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Research Article

FORMULATION, *IN-VITRO* AND *IN-VIVO* EVALUATION OF NANOEMULSION GEL FOR TRANSDERMAL DRUG DELIVERY OF NIMODIPINE

SHEELA A YADAV[1,2*], SUSHILKUMAR S PODDAR[3]

[1]Department of Pharmaceutical Science, Nims Institute of Pharmacy, Nims University, Jaipur - 303 121, India. [2]Department of Pharmaceutics, HK College of Pharmacy, Mumbai - 100 102, India. [3]Department of Pharmaceutics, Prin. K.M. Kundnani College of Pharmacy, Mumbai - 400 005, India. Email: sheel.ved05@gmail.com

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ABSTRACT

Objective: The objective of this study was to develop and evaluate the potential of nanoemulsion (NE) as drug carrier system for transdermal delivery of nimodipine.

Methods: Nimodipine NE was developed through titration method. This was then formulated in to gel. Transdermal *in-vitro* permeation of nimodipine through wistar rat abdominal skin was determined with Franz diffusion cell. The *in-vitro* skin permeation profile of optimized formulation was compared with NE gel (NEG), control or drug loaded neat components.

Result: Significant increase in the steady state flux (J_{ss}), permeability coefficient (k_p) and enhancement ratio was observed in the NE formulation and were compared with other formulations. The highest value of the permeability coefficient was obtained in the optimized NE formulation consisting of 0.008% w/w of nimodipine, 8.00% w/w of triacetin:isopropyl myristate (1:1), 32.00% w/w of S_{mix} (2:1 Tween 80 and PEG-400) and 60.00% w/w of distilled water. The bioavailability studies in wistar rat showed about 3 times improvement for transdermal administration of NEG compared with an oral suspension. The present work also evaluated the transdermal product on blood pressure of methyl prednisolone acetate induced hypertensive rats.

Conclusion: The results of the present investigation suggested that NE could be a potential vehicle for improved transdermal delivery of nimodipine.

Keywords: Nimodipine, Nanoemulsion, Gel, Transdermal delivery, Anti-hypertensive.

INTRODUCTION

Transdermal drug delivery system (TDDS) helps entry of a drug into the systemic circulation via permeation through skin layers at a controlled rate. The TDDS are easy to apply and remove as and when desired [1,2]. This approach of drug delivery is more pertinent in the case of chronic disorders, such as hypertension, which require long-term dosing to maintain therapeutic drug concentration.

Nanoemulsions (NE) have received a growing attention as colloidal drug carrier for pharmaceutical applications. Typically, NE consists of oil, surfactant, co-surfactant and aqueous phase. It is transparent, thermodynamically stable with droplet diameter usually within the range of 10-100 nm and does not have the tendency to coalesce. NE has several advantages such as enhanced drug solubility, good thermodynamic stability and often better therapeutic effect compared to conventional formulations. In addition, it can accommodate both hydrophilic and lipophilic drugs [3,4]. Numerous studies have been conducted in the recent past that showed the significance of these systems for dermal and transdermal delivery both *in vitro* [5-15] and *in vivo* [16-21].

Nimodipine, an antihypertensive drug belonging to the class of calcium channel blocker, has been found to be a good candidate for transdermal drug delivery. The bioavailability of nimodipine from oral formulations is only 13% due to hepatic first-pass metabolism. The drug has a biological half-life of 1-2 hrs only, which makes frequent dosing necessary to maintain the drug within the therapeutic blood levels for extended periods [22].

Conventional tablets of nimodipine, currently available are limited by the unreliable oral absorption and need for frequent dosing, which leads to adverse effects and poor patient compliance. TD system is potential to present a better alternative to the oral therapy.

Certain reports of success have been reported [23]. However, no work has been reported on the Nimodipine TDDS through nanoemulaion. In the current work, this option would be exploited.

