

Effect of Topical Application of Nano Retinol on Mild to Moderate Acne Vulgaris

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ABSTRACT

Background: Acne vulgaris is a common skin condition with substantial cutaneous and psychologic disease burden. Estimates of acne prevalence vary substantially given the absence of a universally accepted or grading schema. **Aim of the Work:** The aim of work is to assess the efficacy and tolerability of topical application of Nano retinol in the treatment of mild to moderate facial acne vulgaris in comparison to classic retinoids. **Patients and Methods:** This study was conducted as a prospective, split face comparative clinical study on 30 female patients participated for treatment of facial acne vulgaris. All patients recruited from outpatient clinic of dermatology department, Ain Shams University in the period from November 2015 to December 2016. History and clinical examinations were done for each patient. **Results:** reduction in the total and inflammatory acne lesion count were reported to be significantly greater in the nano formulation as compared to the conventional formulation. local adverse events were significantly less in the nano formulation as compared to the conventional formulation. **Conclusion:** nanosomal retinol with iontophoresis is more effective and better tolerated than its conventional formulation with nearly no side effects and no precautions for use. **Recommendations:** More studies are needed on a wider scale, greater number of patients with different grades of acne vulgaris to support our findings.

Keywords: topical application, nano retinol, acne vulgaris.

INTRODUCTION

Acne vulgaris is a common inflammatory skin condition, although often perceived as a self-limited disease of adolescence, its prevalence remains high into adulthood. Nearly ninety percent of teenagers have acne vulgaris, and half of them continue to experience symptoms as adults ⁽¹⁾. By the age of forty years, one percent of men and five percent of women still have lesions ⁽²⁾.

While several different acne-grading scales have been used in clinical trials, no standard method for acne grading has been adopted into practice. The basic acne severity index is proposed method for assessing acne severity based upon lesion type, number and location ⁽³⁾.

Acne vulgaris is categorized broadly into mild, moderate, severe forms. Lesions may persist on the face, chest or back areas with the greatest density of pilosebaceous unit ⁽⁴⁾. Mild acne is typical limited to the face and is characterized by non-inflammatory closed and open comedones with few inflammatory lesions. An increasing number of inflammatory papule and pustule on face and often-mild trunkal disease characterizes moderate acne. Finally, acne is considered severe when nodules and cysts are present. In these cases, facial lesions are often accompanied by widespread trunkal disease ⁽⁵⁾.

Acne vulgaris develops in the pilosebaceous unit that produces the comedones this results from the interaction between numbers of factors: the abnormal development and differentiation of follicular cells of the pilosebaceous unit. The increased cornification keratinization of follicular cells due to deposition of keratin within them enhanced sebaceous activity with hyperseborrhea (oily skin), hypercolonization and growth of bacterium propionibacterium acne within the follicles and inflammation and immunological reaction. Hyper cornification of the pilosebaceous duct results from the presence of androgens, local cytokines, and abnormalities of the sebaceous lipids ⁽⁶⁾.

Guidelines in treatment of acne vulgaris focuses on acne severity and degree of inflammation. Treatment options include proper skin care, topical and oral antimicrobials, topical and systemic retinoid, benzoyl peroxide and oral contraceptives for female patients. These treatments may use in combination to achieve disease resolution ⁽⁷⁾.

Retinoids influence proliferation and differentiation of cells and reverse the abnormal desquamation by increasing the follicular epithelial turn over and accelerating the shedding of corneocytes, which leads to an expulsion of mature comedones and suppression of micro comedone formation.

The change of the follicular milieu of sebaceous gland apparatus by restoration of normal cornification promotes an inhospitable environment of propionibacterium acne. Various in vivo and in vitro studies demonstrate also direct immunomodulatory activity of topical retinoid ⁽⁸⁾.

The major adverse effects of topical retinoids are local skin irritation, including peeling, dryness, burning sensation and itching they can increase sensitivity of skin to ultraviolet light ⁽⁹⁾.

Nanotechnology is a new branch of engineering consisting of the usage of Nano scale particles (100nm and smaller). Nano medicine is the application of Nano scale technology for diagnostic and therapeutic purposes in medicine. Nanotechnology applied to dermatology; represent one of most advanced field for which increasing interest, both economic and scientific is rising ⁽¹⁰⁾.

Applications of Nano medicine in dermatology include new direction in medical diagnosis, monitoring and treatment ⁽¹⁰⁾. Retinoids compounds represent an example of positive applications of nanotechnology in drug formulation. Retinoids derive from vitA and are successfully used in the treatment of range spectrum of dermatological conditions including acne vulgaris ⁽¹¹⁾.

