



Available online on 15.01.2019 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

© 2011-18, publisher and licensee JDDT, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited



Open Access

Research Article

In-Vivo assessment of glucocorticoid loaded tea tree oil nanoemulsion gel

Md. Sarfaraz Alam^{1*}, Rohit Saraswat², Pankaj Sharma³, Md. Salahuddin Ansari¹

¹ PhD Research Scholar, School of Pharmacy, OPJS University, Rawatsar Kunjla, Near Sankhu Fort, Rajgarh (Sadulpur) -Jhunjhunu Road, Churu – Rajasthan-331303, India

² Head, School of Pharmacy OPJS University, Rawatsar Kunjla, Near Sankhu Fort, Rajgarh (Sadulpur) -Jhunjhunu Road, Churu – Rajasthan-331303, India

³ Head, School of Pharmacy, Apex University, Jaipur, Rajasthan, India

ABSTRACT

Optimized formulations were subjected to various *in vivo* studies like anti-inflammatory activity, Nickel induced dermatitis, irritation study and Acute and repeated dose dermal toxicity studies. Clobetasol propionate (CP) has anti-inflammatory, immunomodulatory, and antiproliferative activity. The aim of the present work was to test the hypothesis that the addition CP in nanoemulsions would result in enhancement CP delivery and leading to better antipsoriatic activity. Nanoemulsions were prepared by aqueous phase titration method, using Tea Tree oil, Tween 20, Transcutol P, and distilled water as the oil phase, surfactant, co-surfactant and aqueous phase, respectively. We developed a topical O/W nanoemulsion in which drug is incorporated in disperse phase of oil and evaluated its efficacy against different types of *in vivo* studies. It was also found that the significantly increased their anti-inflammatory activity. It was reported that CP-loaded nanoemulsion significantly increased NTPDase (Nucleoside triphosphate diphosphohydrolases) activity in lymphocytes. This membrane protein is responsible for the hydrolysis of extracellular ATP (Adenosine triphosphate) which is responsible for cell proliferation, differentiation and inflammatory processes. *In vivo* irritation studies did not show any irritation in spite of having high amount of surfactant. Group treated with CP loaded nanoemulsion gel showed no evident toxicity even on repeated exposure. On the basis of above *in vivo* study we conclude that developed nanoemulsion is safe for human.

Keywords: Clobetasol propionate, *In-vivo* study, Nanoemulsion, Anti-inflammatory study, Toxicity study

Article Info: Received 10 Nov 2018; Review Completed 20 Dec 2018; Accepted 02 Jan 2019; Available online 15 Jan 2019



Cite this article as:

Alam MS, Saraswat R, Sharma P, Ansari MS, *In-Vivo* assessment of glucocorticoid loaded tea tree oil nanoemulsion gel, Journal of Drug Delivery and Therapeutics. 2019; 9(1):108-113 DOI: <http://dx.doi.org/10.22270/jddt.v9i1.2178>

*Address for Correspondence:

Md. Sarfaraz Alam, PhD Research Scholar, School of Pharmacy, OPJS University, Rawatsar Kunjla, Near Sankhu Fort, Rajgarh (Sadulpur) -Jhunjhunu Road, Churu – Rajasthan-331303, India

INTRODUCTION

Topical corticosteroids are one of the most commonly used and most beneficial for dermatologic disorders. The clinical value of the topical corticosteroids in the treatment of psoriasis and other skin disease is related with their vasoconstrictive, anti-inflammatory, immune suppressive, and antiproliferative properties¹. Regardless of its clinical usefulness, the application of topical glucocorticoid is limited due to their adverse effects, such as skin atrophy, steroid acne, hypo pigmentation, allergic contact dermatitis and their poor absorption through skin². The recent advancement on the corticosteroids are to develop the strategies to enhance the benefit-risk ratio of glucocorticosteroids specially increase their absorption and should be free from the any histopathological change in the natural structure of skin³. In last two decades, nano sized based formulation has emerged as an excellent tool in the drug delivery system especially the nanoemulsions for