METHODS

Components

Nimodipne was a gift sample from USV (Mumbai, India). Oleoyl macrogol-6 glycerides/glycerides (labrafil 1944CS), propyleneglycoldicaprylate/dicaprate (labrafac PG), PEG-8 caprylic/capric glycerides (labrasol), propylene glycol monocaprylate (Capryol PGMC), diethylene glycol monoethylether (transcutol P) were courtesy Gattefosse SAS (France). Castor oil, olive oil and soybean oil were purchased from Genuine chemicals (Mumbai, India). Triacetin (glycerin triacetate), tween 80, tween 20 and polyethylene glycol 200 (PEG-200) were purchased from Ozone chemicals (Mumbai, India). PEG-400, propylene glycol n-butanol were purchased from E-Merck (Mumbai, India). Isopropyl myristate (IPM) was purchased from standard deviation Fine chemicals (Mumbai, India). High-performance liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from Finar chemical (Ahmedabad, India). Water was obtained from Mili Q water purification system (Milipore, MA). All other chemicals and solvents procured from the local market in Mumbai were of analytical grade.

Preparation of NE

Details of the screening and selection procedure for oils, surfactants and co surfactants have been presented in our previous report [24]. Various NE were prepared by way of the aqueous phase titration (spontaneous emulsification) method. The composition of NE was selected on the basis of the pseudoternary phase diagram. NE formulation of nimodipine was prepared by dissolving 0.008% w/w of nimodipine in the oil phase triacetin+IPM (1:1) followed by incorporation of required quantity of surfactant mixture Tween 80 and PEG-400 (S_{mix})

and imparting agitation with a vortex mixer (Dolphin, Mumbai, India). The final preparation was made up to volume by slow addition of water with continued mixing. The composition of the NE is given in Table 1. Nimodipine dissolved in Tween 80 was prepared to serve as a control. To study the permeation from neat components (oil and S_{mix}), the same amount of drug dissolved in the surfactant mixture was used. While for oil, drug saturated oil phase was used.

In-vitro permeation studies

Preparation of rat skin

The animal protocol to carry out skin permeation study was approved by the Institutional Animal Ethics Committee (Approval No. OCP/CPCSEA/IAEC/2012/004). Male wistar rats weighing between 200 and 250 g were sacrificed with prolonged ether anesthesia, hair on the skin was removed with a depilatory. The abdominal skin of each rat was excised. Subcutaneous tissue was carefully removed, and dermis side was wiped with isopropyl alcohol to remove residual adhering fat.

Permeation studies

The skin permeation rates of nimodipine from various NE were determined to evaluate the effect of a range of factors. The effect of the S_{mix} on the permeation of nimodipine through the skin was evaluated. The permeation studies were performed using Franz diffusion cell apparatus. The effective diffusion area in the cell was 0.706 cm^2 and receptor volume was 15 ml. The skin samples were mounted between the donor and receptor compartments of the diffusion cell with the stratum corneum side facing upward. The receptor compartment was filled with 10% Tween 80- phosphate buffer (pH 7.4) and magnetically stirred at 600 rpm. The diffusion cell was maintained at 37°C using a recirculating water bath. The test NE samples (1 ml) were placed into the donor compartment and sealed with soft paraffin film to provide occlusive condition and to prevent the evaporation of water from the formulation. Samples from the receptor compartment were withdrawn at regular intervals (2, 4, 6, 8, 10, 12, 24 and 48 hrs) filtered through 0.45 µm membrane filter and analyzed for drug content employing HPLC at 238 nm.

Data analysis

The cumulative amount of nimodipine that got permeated through the skin (Q, µg/cm^2) was plotted as a function of time (t, h). The drug flux (permeation rate) at steady state (J_{ss}, µg/cm^2/h) was calculated from the slope of the linear portion of the curve. Cumulative amount of drug permeated through the skin (µg/cm^2) was plotted as a function of time for each formulation. Drug flux (permeation rate) at steady state (J_{ss}) was calculated by dividing the slope of the linear portion of the graph (Table 2) with the diffusion cell area (µg/cm^2/h). Permeability coefficient (K_p) was calculated via dividing J_{ss} by the initial concentration of the drug in the donor cell (cm/h).