Major issue for retinoid therapy is local erythema, peeling, dryness and pain. Skin irritation side effects have been reduced by nanoparticle encapsulation ⁽¹²⁾.

Improved stability and controlled release have been the primary focal points of Nano particle delivery for retinoid therapy ⁽¹²⁾. A major advantage of such delivery system is the better tolerability of irritating retinoid. Improving patient compliance as well as the avoidance of systemic side effects ⁽¹³⁾.

Aim of the Work

The aim of work is to assess the efficacy and tolerability of topical application of Nano retinol in the treatment of facial mild to moderate acne vulgaris in comparison to classic retinoids.

PATIENTS AND METHODS

This comparative study included 30 female patients participated for treatment of facial acne vulgaris. All patients recruited from outpatient clinic of dermatology department, Ain Shams University in the period from November 2015 to December 2016. History

and clinical examinations were done for each patient.

Study design

A Prospective, split face comparative clinical study.

Inclusion criteria

Patients with facial mild to moderate acne vulgaris according to ⁽¹⁴⁾.

Exclusion criteria was:

- a- Pregnant females at the time of enrollment and planning pregnancy during the study period.
- b- Patient with skin condition of the face such as open or incomplete wound at the affected site or acute or chronic eczema, rosacea, perioral dermatitis, atopic or seborrheic dermatitis or psoriasis.
- c- Patients with known hypersensitivity to preparations containing retinoid.
- d- Patients who take any other medications systemic or topical for acne vulgaris concomitantly along with the study medication.
- e- Patients who use peeling agents, abrasive cleansers, strong drying agents, astringents or irritant products (aromatic and alcoholic agents) concomitantly along the study as they produce irritant effects.
- f- Patients who use comedogenic cosmetics that they can exacerbate acne lesions.

All the patients were subjected to the following:

- 1- Written informed consent included detailed information about the treatment, alternative treatment options, possible complications, and the effect of the treatment.
- 2- Detailed history including personal history, present history emphasizing on pregnancy and drug history.
- 3- **General examination.**
- 4- **Dermatological examination** was done for all patients before starting to:
 - a. Exclude the presence of any other skin problems e.g. rosecea and seborrheic dermatitis.
 - b. Determine Efficacy of the medication by counting the number of inflammatory, non-inflammatory and total lesions at first and last session ⁽¹⁵⁾.
 - c. Photography of the face was done with (Nikon) digital camera (16 mega pixel resolution). The photos were taken before

starting treatment (baseline) and at the end of the treatment session (after 6 weeks).

Treatment methodology:

- 1: The face was divided into 2 sides.
- 2: Application step:
 - (a): thin film of topical retinoid cream was applied by the patient to the left side of the face at night every day using finger tips after washing the face with non-abrasive cleanser, avoiding the eyes and lips.
 - (b): On the right side 1 ml of retinol Nano Meso Liposomal Solution (lipoceutical nanosomes solutions; Lipoceutical nanosome &Technology Sesderma,



Figure (1): Nanosomal retinol

It was applied to the skin and absorption was optimized by iontophoresis device. The device was moved gently on the applied areas until the solution was absorbed by the skin. The iontophoresis was adjusted on (negative mode, 5mA, for 10 mins (iontocare, Signstek, Ion Galvanic Elf, China).

The role of nano retinol aided by iontophoresis was to enhance drug permeation by

applying electric current that causes tissue structural changes such as micro pore formation in the stratum corneum, that assist the transport of drugs across the stratum corneum ⁽¹⁶⁾. Besides the use of the small particles of nanosomal retinol with an average size 178.4nm for deeply penetration into the skin layers.

Evaluation of the response

1. Clinical photography of the face before starting treatment (baseline) and at the end of the treatment sessions (after 6 weeks).
2. The global assessment of efficacy is rated by assessment on a five pointed rated scale depend on count of lesions as:
 - Excellent (76 to 100 percent improvement),
 - Good (51 to 75 percent improvement)
 - Fair (26 to 50 percent improvement)

Valencia, Espania).It contains retinol nanosomes that are unilamellar, flexible, with an averge size of 178, ($\pm 14.0\text{nm}$) prepared in a liposomal solution and filtered through 200nm cellulose membranes (in a sterile atmosphere) and later on they are packed on apyrogen flasks which have been sterilized by autoclave. Polydispersion index (P.I.) is 0.005 (< 0.12) confirming the homogenous and unique nature of the solution and the absence of residual products that could confer instability to the formulation. It was applied once per week for six weeks.



Figure (2): Iontophoresis device

Poor (up to 25 percent).