delivery of the poorly water soluble drug to the dermis of the skin with an intention to decreasing the side effects via decrease in dose^{4,5}. In such cases, the particle size is decrease to its sub-micron level upto 10-200 nm to increase the absorption and therapeutic application of the drug in the target tissue. It may permit reproducible and long-term release of the drug at the site of action^{6,7}. Clobetasol propionate (CP) is a potent, highly hydrophobic corticosteroid used for the treatment of skin ailments such as atopic dermatitis and psoriasis⁸. Hence oil/water (O/W) nanoemulsion is one of the best approaches in which drug present in oil phase. But, in nanoemulsion there is high amount of surfactant and cosurfactant are used which has irritation potential so, researcher must assess the irritation potential before application^{9,10}. The reported article of Senyigit showed that the incorporation of CP into lecithin/chitosan nanoparticles induced an accumulation of CP especially in the epidermis without any significant permeation across pig ear skin¹¹. The psoriasis may occurs

because of the delayed hypersensitivity reaction, mediated by T lymphocyte to an antigen protein or a hapten linked to a protein also, the extracellular ATP (Adenosine triphosphate), which is able to regulate the cell - cell interactions and play important role in the processes of cell activation, differentiation, development, proliferation, and death, as well as effector lymphocyte response^{12, 13}. NTPDase (ecto-nucleoside triphosphate diphosphohydrolase; CD39) is an integral membrane protein that metabolizes extracellular. Taking all these considerations into account, *in vivo* protocol was developed based on the induction of contact dermatitis in rats using the *in a* dispersion of nickel sulfate in solid Vaseline at 5%, carrageenan induce inflammation, their irritation study, acute and repeated dose dermal toxicity studies¹⁴. Inflammation is the one of the most significant clinical symptom related with psoriasis and other skin diseases. So, it was also investigated in these studies. High amount of surfactant and co-surfactant was used in nanoemulsion, which has irritation potential so, irritation study was also performed. In such type of skin disease needs long terms treatment so acute and repeated dose dermal toxicity studies were also performed.

MATERIALS AND METHODS

Materials

CP was obtained as a gift sample from Ranbaxy Research Laboratory (Gurgaon, India). Tea Tree Oil (TTO) was a gift sample from Natural Aroma Products Pvt. Ltd. New Delhi, INDIA. PEG 400, Tween 80, Tween 20 and ethanol was purchased from Merck (Merck, India). Caprylocaproyl macrogol-6 glycerides (Labrasol), diethyleneglycol monoethyl ether (Transcutol P) and Plurol Oleique were obtained as a kind gift sample from Gattefosse (Mumbai, India). All other chemicals were of analytical grade.

Preparation of optimized nanoemulsion

On the basis of solubility studies Tea Tree oil (TTO), Tween 20 and Transcutol P were selected as oil, surfactant and co-surfactant respectively. Different combination of surfactant and cosurfactant were prepared commonly known as Smix. To find out the nanoemulsion region pseudo ternary phase diagrams were constructed for each Smix ratio. Finally Smix(1:1) was selected for the preparation of nanoemulsion. A number of nanoemulsion formulations were prepared based on the different ratio of oil: Smix (1:1): water and evaluated for their physical stability studies. Considering the irritation potential of surfactants, Smix ratio containing minimum percentage of surfactant was selected for the preparation nanoemulsion. Optimized nanoemulsion was prepared by dissolving 0.05% (w/v) of CP in 15% (v/v) TTO then 35% (v/v) mixture of Tween-20 and ethyl alcohol (1:1 v/v) were added slowly in oil phase. Then remaining amount of distilled water was added slowly to get the final preparation of 100% (v/v) [data unpublished].