Preparation of NE gel (NEG)

The NE was insufficiently viscous for its adequate retention on the skin. Therefore, the optimized NE was converted into a gel. NEG was prepared by dispersing 1% w/w of carbapol 934 in sufficient quantity of distilled water. The dispersion was stored for 24 hrs for complete swelling of the carbopol. Nimodipine NE was added slowly to the carbapol dispersion. Triethanolamine (0.5% w/w) was added to neutralize carbapol resulting in gel formation. Formula is shown in Table 1.

In-vivo studies

The animals used for *in-vivo* experiments were adult male or female wistar albino rats (200-250 g) procured from the central animal house of the Oriental college of Pharmacy (Sanpada, Navi Mumbai, India). They were kept under standard laboratory conditions, at 25±1°C and 55±5% relative humidity with a 12 hr light/dark cycle. They were housed in polypropylene cages, 4 per cage, with free access to a standard laboratory diet (Lipton feed, Mumbai, India) and water ad libitum. Guidelines of the institutional animal ethics committee were followed for the experiments.

Pharmacokinetic evaluation of NEG on animals

The bioavailability of nimodipine from NE based gel (8 mg in 1 g of NEG) was compared with an oral suspension. The later was prepared by suspending 8 mg of nimodipine in 5 ml of water (containing 0.5% w/v of sodium carboxy methyl cellulose). The animals were selected after superficial examination of the skin surface for abnormalities. Rats weighing between 200 and 250 g were shaved on the abdomen for the study. Before the application of the gel, rats were kept under observation for 24 hrs for any untoward effect of shaving; they were fasted over this period. They were divided into 2 groups (n=6). Group I was administered nimodipine orally through feeding tube followed by rinsing with 10 ml water and Group II received NEG dermally. The gel was applied over a skin surface area of 4 cm^2 and was covered with a water impermeable back up membrane which was further fixed with the help of an adhesive membrane. Blood samples from marginal ear vein were collected at different time intervals (2, 4, 6, 8, 10, 12, 24, and 48 hrs). All the samples were allowed to clot, centrifuged in tubes and stored at −20°C until HPLC analysis. The amount of nimodipine in the samples was estimated using HPLC (Gannu *et al*. 2009).

Efficacy of NEG against hypertension in rats

A blood pressure (BP) measuring instrument (Digital stoelting) with a non-invasive tail cuff and a digital BP display panel was used. The rats were trained to stay in the rat holders in a calm and non-aggressive state during BP measurement. After recording the initial BP of rats, they were divided into 4 groups of 6 each. Group I was taken as control. Hypertension was induced in the remaining 3 groups by subcutaneous injection of methyl prednisolone acetate (MPA) (20 mg/kg/week). 2 weeks later, all the rats with a minimum mean BP of 150 mm Hg were selected. After MPA treatment, group II served as toxic control and received no further treatment. Group III received nimodipine orally (8 mg in 5 ml Water). Group IV receives NEG (1 g). BP was recorded at different time intervals (2, 4, 6, 8, 10, 12, 24 and 48 hrs).

Skin irritation test

This test was performed using six rats. Hair of rats on the dorsal side was removed with depilatories and NEG formulation (1 g) was applied. The development of erythema was monitored for 7 days.

Table 1: Composition of the selected NE and NEG formulations

Formulation code	Nimodipine[a]	Oil[a]	S_{mix}[a]	Water[a]	Carbapol 934[a]	Triethanolamine[a]
NF1	0.008	11.11	44.44	44.44[b]	-	-
NF2	0.008	10.00	40.00	50.00[b]	-	-
NF3	0.008	09.09	36.36	54.55[b]	-	-
NF4	0.008	08.00	32.00	60.00[b]	-	-
NF5	0.008	11.11	44.44	44.44[c]	-	-
NF6	0.008	10.00	40.00	50.00[c]	-	-
NF7	0.008	09.09	36.36	54.55[c]	-	-
NF8	0.008	08.00	32.00	60.00[c]	-	-
NEG	0.008	08.00	32.00	58.15[b]	1	0.5

[a]% w/w of components, [b]S_{mix} 1:1, [c]S_{mix} 2:1, NF: Nanoemulsion formula, NEG: Nanoemulsion gel, NE: Nanoemulsion

Histopathological examination of skin specimens

Abdominal skin of wistar rat was treated with optimized NEG formulation. After 48 hr, they were sacrificed, and the skin samples from treated and untreated (control) area were taken. Each specimen was stored in 10% formalin solution in phosphate buffer saline (pH 7.4). Specimen was cut into sections vertically. Each section was dehydrated using ethanol, embedded in paraffin for fixing and stained with hematoxylin and eosin. The samples were then observed under optical microscope (Motic, Japan) and compared with control sample.

