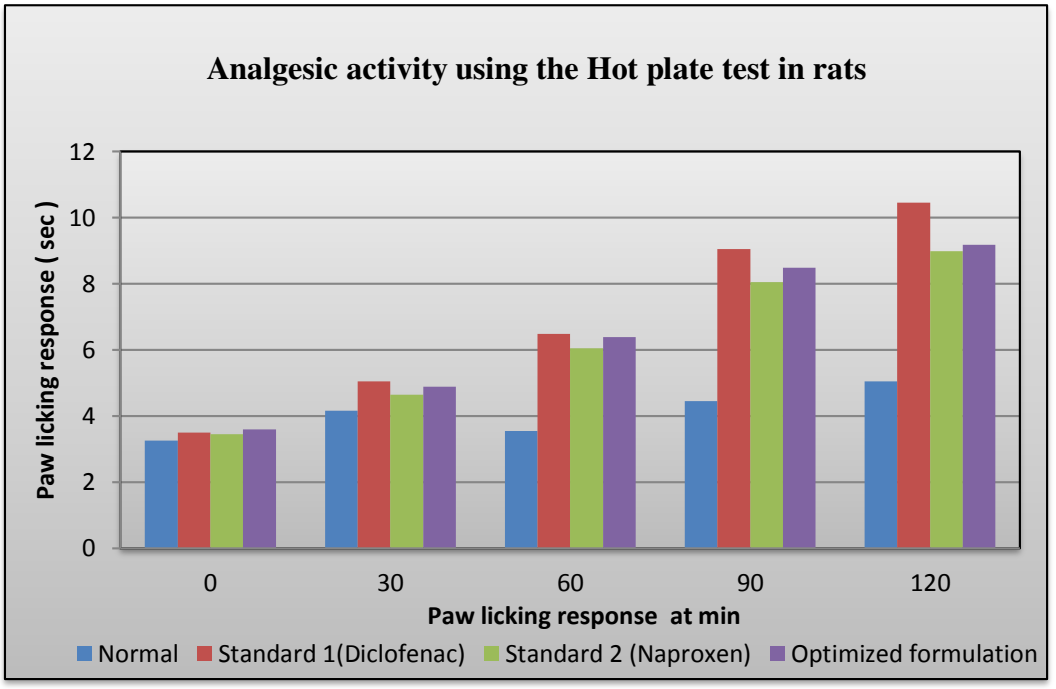

Figure 3: Analgesic activity using the Hot plate test in rats

Discussion

In present study it was observed that anti-inflammatory effect after topical application of optimized NANEs of Naproxen to the experimental rats shows significantly inhibits the edema size induced by carrageenan injection into the subplantar area of the right hind paw for each rat. In case of analgesic effect by tail-flick and hot plate test, topical treatment of the rats with NANEs of Naproxen significantly ($P < 0.01$) increased latency to flick tail and reduced paw licking response as compared to normal animals respectively. After carrying out the experiments for evaluation of both the analgesic and the anti-inflammatory effects of optimized NANEs of Naproxen, it is clear that all the studied have an acceptable analgesic and anti-inflammatory effect.

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