Dermatitis induction by 5% nickel sulfate

Contact dermatitis was induced by 5% nickel sulfate in solid Vaseline similar to the procedure adopted by Brum et al. Animals were divided into five sets (n = 8). After tricotomization, all groups received sensitization with nickel sulfate in the abdomen, except the first group which received only solid Vaseline and continued under the same environmental and feeding conditions as the other groups, this being the control group (C). The induction of dermatitis was done 6 days after sensitization by nickel sulfate in solid Vaseline (5 applications with an interval of 72 hours) in each ear after tricotomization. The first group which received only solid Vaseline was euthanized 72 h after the last application

of the sensitization agent. The second group was induced to allergic contact dermatitis, which was not managed, and the rats were euthanized 72 h after the last application of nickel sulfate, being the positive control (D). The third group received the topical administration in each ear of the placebo nanoemulsion the fourth received topical administration in each ear of the CP loaded nanoemulsion and fifth group received marketed preparation. Dose of application for CP loaded nanoemulsion and marketed formulation was equal to 1 microgram of CP. Group 4 & 5 were treated daily with 0.5 gram of the drug loaded nanoemulsion and marketed formulation for 5 days on days 1, 3, and 5. All formulations were applied uniformly throughout the ear tissue with massage in order to obtain a better drug penetration. After the completion of each treatment, the animals were euthanized and the blood was collected by cardiac puncture to determine the NTPDase activity^{15,16}. For NTPDase activity of lymphocytes, mononuclear leukocytes were isolated from rat blood collected with EDTA and separated using Ficoll-Hypaque density gradients as described by Memon et al. After the isolation of mononuclear cells, NTPDase activity was determined by colorimetric assay in compliance with Leal et al. All samples were run in duplicate or triplicate, and specific activity is reported as nmol Pi released/ min/mg protein. Protein was measured by the Coomassie Blue method using bovine serum albumin as the standard as described by Bradford et al¹⁷.

In vivo anti-inflammatory study

The protocol to carry out *in vivo* anti-inflammatory efficacy studies was approved by the Institutional Animal Ethics Committee (IAEC) of Jaipur National University, Jaipur Rajasthan. The committee's guidelines were followed for the studies. The anti-inflammatory activity of optimized formulations (Nanoemulsion gel A2) was evaluated using the carrageenan induced hind paw edema method developed by Winter et al. The edema of paw was measured with the help of digital plethysmometer. Young Wistar rats, weighing 150-250 g, were randomly divided into four groups, each containing six rats. One group out of four was subjected to optimized placebo nanoemulsion gel, optimized formulations (Nanoemulsion gel A2), marketed formulation and control. The digital plethysmometer 7140 is a volume meter; designed for accurate measurement of the rat paw swelling. It consists of water filled Perspex cell into which the rat paw is dipped. A transducer of original design, which records small difference in water level caused by volume displacement; operates on a graphic LCD read out which shows the exact volume of the paw (control or treated). The measuring cell consists of two vertical inter-connected Perspex tube; the larger one is used to measure the displacement of water. The water level in the smaller tube, which contains the transducer, follows the change in level of water in the larger tube. Therefore proportional changes in water level occur when something is dipped in. Its conductance is linearly proportional to the water level but is also affected by water conductivity, which in turn depends on its ions contents and temperature. A sophisticated electronic circuit monitored by the compensator electrodes, corrects the level of electrons. Thus the reading generates signal proportional to the water-level only and hence to the volume of the dipped object. The rats were given free access to water and food. The rats were kept under observation for 24 h. The backside of rats was shaved 12 h before starting the experiment¹⁸. The optimized placebo nanoemulsion gel, optimized formulations (Nanoemulsion gel A2) and marketed formulation were applied on the shaved backs of all animals of group no. 2, 3 and 4 respectively. Right hind paw edema was induced in all three groups of animals by sub

planter injection of 0.1 ml of a 1% w/v homogeneous suspension of carrageenan in distilled water. In animals of group no. 2, 3 and 4 carrageenan was injected half an hour later the application of formulations. The swelling of the injected paw was measured immediately (0 h) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 12 and 24 h after injection using a digital plethysmometer¹⁹. The amount of paw swelling was determined from time to time and expressed as percent edema relative to the initial hind paw volume. The mean values of percentages were determined for each time interval. Percentage inhibition of edema produced by each group was calculated against the respective control group using the following formula:

$$\% \text{ Inhibition} = \frac{\% \text{ Edema (Control)} - \% \text{ Edema (Formulation)}}{\% \text{ Edema (Control)}}$$