RESULT AND DISCUSSION

The excipients selected were pharmaceutically acceptable ingredients. NE containing 0.008% w/w nimodipine were prepared using triacetin+IPM (1:1) as the oil phase, Tween 80 as the surfactant and PEG-400 as the cosurfactant employing phase titration (spontaneous emulsification) method (Table 1). The construction of phase diagrams made it easy to find out the concentration range of components for the existence range of NE. No change was found in the phase behavior of the pseudoternery phase diagram when the nimodipine was loaded in the formulations. The ratio of Tween 80 and PEG-400 (S_{mix}) was selected at 2:1 and 1:1 [25].

***In-vitro* skin permeation studies**

The *in-vitro* permeation profile is an important tool that predicts in advance how a drug would behave *in vivo* [26]. The effect of the S_{mix} on the skin permeation of nimodipine was evaluated. Different NE was prepared for this purpose with two different S_{mix} ratios *viz.* 2:1 and 1:1. The composition of NE has been shown in Table 1. The permeation parameters of the tested NE formulations are presented in Table 2. The permeation profiles of nimodipine through rat skin from various vehicles are shown in Figs. 1 and 2.

Table 2: Permeability parameters of NF (mean±SD, n=3)

Formulation code	J_{ss} (µg/cm²/h)	Kp (cm/hr×10⁻³)	ER
NF1	160.92±1.21	20.1±0.24	2.7±0.93
NF2	163.94±2.12	20.41±0.98	2.4±0.98
NF3	167.85±1.98	20.98±1.10	2.9±1.02
NF4	212.10±0.79	26.52±0.98	3.6±0.79
NF5	167.92±0.97	21.10±0.45	2.9±0.72
NF6	173.80±1.56	21.70±1.54	3.0±1.67
NF7	174.90±0.98	21.86±0.87	3.0±0.65
NF8	173.19±1.72	21.60±1.23	3.0±1.09
Neat oil	46.69±0.99	05.80±0.65	0.5±0.25
Neat surfactant	53.07±0.23	06.6±0.55	0.6±0.53
Control	87.18±1.78	07.21±1.51	-

J_{ss}: Flux, K_p: Permeability coefficient, NF: Nanoemulsion formula, ER: Enhancement ratio, SD: Standard deviation

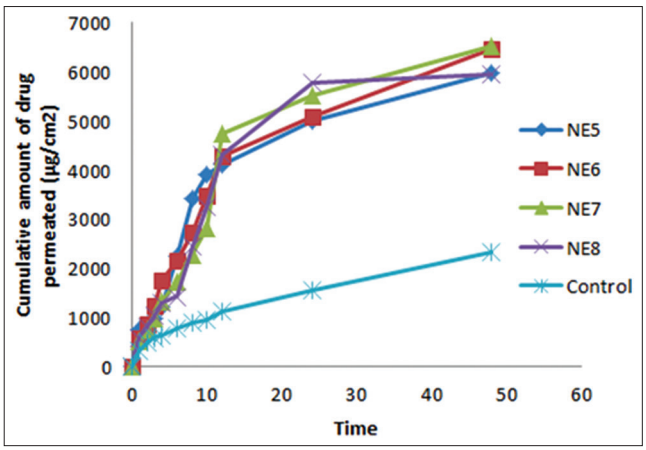

Fig. 1: Permeation profile of nimodipine through excised rat skin from nanoemulsions formulated with S_{mix} 2:1 (mean ± standard deviation, n= 3)