No improvement zero percent).

3. The assessment of tolerability was done on a four-point rating scale (Excellent - no adverse event reported; Good - mild adverse event(s) reported which subsided with or without medication and did not necessitate stoppage of study medication; Fair - moderate to severe adverse event(s) reported which subsided with or without medication and did not necessitate stoppage of study medication; Poor - severe or serious adverse event(s) which necessitated stoppage of study medication). ⁽¹⁷⁾.

The study was done after approval of ethical board of Ain Shams University and an informed written consent was taken from each participant in the study.

Statistical analysis: Analysis of data was done using SPSS (Statistical Package for Social Science) program version 18.

Student t test was used to compare quantitative data between two independent groups and Paired samples t test was used to compare quantitative data for the same group before and after treatment. Chi-Square test was

used to compare qualitative data between different groups. P value ≤ 0.05 was considered statistically significant.

Results

This study included 30 female patients with acne vulgaris aged 15 to 27 with a mean \pm SD (18.77 \pm 2.71).

Twenty four patients (80%) had moderate acne and six (6%) patients had mild acne vulgaris.

There was no statically significant difference between the 2 sides treated as

regards number of total acne lesions, non-inflammatory and inflammatory lesions before treatment.

A) Efficacy of Nano retinol treatment on the right side:

There was a highly statistical significant difference between number of total, inflammatory and non-inflammatory lesions mean count before and after treatment (table 1).

8patients were rated as excellent, 12 as good and 10 as fair as regards total lesion count.

Table (1): Change in number of lesions after treatment in Nano group.

	Mean	SD	t*	P value
Total number before treatment	14.67	6.08	10.91	<0.001 (HS)
Total number after treatment	5.43	3.71		
Difference	9.23	4.64		
Non-inflammatory before treatment	2.53	2.52	4.60	0.001 (HS)
Non-inflammatory after treatment	1.27	1.89		
Difference	1.27	1.51		
Inflammatory before treatment	12.13	5.91	8.86	<0.001 (HS)
Inflammatory after treatment	4.13	3.38		
Difference	8.00	4.95		

*Paired samples t test

(HS): highly significant

B) Efficacy of Conventional Topical retinoids treatment on the left side

There was a highly statistical significant difference between total, non-inflammatory and inflammatory lesions mean count before and after treatment (table 2). 3 patients were rated as good, 19 as fair and 8 as poor as regards total lesion count.

Table (2): Change in number of lesions after treatment in topical Retinoid group.

	Mean	SD	t*	P value
Total number before treatment	14.40	5.88	10.53	<0.001 (HS)
Total number after treatment	9.60	4.68		
Difference	4.80	2.50		
Non-inflammatory before treatment	3.20	3.72	3.55	0.001 (HS)
Non-inflammatory after treatment	1.87	2.24		
Difference	1.33	2.06		
Inflammatory before treatment	11.20	5.39	6.53	<0.001 (HS)
Inflammatory after treatment	7.73	3.75		
Difference	3.47	2.91		

*Paired samples t test

(HS): highly significant

Comparison of Nano and topical retinoid as regards total number of lesions after treatment:

There was a highly statistical significant difference between the 2 sides treated as regards number of total acne lesions with better results on Nano side (table 3) (figure 3).

Table (3): Comparison of Nano and topical retinoids as regards total number of lesions after treatment.

	Total number after treatment				t*	P value
	Minimum	Maximum	Mean	SD		
Nano	1.00	13.00	5.43	3.71	3.82	< 0.001 (HS)
Topical retinoids	2.00	17.00	9.60	4.68		

*Independent samples t test
(HS): highly significant

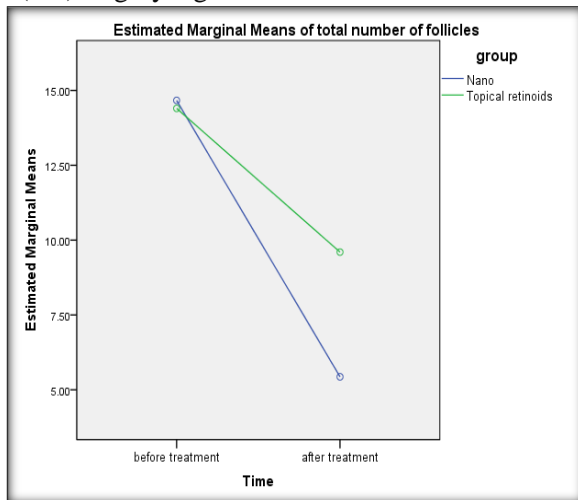


Figure (3): Difference between Nano and topical retinoids as regards change in total number of acne lesions after treatment.