Where,

Mean edema =

$$\% \text{ Paw Edema} = \frac{\text{Mean of final paw volume} - \text{Mean of initial paw volume}}{\text{Initial Paw Volume}} \times 100$$

In vivo skin irritation test

All the materials used for preparation of nanoemulsion fall under generally regarded as safe (GRAS) category. Concentration of all materials is very critical issue for this formulation. Large amount of surfactants is usually irritant to the skin. Therefore skin irritation test was performed to confirm concentration of materials used for nanoemulsion preparation is safe. Therefore skin irritation test was performed to confirm the safety of optimized nanoemulsion gel of CP. Van-Abbe et al. mentioned that a value between 0 and 9 indicates that the applied formulation is generally non irritant to human skin. Skin irritation test was performed on optimized formulation, Placebo nanoemulsion and marketed cream on wistar rats weighing 180–200 g. Wistar rats were divided into 3 groups: placebo nanoemulsion gel, optimized nanoemulsion gel of CP and Marketed preparation were applied to each group, one group contain 6 rats. The animals were kept under standard laboratory conditions, temperature at $25 \pm 1^\circ\text{C}$ and relative humidity ($55 \pm 5\%$). The animals were housed in polypropylene cages, six per cage, with free access to standard laboratory diet and water as mention above. A single dose of 10 mg of placebo nanoemulsion gel, optimized nanoemulsion gel of CP and Marketed preparation were applied to the left ear of the rat and the right ear as a control. The development of erythema was monitored for 14 days using the reported method^{20, 21}.

In vivo Acute and repeated dose dermal toxicity studies

Acute and repeated dose dermal toxicity studies were performed in accordance with 2002 and OECD, 1981. Briefly, albino Wistar rats weighing between 200 and 300 g were randomized and assigned to the treatment and control groups (each group contained six rats). Test formulations were applied uniformly over depilated skin (approximately 10% of the body surface area). A 100 times higher dose of human exposure was selected for acute dermal toxicity study while usable dose was selected for repeated dose dermal toxicity study. For acute dermal toxicity, exposure period comprised of 24 h and the observation period was of 14 days²². For repeated dose dermal toxicity, the animals were treated for 6 h per day on a 6-day per week basis, for a total period of 28 days. Animals were observed and individual records were maintained throughout the study period for any changes in vital physiological functions of respiratory, circulatory, autonomic and central nervous system and pharmacological signs such as tremors, convulsions,

salivation, diarrhea, lethargy, sleep somatomotor activity and behavior pattern. At the conclusion of study period, blood samples were collected for hematology and biochemistry determinations. All the animals in each experimental group were humanely sacrificed using ether anesthesia. The animals were dissected and major organs namely liver, kidney, heart, lung and spleen were collected and weighed carefully in order to calculate organ to body weight ratios. The tissue samples were preserved in formalin (10% buffered neutral) and processed for histopathology by cutting sections of 5 μm thickness and staining with hematoxylin and eosin²³.

RESULT AND DISCUSSION

Contact dermatitis

The in vivo NTPDase activity of lymphocytes after each treatment is shown in (Fig.1). A significant increase in NTPDase activity was observed in lymphocytes of the group treated with CP-loaded nanoemulsion, in relation to ATP and ADP (Adenosine Diphosphate) (Fig. 1A and 1B, respectively), compared to all other groups ($p < 0.05$). The ADP and the ATP hydrolysis values for the group treated with CP loaded nanoemulsion and the control groups were not statistically different. The higher NTPDase activity may be associated with the high levels of extracellular ATP resulting from the inflammatory process, which occurs in cases of allergic contact dermatitis. During the dermatitis, these high levels of ATP would have an affinity for drug loaded nanoemulsion. Data were analyzed statistically by one-way ANOVA followed by the Tukey-Kramer test (1A) and Kruskal-Wallis Test (1B), $p < 0.05$.