The content of S_{mix} in the NE formulation was found to affect the skin permeation rate of nimodipine directly. As the content of S_{mix} 2:1 increased, the skin permeation decreased. This might be due to a decreased thermodynamic activity of a drug in the NE at the higher content of surfactant [27]. The thermodynamic activity of a drug in the formulation is a significant driving force for the release and penetration of the drug into the skin. With increased surfactant concentration, affinity to the vehicle might have become greater leading to observed slow release of the drug/or a poor transfer from the vehicle to the skin [28]. Another possible reason that could have an additive effect was the hydration effect of water [29]. When the water content was increased in the formulation, the hydration of stratum corneum would have increased. It is the water in NE that could hydrate the skin causing the corneum cells to swell, thus making the channels for drug passage wider. Therefore, with the increased amount of the water in the system, the cumulative permeation amount might have improved.

Permeation of nimodipine was also considered from the neat oil phase as oil has permeation enhancing properties. However, as Fig. 3 shows, a relatively lower flux value (46.69 µg/cm²/h) was obtained when compared to NE formulations, which might be ascribed to the greater affinity of the drug for the oil phase because of its lipophilic nature and therefore lesser partitioning of the drug from the vehicle to the skin ($p<0.01$). Permeation was also carried out from neat surfactant in order to investigate whether the NE had any superior effect. Comparatively far lower flux (53.07 µg/cm²/h) could be attained with

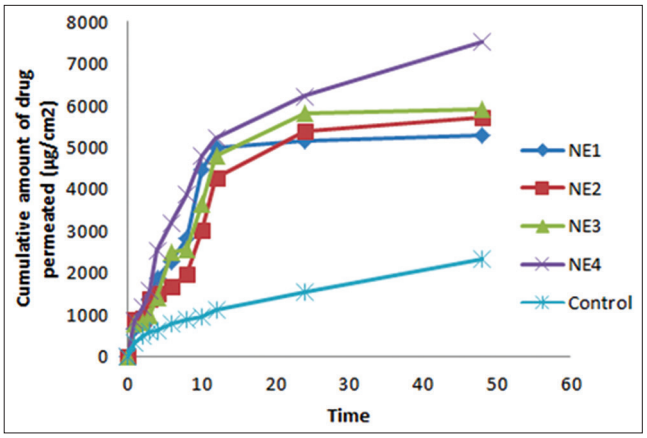

Fig. 2: Permeation profile of nimodipine through excised rat skin from nanoemulsions formulated with S_{mix} 1:1 (mean ± standard deviation, n= 3)

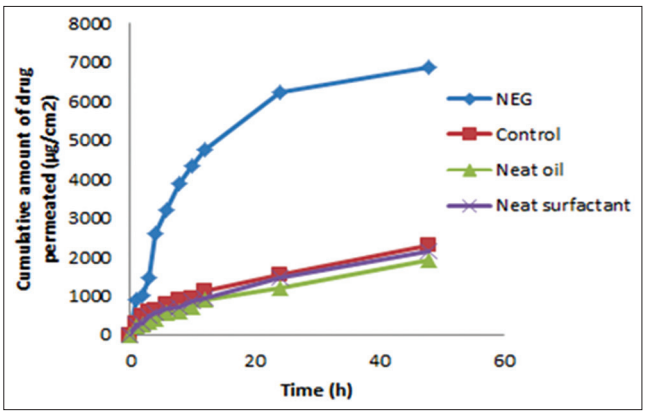

Fig. 3: Comparison of permeation profile of nimodipine through excised rat skin from optimized nanoemulsion gel with control and drug loaded neat component

Tween 80 in contrast with the NE and this was statistically significant ($p<0.01$) (Table 2). Again drug release from the vehicle might have played a crucial role.

Among the formulations tested, the formulation NF4, which was composed of 0.008% nimodipine, 8.00% oil triacetin: IPM (1:1) and 32.00% S_{mix} (1:1) and 60% of water, showed the most favorable permeation profile (Fig. 2). The skin permeation rate of nimodipine from this NE was as high as 212.1 µg/cm^2/hr.