Comparison of Nano and topical retinoids as regards number of non-inflammatory lesions after treatment: There was no statistically significant difference between the 2 sides treated as regards number of non-inflammatory acne lesions (table 4) (figure 4).

Table (4): Comparison of Nano and topical retinoids as regards number of non-inflammatory lesions after treatment.

	Non-inflam. lesions s after treatment				t*	P value
	Minimum	Maximum	Mean	SD		
Nano	0.00	9.00	1.27	1.89	1.12	0.27 (NS)
Topical retinoids	0.00	8.00	1.87	2.24		

*Independent samples t test
(HS): highly significant

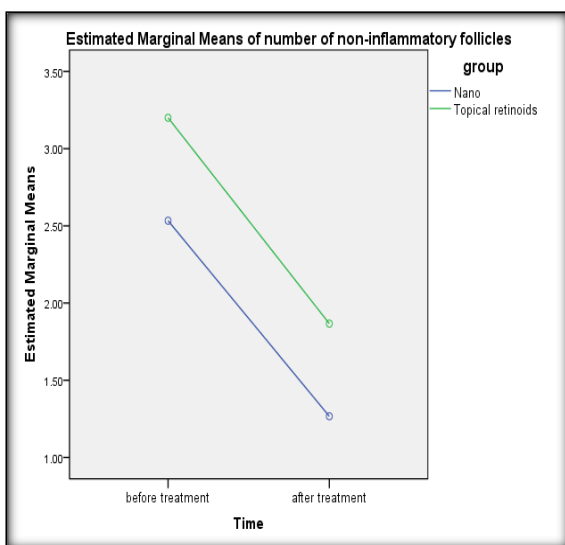


Figure (4): Difference between Nano and topical retinoids as regards change in number of non-inflammatory lesions after treatment.

Comparison of Nano and topical retinoids as regards number of inflammatory lesions after treatment:

There was a highly statistical significant difference between the 2 sides treated as regards number of inflammatory acne lesions with better results on Nano side (table 5) (figure 5).

Table (5): Comparison of Nano and topical retinoid as regards number of inflammatory lesions after treatment.

	Inflammatory lesions after treatment				t*	P value
	Minimum	Maximum	Mean	SD		
Nano	0.00	11.00	4.13	3.38	3.91	<0.001 (HS)
Topical retinoids	1.00	16.00	7.73	3.75		

*Independent samples t test

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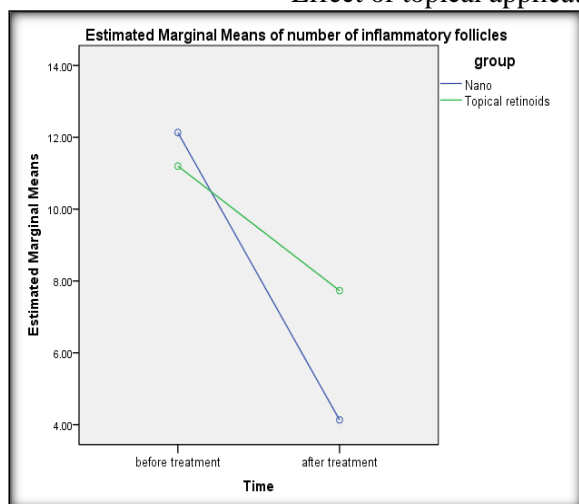


Figure (5): Difference between nano and topical retinoids as regards change in number of inflammatory lesions after treatment.

Comparison of Nano and topical retinoids as regards frequency of side effects:

There was a highly statistical significant difference between the 2 sides as regards frequency of side effects with more on left side treated with conventional topical retinoid (table 6). Global assessment of tolerability showed that Nanoretinol was significantly better tolerated as compared to the conventional topical retinoids. In the Nano group, 27 (90%) patients were rated to have an “excellent” tolerability to the study medication, 3 patients (10%) were rated as having a “good” tolerability and none of the patients was rated to have a “poor” tolerability. While in the topical retinoid group only two patients (6.7%) were rated to have a “good” tolerability and 28 patients (93.7%) were rated to have a “fair” tolerability and none of the patients were rated to have a “poor” tolerability to the study medication.

Table (6): Comparison of Nano and topical retinoids as regards frequency of side effects.