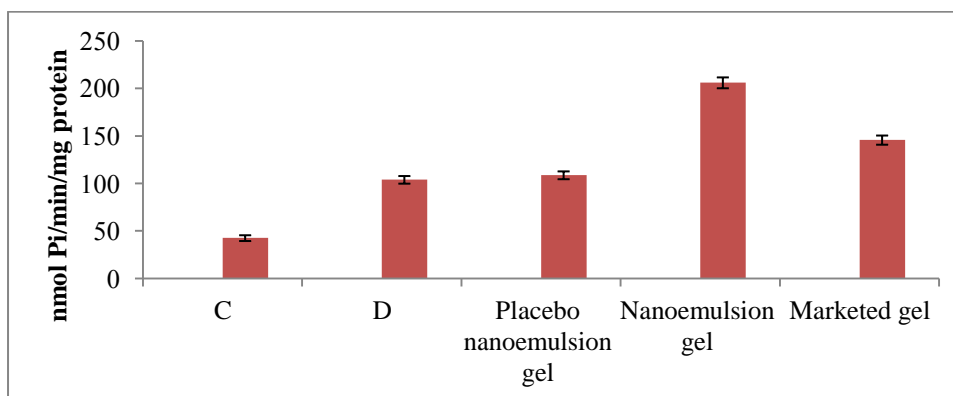
P2X7 purinergic receptors, leading to a Th1 pattern of immune response with the production of inflammatory cytokines. The NTPDase would act by decreasing the levels of ATP, which in low concentration would bind to the P2Y receptors, reversing the pattern of immune response to Th2 with the release of anti-inflammatory cytokines. Thus, it is possible that the increased hydrolysis of adenine nucleotides also leads to an increase in the extracellular adenosine concentration, which has immunosuppressive and anti-inflammatory effects. Adenosine plays a central and direct role in the regulation of inflammatory responses and in limiting inflammatory tissue destruction. In this context, the higher NTPDase activity in the treatment with the CP-loaded nanoemulsion, at intervals of 48 h, could be related to the higher anti-inflammatory effect in comparison with placebo nanoemulsion and marketed preparation. Therefore, the best result observed for the CP loaded nanoemulsion at 0.05%, even after a longer interval of time, may be related to the slower CP release as found and its accumulation in the hair follicles in the form of nanosize (data unpublished). This result demonstrates that the nanoemulsion formulation, which did not appear to stimulate an inflammatory or immune response using the contact dermatitis model.

In vivo anti-inflammatory study

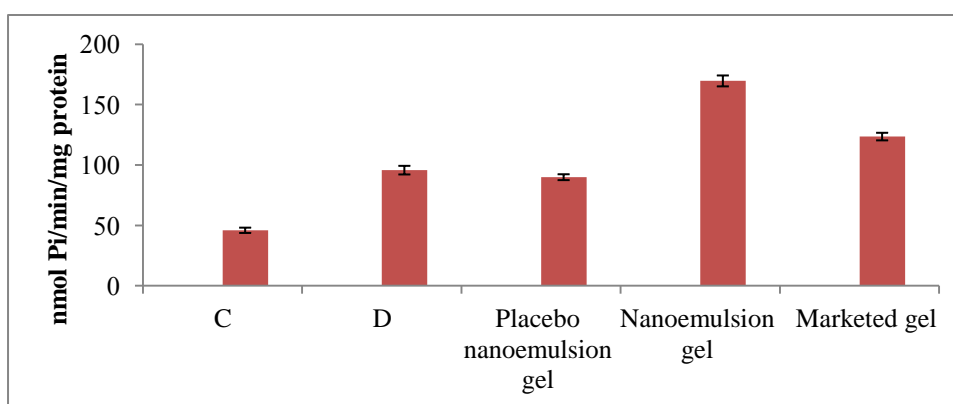
The anti-inflammatory effects of placebo nanoemulsion gel, optimized drug loaded nanoemulsion gel and marketed conventional gel with the control. The anti-inflammatory activity of optimized nanoemulsion was evaluated using the carrageenan-induced hind paw edema method using digital Plethysmometer. The rat's left footpad became edematous soon after injection of carrageenan and reached its peak at 12 h (39.31 %). Mean percent edema and % inhibition of inflammation of all the three groups were calculated and mentioned in (Table 1). Inhibition of edema was found to be highest in the groups in which optimized nanoemulsion was applied. The optimized nanoemulsion inhibited edema ($P < 0.05$) 84.51% up to 12 h. In case of placebo