Surfactant present in the NE might cause increased membrane fluidity, solubilisation or extraction of lipid present in the stratum corneum leading to alterations in the tight junction properties [30] which might have become the cause of improved permeation. The nanosized droplets in NE lead to an enormous increase in the interfacial area, which influences transport properties of the drug through the interface [31]. It is assumed in addition that the low interfacial tension and the continuously and spontaneously fluctuating interfaces of NE are supposed to facilitate the transfer of the drug to the skin.

Pharmacokinetic evaluation of gel

Fig.4 shows the blood plasma levels of nimodipine after transdermal and oral administrations. The pharmacokinetic parameters recorded in Table 3 were calculated from the blood plasma concentrations of the drug. The results reveal that nimodipine was released and permeated better from NE transdermal gel as compared to the oral suspension. The maximum drug concentration, C_{max}, after oral administration was 41.2±3.87ng/ml and T_{max} was 2±0.5 hrs. For the NEG, C_{max} and T_{max} were 46.7±11.46ng/ml and 12±3.54 respectively. All the pharmacokinetic parameters obtained with nimodipine NEG were significantly different from those obtained with oral nimodipine administration. The overall mean value of AUC_{0-t} by transdermal route was 3 times higher than that of the oral route, and the difference was found to be statistically significant ($p<0.05$) demonstrating improved bioavailability of nimodipine from NEG. This could be due to avoidance of first-pass hepatic metabolism for transdermal route. Though, oral route hinted faster onset, the formulated transdermal product would lead to better therapy.

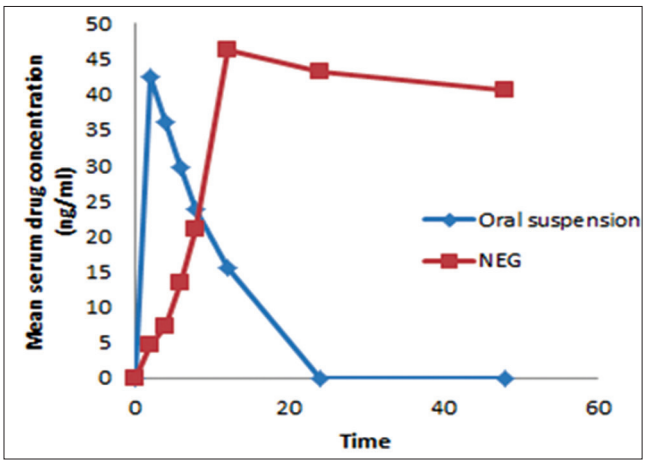

Fig. 4: Nimodipine concentrations in the rat serum after transdermal and oral treatment

Table 3: Pharmacokinetic parameters of Nimodipine oral suspension and transdermal gel administration

Formulasion	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-t} (ng h/ml)	$AUC_{0-\infty}$ (ng h/ml)
Oral suspension	41.2±3.87	2±0.5	424±15.39	472±79.3
Transdermal gel	48.7±11.46	12±3.54	1272±145.21	1521±307.59

Efficacy of transdermal gel against hypertension in rats

The result in Tables 4-7 indicated that the administration of MPA produced significant hypertension in rats. The oral administration of nimodipine significantly ($p<0.05$) controlled the hypertension initially, with the maximum effect observed at 2 hrs, but after 2 hrs the BP started rising until at 48 hrs when it was the same as the initial value. In contrast, the administration of nimodipine through transdermal gel resulted in a gradual decrease of BP, with the maximum effect observed at 12 hrs ($p<0.05$). Despite the fact that the gel produced a peak effect at 12 hrs, it started exhibiting effect ($p<0.05$) right from the 1st hr and continued for 48 hrs. This clearly indicated that the transdermal gel released the drug *in-vivo* gradually over a period, which results in prolonged control of hypertension for 48 hrs. Oral nimodipine acted quickly and drastically, but then its effect dropped off also quickly.

Skin irritation test

The test results are shown in Tables 8 and 9.

The skin irritation test of the transdermal formulations showed a skin irritation score (erythema and edema) of <2. Acoording to Draize *et al*., materials producing score of 2 or less are considered negative (no skin irritation) [32]. Hence, the developed transdermal formulations were free of skin irritation.