		Group				X ^{2*}	P value
		Nano		Topical Retinoids			
		N	%	N	%		
Dryness	Yes	0	.0%	30	100.0%	60.00	<0.001 (HS)
	No	30	100.0%	0	0.0%		
Photosensitivity	Yes	0	0.0%	30	100.0%	60.00	<0.001 (HS)
	No	30	100.0%	0	0.0%		
peeling	Yes	3	10.0%	30	100.0%	49.09	<0.001 (HS)
	No	27	90.0%	0	0.0%		
Burning	Yes	0	0.0%	30	100.0%	60.00	<0.001 (HS)
	No	30	100.0%	0	0.0%		

*Chi Square test



(a)



(b)

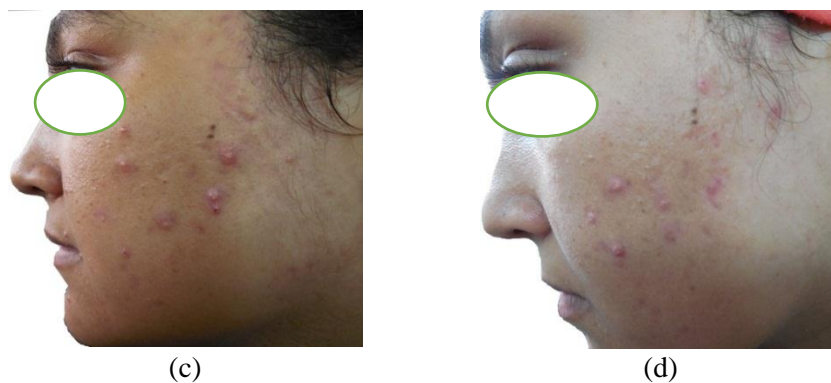


Figure (6): a) Nano retinol (before); b) Nano retinol (after); c) Topical retinoids (before); d) Topical retinoids (after).

DISCUSSION

Acne vulgaris is a chronic inflammatory human skin disease, characterized by areas of skin with seborrhoea, comedones, papules, nodules, and possibly scarring with lesions occurring on face, neck, and back⁽¹⁸⁾.

In this study, we aimed to compare efficacy and tolerability of Nano retinol versus conventional formulation of retinoid in the treatment of mild to moderate facial acne vulgaris.

Topical retinoids was applied to the left side of the face and showed that there was a highly statistical significant difference on acne lesions between total, inflammatory and non-inflammatory mean count before and after treatment. This finding is in agreement with **Bershad et al.**⁽¹⁹⁾ who used topical retinoids as monotherapy in the treatment of acne and showed not only significant reductions in comedones, but also a significant reduction in papulopustular lesions⁽²⁰⁾ used topical retinoids combined antimicrobial agents vs antimicrobials alone in the treatment of acne and there was a reduction in inflammatory and noninflammatory acne lesions faster and to a greater degree than antimicrobial agents alone.

Topical retinoids play a vital role in the treatment of mild to moderate acne through multiple actions. They act on the primary lesion – the microcomedo⁽²¹⁾, by regulating the rate of differentiation and proliferation of the follicular keratinocytes, they inhibit the formation of and reduce the number of microcomedones. Topical retinoids promote normal follicular keratinocyte desquamation and thus help mitigate against the development of a propitious microenvironment for *P. acnes*

⁽²²⁾. Also topical retinoids have direct anti-inflammatory properties. The role of retinoid as anti-inflammatory agents is also supported by studies that demonstrated the ability to reduce TLR2 expression and function⁽²³⁾.

However, it causes a high incidence of side effects including sensitivity to sunlight, irritation, and erythema, resulting in low patient compliance⁽²⁴⁾.

In our study we applied Nano retinol with iontophoresis on the right side of the face and results showed a highly statistical significant difference between number of total inflammatory, and non-inflammatory lesions mean count before and after treatment.

Conventional formulations are limited by poor solubility, high chemical/photochemical instability and the irritation. Interestingly, lipid nanoparticles enable the administration of retinoids in aqueous media, providing drug stabilization and controlled release⁽²⁵⁾.

The present study evaluated this novel nano formulation of retinoids with the conventional formulation in patients with mild to moderate acne vulgaris of the face for 6 weeks. Evaluation of the mean change in the lesion count in this clinical study indicated that the nano formulation is significantly better in reducing the inflammatory and the total lesion count as compared to the conventional formulation. This finding is in agreement with **Chandrasekhar et al.**⁽¹⁷⁾ who compared tretinoin nanogel with conventional one and showed significant reduction in total lesions, and inflammatory lesions in the nano formulation group.

The incorporation of retinoids into solid lipid nanoparticles significantly improved

their effects for the treatment of acne compared with commercial formulations ⁽²⁶⁾.