nanoemulsion inhibited edema ($P < 0.05$) 33.9% up to 12 h. This action may be due to Tea Tree oil present in nanoemulsion, as this oil shows anti-inflammatory activity based on the anti-inflammatory studies, it can be concluded that CP optimized

nanoemulsion shows maximum inhibition of edema than the placebo²⁴. Infected marketed preparation has greater activity than placebo due to presence of drug.



(A)



(B)

Figure 1: ATP (A) and ADP (B) hydrolysis in lymphocytes obtained from the control group (C), contact dermatitis group (D), groups with dermatitis treated with Placebo nanoemulsion, drug loaded nanoemulsion and marketed gel preparation.

Table 1: Anti-inflammatory effects of Placebo nanoemulsion gel and Drug loaded nanoemulsion gel and in carrageenan-induced rat paw edema

Group	Formulation	N	Mean Wt. \pm SD (g)	Time (h)	Mean % Edema \pm SD	% Inhibition
I	Control (carrageenan only)	6	180.0 \pm 12.2	1	28.72 \pm 2.33	
				2	41.20 \pm 4.24	
				3	74.45 \pm 4.32	
				6	58.14 \pm 3.05	
				12	39.31 \pm 3.57	
II	Placebo nanoemulsion	6	200.0 \pm 15.5	1	26.9 \pm 2.81	6.27
				2	36.31 \pm 2.01	11.86
				3	62.84 \pm 4.16	15.59
				6	45.11 \pm 1.88	22.37
				12	25.99 \pm 1.25	33.9
III	Drug loaded nanoemulsion	6	190 \pm 9.5	1	25.43 \pm 4.16	11.39
				2	32.16 \pm 1.01	21.94
				3	41.34 \pm 1.97	44.47
				6	14.97 \pm 1.05	74.23
				12	6.09 \pm 1.11	84.51
IV	Marketed preparation	6	195 \pm 10.31	1	26.4 \pm 3.12	8.013
				2	35.91 \pm 4.16	12.83
				3	62.87 \pm 3.37	15.55
				6	41.59 \pm 3.05	28.42
				12	21.22 \pm 3.55	46.03

N = Number of rats in each group; SD = Standard deviation

***In vivo* skin irritation test**

The mean values of skin irritation score for placebo nanoemulsion gel, optimized drug loaded nanoemulsion gel and marketed conventional gel were found to be 1.50 ± 0.54 , 1.66 ± 1.03 and 1.00 ± 0.89 respectively (Table 2). From these results which were based on 14 days test it can be concluded

that optimized nanoemulsion was safe to be used as topical drug delivery system. It clearly indicated that nanoemulsion has more skin irritation potential due to high amount of surfactant in comparison to placebo nanoemulsion because drug itself may has irritation potential. Overall all the formulation have low irritation score hence it is safe for human use.

Table 2: Skin irritation score of the Placebo nanoemulsion and Drug loaded nanoemulsion.

S. No	Group	Score after (days) 1	Score after (days) 2	Score after (days) 3	Score after (days) 4	Score after (days) 7	Score after (days) 14	Mean score \pm SD
1	Placebo Nanoemulsion gel	1	2	1	2	1	2	1.50 ± 0.54
2	Drug loaded Nanoemulsion gel	2	0	2	3	2	1	1.66 ± 1.03
3	Marketed gel	2	1	0	1	0	2	1.00 ± 0.89

***In vivo* Acute and repeated dose dermal toxicity studies**

Dermal toxicity studies provided detailed insight on any possible health hazards likely to arise from repeated exposures over a limited period of time. Acute as well as repeated application of test formulations did not reveal any abnormal changes in cage side observations. There was no mortality in any of the study groups. All study groups showed normal weight and exhibited no toxic effects during the study period. Histopathology of all major organs such as

liver, kidney, spleen, heart and lung did not reveal any distinct pathological alterations (Fig.2).