Histopathological examination

As visible in the photomicrograph (Figs. 5 and 6) no significant alteration was seen with the treatment of the tween 80/PEG 400 nanoemulsion gel based formulation to the skin. It was found that stratum corneum remained intact.

This study thus, showed that the formulated NE would be a safe carrier for the topical delivery of nimodipine.

CONCLUSION

A novel NE transdermal gel formulation of nimodipine has been successfully attempted. Results have revealed that proper management

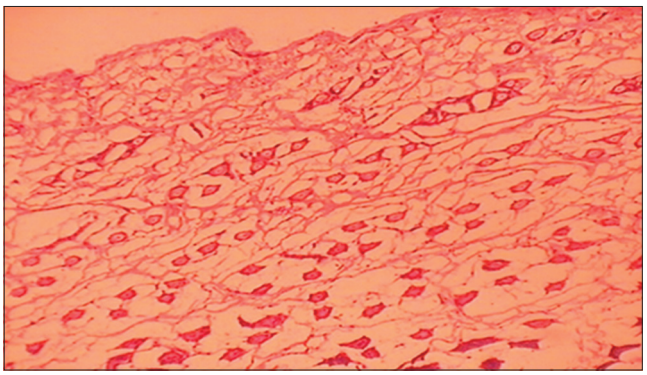

Fig. 5: Photomicrograph of control skin

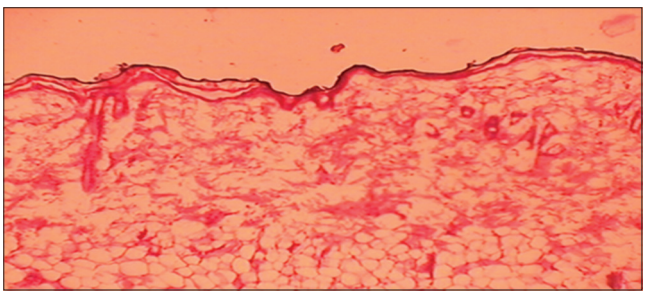

Fig. 6: Photomicrograph of treated skin

Table 4: Reduction in blood pressure level of rats after treatment with NEG without drug (control)

Group	Treatment	Rats	Initial blood pressure level (mmHg±SD)	**Blood pressure at varying time points (mmHg±SD)							
			0	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	24 hrs	48 hrs
A	Control*	1	96±0.9	96±0.7	97±0.7	96±0.9	96±0.9	96±0.8	96±0.9	96±0.4	96±0.6
		2	96±0.7	96±0.8	96±0.7	96±0.8	96±0.8	96±0.7	96±1.2	96±0.3	96±0.9
		3	95±0.5	95±0.7	95±0.7	95±0.9	95±0.9	95±0.9	95±0.6	95±0.5	95±0.7
		4	96±0.3	96±0.5	96±0.6	96±0.8	96±0.7	96±0.9	96±0.5	96±0.5	96±0.6
		5	96±0.7	96±0.6	96±0.8	96±0.9	96±0.6	96±0.7	96±0.5	96±0.7	96±0.6
		6	96±0.6	96±0.5	96±0.9	96±0.8	96±0.8	96±0.8	96±0.7	96±0.6	96±0.6

*No treatment was given, **Mean of three observations±SD, SD: Standard deviation, NEG: Nanoemulsion gel

Table 5: Reduction in blood pressure level of rats after treatment with NEG without drug (placebo control)

Group	Treatmeent	Rats	Initial blood pressure Level (mmHg±SD)	**Blood pressure at varying time points (mmHg±SD)							
			0	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	24 hrs	48 hrs
A	Placebo/ toxic control*	1	157±1.2	152±0.7	155±0.2	151±0.9	156±0.7	153±0.6	152±0.9	155±0.8	152±0.7
		2	158±0.9	155±0.8	155±0.3	158±0.4	154±1.2	153±0.7	152±0.6	157±0.4	156±0.6
		3	157±0.8	154±0.8	156±0.4	157±0.4	153±0.9	152±0.5	158±0.7	156±0.7	154±0.7
		4	158±0.1	152±0.7	154±0.4	157±0.3	156±0.8	154±0.7	156±0.6	153±0.4	157±0.6
		5	157±0.2	157±0.5	154±0.9	154±0.3	153±0.3	156±0.6	157±0.7	153±0.6	153±0.5
		6	158±0.4	152±0.4	156±0.7	153±0.4	156±0.4	154±0.5	157±0.6	154±0.5	155±0.7