Nano emulsion gel adapalene 0.1% was compared with conventional gel of adapalene and clindamycin (as phosphate) 1% combination. They showed significant better reduction in total, inflammatory and non-inflammatory lesions with the Nano-emulsion gel as compared to the conventional gel ⁽²⁷⁾.

in comparison of isotretinoin loaded solid lipid nanoparticles with conventional formulation in the treatment of mild to moderate acne vulgaris accompanied with topical administration of clindamycin 2% solution twice a day for 8 weeks on both sides of the face showed Isotretinoin solid lipid nanoparticles had higher efficacy than conventional to clear non-inflammatory and inflammatory lesions ⁽²⁸⁾.

The better efficacy of Nano retinol compared to conventional formulation is attributed to nano technology which an important place in acne therapy ⁽¹⁸⁾. Nanotechnology aids in drug permeation by releasing active substances at specific sites, increasing stability, ensuring adequate contact ⁽²⁹⁾.

Nanoparticles have been used as a drug carrier for transdermal drug delivery system. It has been found that encapsulation of substances in nanoparticles enhances their transdermal penetration and permeation ⁽³⁰⁾.

Nanoparticulate systems not only enhance skin absorption but can allow for drug targeting to the skin and/or its substructures ⁽³¹⁾. The bioavailability of drugs permeating into the skin can be enhanced by using Nano carriers because the small particulate size ensures close contact to the SC ⁽³²⁾. Nano-sized particles can make close contact with superficial junctions of the SC and the furrows between the corneocyte islands, allowing superficial spreading of the active agents. Following the evaporation of water from the nanosystems applied to the skin surface, particles form an adhesive layer occluding the skin. Hydration of the SC thus increases to reduce corneocyte packing and widen the inter-corneocyte gaps, subsequently enhancing drug transport ⁽³³⁾.

The enhanced efficacy of the nanotechnology formulation as compared to the conventional formulation is also attributed

to the advanced nanotechnology which allows increased concentration of the active drug in the pilosebaceous unit ⁽³⁴⁾.

Another reason for improved efficacy of the nano formulation is the enhanced photostability in the novel formulation as compares to conventional one. **Sungho and Seung-Cheol**, ⁽³⁵⁾ investigated the effect of nanoliposomes on the stabilization of incorporated retinol under a variety of conditions such as temperature, UV light, and time. Retinol was efficiently incorporated into Nano liposomes and the stability of incorporated retinol in nanoliposomes was significantly enhanced under both dark and UV light during the whole period of storage, compared to that of bare retinol. The retinol in nanoliposomes was not considerably affected by UV light, implying that nanoliposomes could act as a barrier for retinol against UV light to some extent.

Local adverse effects in our study were significantly lower in the nano formulation as compared to the conventional one. This finding is in agreement with **Chandrasekhar et al.** ⁽¹⁷⁾ who used nano formulation compared to conventional formulation and showed that local adverse events (erythema, dryness, peeling, and burning) were significantly less in the nanogel as compared to the conventional one.

When they compared the nano-emulsion gel with conventional gel of adapalene. The nano form showed significantly lower incidence and lesser intensity of adverse events like local irritation and erythema ⁽²⁷⁾.

In the present study global assessment of tolerability showed that Nano formulation was significantly better tolerated as compared to the conventional one. Ninety percent of patients were rated excellent tolerability compared to conventional one as none of the conventional group patient rated to excellent tolerability. Ten percent of patients rated good and none of them rated poor when compared to the conventional group as 6.7% rate good and 93.7% rated fair tolerability. This finding is in agreement with a comparative study that used liposomal tretinoin 0.001 mg in 20 patients with uncomplicated acne received liposomal tretinoin on one side of the body vs commercial preparation with either 0.25 mg Or

0.005 mg tretinoin on the other side. The liposomal tretinoin is better tolerated than commercial one⁽³⁶⁾.

A major advantage of such delivery systems is the better tolerability of irritating retinoid improving patient compliance as well as the avoidance of systemic absorption and side effects⁽³⁷⁾.

Retinoid-loaded nanostructured systems have decreased the adverse effects of these molecules and protected them against degradation.⁽³⁸⁾

SLN's loaded with all-trans-retinoic acid (ATRA) were significantly less irritant than commercial retinoid cream.⁽³⁹⁾

This better tolerability profile of the novel formulation is also attributable to the nano technology. The reduced incidence of skin irritation is hypothesized to be the result of encapsulation of retinol in the nano formulation which reduces the contact of the acidic function (-COOH) of retinol (the triggering factor for topical adverse events) with the stratum corneum⁽⁴⁰⁾.