However, examination of application site skin area demonstrated dermal changes such as mild keratosis on repeated application of CP gel. Repeated exposure of CP gel showed slight collagenous mass infiltration in the subdermal layer, but no inflammatory reaction. No such reaction was observed when CP gel was applied to the skin for shorter periods.

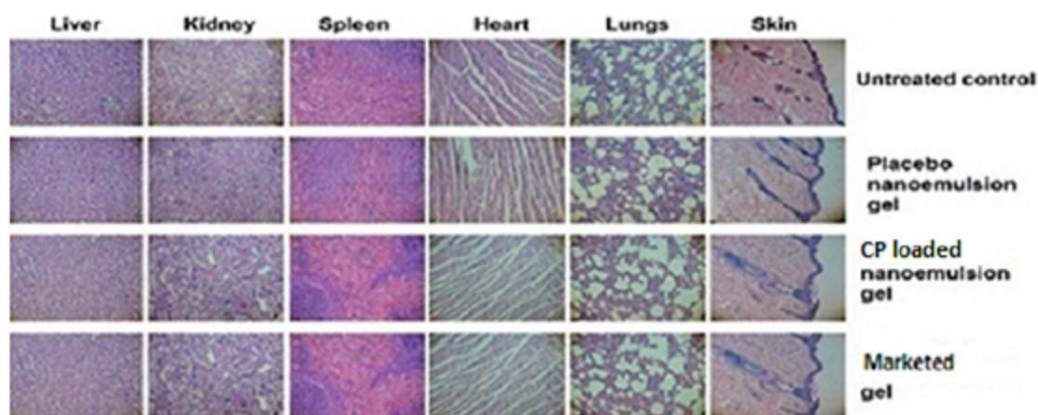


Figure 2: Histopathology of all major organs such as liver, kidney, spleen, heart and lung

CONCLUSION

On the basis of above *in vivo* study we conclude that developed nanoemulsion is safe for human use because it has good anti-inflammatory action and did not show any irritation to the skin. Although nanoemulsion contain high amount of surfactant in comparison to market gel. Surprisingly, group treated with CP loaded nanoemulsion gel showed no evident toxicity even on repeated exposure, demonstrating significantly improved safety.

CONFLICTS OF INTEREST

All authors declared conflicts of interest none.

ACKNOWLEDGMENT

We are thankful to School of Pharmacy, OPJS University, Churu, Rajasthan, India – 331303.