NEG: Nanoemulsion gel, *After induction of hypertension no treatment was given, **Mean of three observations±SD, SD: Standard deviation

Table 6: Reduction in blood pressure level of rats after oral treatment with nimodipine suspension (positive control)

Group	Treatment	Rats	Initial blood pressure level (mmHg±SD)	**Blood pressure at varying time points (mmHg±SD)							
			0	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	24 hrs	48 hrs
A	Positive control*	1	154±0.9	88±0.4	98±0.4	106±0.3	112±0.1	122±0.7	132±0.4	139±0.3	152±0.5
		2	152±0.8	90±0.4	97±0.3	110±0.2	120±1.2	129±0.6	135±0.3	142±0.6	155±0.6
		3	156±0.8	87±0.4	99±0.5	113±0.3	121±0.9	130±0.4	135±0.4	144±0.5	156±0.4
		4	155±0.2	87±0.6	97±0.8	108±0.5	125±0.8	134±0.4	137±0.6	142±0.4	147±0.5
		5	156±0.3	88±0,9	92±0.3	109±0.5	123±0.3	134±0.5	141±0.4	151±0.4	157±0.8
		6	156±0.5	83±0.4	98±0.8	109±0.3	126±0.4	137±0.3	139±0.7	149±0.7	153±0.9

*Formulation with nimodipine suspension, ** Mean of three observations±SD, SD: Standard deviation

Table 7: Reduction in blood pressure level of rats after treatment with nimodipine nanoemulsion gel formulation (Formulation control)

Group	Treatment	Rats	Initial blood pressure level (mmHg±SD)	**Blood pressure at varying time points (mmHg±SD)							
			0	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	24 hrs	48 hrs
A	Formulation nimodipine nanoemulsion gel*	1	157±0.9	132±0.3	122±1.1	112±1.12	111±1.1	107±1.6	99±1.8	102±0.3	111±0.4
		2	158±0.2	141±0.3	137±0.9	121±1.2	113±1.23	109±0.9	101±0.7	103±0.8	102±0.3
		3	157±0.4	132±0.5	129±0.8	121±1.2	126±1.7	115±1.7	103±0.2	111±0.8	114±0.5
		4	156±1.2	139±0.9	129±0.7	111±0.3	111±0.9	108±0.8	98±0.3	102±0.3	112±0.3
		5	157±0.9	136±0.7	128±0.7	113±0.9	113±0.9	105±0.8	99±0.5	102±0.4	110±0.6
		6	158±0.2	138±0.6	126±0.8	112±0.9	112±0.8	106±0.5	99±0.5	101±0.3	109±0.4

*The optimized formulation containing drug, **Mean of three observations±SD, SD: Standard deviation

Table 8: Skin irritation scores of NEG

Rats	Intact skin				Abraded skin			
	24 hr		72 hr		24 hr		72 hr	
	A*	B**	A*	B**	A*	B**	A*	B**
1	1	1	0	0	1	1	1	0
2	0	0	1	0	1	0	2	0
3	1	1	0	1	0	2	1	1

A*=Erythema formation score, B**=Oedema formation score, NEG: Nanoemulsion gel

Table 9: Skin irritation scores of NEG (average)

Rats	Intact skin (i)		Abraded skin (ii)		Combined average (i+ii)
	24 hr	72 hr	24 hr	72 hr	
1	1*	1*	1*	1*	-
2	1	1	1	2	-
3	2	1	2	2	-
Average	1**		1.5**		1.25

*Total of A and B from Table 8; **Average of all six readings of 24 and 72 hr, NEG: Nanoemulsion gel

of oil, polymer and drug could give desirable outcome. The nimodipine NEG developed in this study has shown improved performance and could be an option for effective management of hypertension. The option has a good probability to move further via clinical and scale-up protocols. The end result would benefit the suffering patients at large.

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