CONCLUSION

Nanotechnology is considered to be a new industrial revolution and also proved to be beneficial in many dermatological and cosmetic preparations. The field of nanoparticle drug delivery to the skin has progressed for more safe and targeted delivery of active drug moiety. Nanotechnology is being utilized in cutaneous drug delivery to stabilize compounds, allow controlled release, and to enable targeted drug localization to maximize activity and minimize toxicity. In the treatment of facial acne vulgaris Nanosomal retinol with iontophoresis is more efficacious and better tolerated than its conventional formulation with nearly no side effects and no precautions for use.

Recommendations

More studies are needed on a wider scale, greater number of patients with different grades of acne vulgaris to support our findings.

REFERENCE

- 1- Yentzer BA., Baldwin H, Shalita AR, Webster G and Feldman SK (2010): Optimizing patient adherence: update on combination acne therapy—teens and beyond. *J Drugs Dermatol*, 9(6):6.
- 2-Friedlander SF, Eichenfield LF, Fowler JF *et al.* (2010): Acne epidemiology and pathophysiology. *Semin Cutan Med Surg.*, 29:2–4.
- 3-Bikowski JB (2004): Adopting and innovative acne grading scale into practice, *practical dermatology*, 5(1):48-52.
- 4-Brown SK and Shalita AR (1998): Acne vulgaris. *Lancet*, 351:1871-6.
- 5-Strauss J, Krowchuk DP and Leyden J (2007): Guidelines of care for acne vulgaris management. *J Am Acad Dermatol.*, 6(4):651–63.
- 6-Kurokawa I, Danby FW, Ju Q *et al.* (2009): New developments in our understanding of acne pathogenesis and treatment. *Exp Dermatol.*, 18: 821–32.
- 7-Haider A and Shaw J (2004): Treatment of acne vulgaris. *JAMA.*, 292(6):726–35.
- 8-Wolf JR (2002): Potential anti-inflammatory effects of topical retinoids and retinoid analogues. *adv.*, 19:109-118.
- 9-Yeutzer BA, McClain RW and Feldman SR (2009): Do topical retinoids causes acne to flare? *J Drugs Dermatol.*, 8:799-801.
- 10-Hia J and Nasir A (2011): Photo Nano dermatology: the interface of photobiology, dermatology, and Nanotechnology, 27:2-9.
- 11-Souto EB and Muller RH (2008): Cosmotic features and applications of lipid nanoparticles (sln, Nlc). *Int J Cosmet Sci.*, 30:157-165.
- 12-Castro GA, Coelho AL, Oliveira CA, Mahecha GA, Orefice RL and Ferreira LA (2009): Formation of ion pairing as an alternative to improve encapsulation and stability and to reduce skin irritation of retinoic acid loaded in solid lipid nanoparticles. *Int J Pharm.*, 381:77-83.
- 13-Jenning V, Gysler A, Schafer-Korting M and Gohla SH (2000): Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. *Eur J Pharm Biopharm.*, 49:211-8.
- 14-Lehmann HP, Robinson KA, Andrews JS, Holloway Vand Goodman SN (2002): Acne therapy: a methodologic review. *J Am Acad Dermatol.*, (47): 231–240.
- 15-Roshashlinie R, Aamir S, Ahmad Fadzil M and Adawiyah J (2011): Acne analysis, grading and computational assessment methods: an overview. *Skin Research and Technology*, 18: 1–14.
- 16- Huh CH, Seo K L, and Park J Y (2003): A randomized, double-blinded Placebo-controlled Trial of Vitamin C Iontophoresis in Melasma. *Dermatology*, 206:316-320.
- 17-Chandrashekhhar BS, Anitha M, Mukesh R *et al.* (2015): Tretinoin Nanogel 0.025% Versus Conventional Gel 0.025% in Patients with Acne Vulgaris: A Randomized, Active Controlled, Multicentre, Parallel Group, Phase IV Clinical Trial. *Journal of Clinical and Diagnostic Research*, 9(1): WC04-WC09.
- 18- Tarun G (2014): Current nanotechnological approaches for an effective delivery of bio-active