REFERENCES

- Senyigit T, Padula C, Ozer O, Santi P. Different approaches for improving skin accumulation of topical corticosteroids. *Int J Pharm* 2009; 380:155-160.
- Hengge UR, Ruzicka T, Schwartz RA, Cork MJ. Adverse effects of topical glucocorticosteroids. *J Am AcadDerm* 2006; 54:1-15.
- Baboota S, Alam MS, Sharma S, Kaur SJ, Anil K, Ali J. Nanocarrier-based hydrogel of betamethasone dipropionate and salicylic acid for treatment of psoriasis. *Int J PharmaInvestig* 2011; 1:139-147.
- Marchiori ML, Lubini G, Nora GD, et al. Hydrogel containing dexamethasone-loaded nanocapsules for cutaneous administration: preparation, characterization and in vitro drug release study. *Drug DevInd Pharm* 2010; 36:962-971.
- Christophe H, Ingo A.A.N, Yogeshvar N, Ois-Xavier M, Richard HG. Expert Review: In Vivo Methods for the Assessment of Topical Drug Bioavailability, Online published 2007.
- Alam MS, Baboota S, Ali MS, et al. Accelerated stability testing of betamethasone dipropionate nanoemulsion. *Int J Pharm PharmSci* 2007; 4(4):371-374.
- Kotta S, Khan AW, Pramod K, Ansari SH, Sharma RK, Ali J. Exploring oral nanoemulsions for bioavailability enhancement of poorly water-soluble drugs. *Expert Opin Drug Deli* 2012; 9(5):585-598.
- Gordon ML. The role of clobetasol propionate emollient 0.05% in the treatment of patients with dry, scaly, corticosteroidresponsive dermatoses. *ClinTher* 1998; 20(1):26-39.
- Parveen R, Baboota S, Ali J, Ahuja A, Vasudev SS, Ahmad S. Oil based nanocarrier for improved oral delivery of silymarin: in vitro and in vivo studies. *Int J Pharm* 2011; 413(1-2):245-253.
- Shakeel F, Ramadan W, Gargum HM, Singh R. Preparation and in vivo evaluation of indomethacin loaded true nanoemulsions. *Sci Pharm* 2009; 78:47-56.
- Senyigit T, Sonvico F, Barbieri S, Ozer O, Santi P. Lecithin/chitosan nanoparticles of clobetasol-17-propionate capable of accumulation in pig skin. *J Control Release* 2010; 142:368-373.
- Di Virgilio F, Chiozzi P, Ferrari D, et al. Characterization of NTPDase (NTPDase 1; ecto-apyrase; ectodiphosphohydrolase; CD39; EC 3.6.1.5) activity in human lymphocytes. *Blood* 2001; 97:587-600.
- Burnstock G, Knight GE. Cellular distribution and functions of P2 receptor subtypes in different systems. *Int Rev Cytol* 2004; 240:301-304.
- Ralevic V, Burnstock G. Involvement of purinergic signaling in cardiovascular diseases. *Drug News Perspect* 2003; 16:133-140.
- Fontana MC, Rezer JFP, Coradini K, Leal DBR, Beck RCR. Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. *Eur J Pharm Biopharm* 2011; 79:241-249.
- Seidenari S, Di Nardo A, Giannetti A. Assessment of topical corticosteroid activity on experimentally induced contact dermatitis: echographic evaluation with binary transformation and image analysis. *Skin Pharmacol* 1993; 6(2):85-91.
- Bradford MMA. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72:248-254.
- Koo HJ, Lim KH, Jung HJ, Park EH. Anti-inflammatory evaluation of gardenia extract, geniposide and genipin. *J Ethnopharmacol* 2006; 103(3):496-500.
- Bhadoriya SS, Mishra V, Raut S, Ganeshpurkar A, Jain SK. Anti-inflammatory and antinociceptive activities of a hydroethanolic extract of *Tamarindus indica* leaves. *Sci Pharm* 2012; 80(3): 685-700.
- Van-Abbe NJ, Nicholas P, Boon E. Exaggerated exposure in topical irritancy and sensitization testing. *J SocCosmetChem* 1975; 26:173-187.
- Zulfakar H, Abdelouahab N, Heard CM. Enhanced topical delivery and ex vivo anti-inflammatory activity from a betamethasone dipropionate formulation containing fish oil. *Inflamm Res* 2010; 59:23-30.
- Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera G, Oliveira GC, Rocha-Filho PA. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. *J Nanobiotechnology* 2011; 9:9-44.
- Ali J, Akhtar N, Sultana Y, Baboota S, Ahuja. Antipsoriatic microemulsion gel formulations for topical drug delivery of babchi oil (*Psoralea corylifolia*). *Exp. Clin. Pharmacol* 2008; 30: 277-285.
- Pazyar N, Yaghoobi R. Suppression of Inflammatory Reactions by Terpinen-4-ol, a Main Constituent of Tea Tree Oil, in a Murine Model of Oral Candidiasis and Its Suppressive Activity to Cytokine Production of Macrophages in Vitro. *The Pharmaceutical Society of Japan Biol. Pharm. Bull* 2013; 36(5):838-844.