- drug molecules in the treatment of acne. *Artificial Cells, Nanomedicine, and Biotechnology*; 44 (1): 98-105.
- 19-Bershad S, Kranjac Singer G, Parente JE, Tan M-H, Sherer DW, Persaud AN et al .(2002):** Successful treatment of acne vulgaris using a new method: results of a randomized vehicle-controlled trial of short-contact therapy with 0.1% tazarotene gel. *Arch Dermatol.*, 138:481-9.
- 20-Gollnick H, Cunliffe WJ, Berson D et al. (2003):** Management of acne: a report from a global alliance to improve outcomes in acne. *J Am Acad Dermatol.*,49:S1-37.
- 21-Chivot M (2003):** Treatment of mild to moderate acne. *Ann Dermatol Venereol.*, 130:132-5.
- 22-Millikan LE (2003):** The rationale for using a topical retinoid for inflammatory acne. *Am J Clin Dermatol.*, 4:75-80.
- 23-Tenaud I, Khammari Aand Dreno B (2007):** In vitro modulation of TLR-2, CD1d and IL-10 by adapalene on normal human skin and acne inflammatory lesions. *Exp Dermatol.*, 16 (6):500-506.
- 24-Maneerung, Tokura S, and Rujiravanit R (2008):** Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing.” *Carbohydrate Polymers*, 72(1): 43-51.
- 25-Javier O, Karina V, Javier M and Felipe O (2015):** Lipid nanoparticles for the topical delivery of retinoids and derivatives. *Nanomedicine (Lond.)*,10(2): 253-269.
- 26-Katare OP, Raza K, Singh B and Dogra S (2010):** Novel drug delivery systems in topical treatment of psoriasis: rigors and vigors. *Indian J. Dermatol. Venereol. Leprol.*,76(6): 612-621
- 27-Prasad S, Mukhopadhyay A, Kubavat A et al. (2012):** Efficacy and safety of a nano-emulsion gel formulation of adapalene 0.1% and clindamycin 1% combination in acne vulgaris: a randomized, open label, active-controlled, multicentric, Phase IV clinical trial. *Indian J. Dermatol. Venereol. Leprol.*, 78(4), 459-467.
- 28-Pouran L, Navid M, Danial B, Mahmoud R and Shiva G (2013):** The efficacy of isotretinoin-loaded solid lipid nanoparticles in comparison to Isotrex® on acne treatment. *Nanomed J.*, 1(1):38-47.
- 29-Gupta S, Basal R, Gupta S, Jindal N and Jindal A (2013):** Nanocarriers and nanoparticles for skin care and dermatological treatments. *Indian Dermatol Online J.*, 4: 267-272.
- 30-Rastogi R, Anand S and Koul V (2010):** Electroporation of polymeric nanoparticles: an alternative technique for transdermal delivery of insulin. *Drug Dev Ind Pharm.*, 36(11):1303- 1311.
- 31-Schafer-Korting M, Mehnert W, Korting HC (2007):** Lipid nanoparticles for improved topical application of drugs for skin diseases. *Adv Drug Deliv Rev.*, 59:427-43.
- 32-Souto EB, Almeida AJ and Müller RH (2007):** Lipid nanoparticles (SLN®, NLC®) for cutaneous drug delivery: structure, protection and skin effects. *J. Biomed. Nanotechnol.*, 3: 317-331.
- 33- Smijs TGM and Bouwstra JA (2010):** Focus on skin as a possible port of entry for solid nanoparticles and the toxicological impact. *J. Biomed. Nanotechnol.*, 6: 469-484.
- 34-Liu J, Hu W, Chen H, Ni Q, Xu H and Yang X (2007):** Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery. *Int J Pharm.*,328:191- 95.
- 35-Sungho Ko and Seung-Cheol L (2010):** Effect of nanoliposomes on the stabilization of incorporated retinol. *African Journal of Biotechnology*, 9 (37): 6158-6161.
- 36-Ming Fa and Chao-H (2011):** Delivery of pharmaceutical agents to treat acne vulgaris:current status and perspectives.journal of medical and biological engineering,32(4):215-224.
- 37-Papakostas D, Rancan F, Sterry W, Blume-Peytavi U and Vogt A (2011):** Nanoparticles in dermatology. *Arch Dermatol Res.*,303:533-50.
- 38-Raza K, Singh B, Singal P, Wadhwa S and Katare OP (2013):** Systematically optimized biocompatible isotretinoin-loaded solid lipid nanoparticles (SLNs) for topical treatment of acne. *Colloids Surf. B Biointerfaces*, 105: 67-74.
- 39-Castro GA, Oliveira CA, Mahecha GA, Ferreira LA (2011):** Comedolytic effect and reduced skin irritation of a new formulation of all-trans retinoic acid-loaded solid lipid nanoparticles for topical treatment of acne. *Arch Dermatol Res.*, 303:513-20.
- 40-Yamaguchi Y, Nagasawa T, Nakamura N, Takenaga M, Mizoguchi M, Kawai S et al (2005):** Successful treatment of photo-damaged skin of nano-scale at RA particles using a novel transdermal delivery. *J Control Release*, 104:29-40.