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POLYPHENOLS AS ADJUVANT IN TOSSING OUT ACNE VULGARIS (PUSTULES): A NOVEL FORMULATION STRATEGY

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ABSTRACT: Acne vulgaris is a skin condition in which people lose their self-esteem and ultimately lead to depression. It is more common among the adolescent age groups. According to GBD (Global burden of disease) study, about 85% of the populations are being affected. Various medications are available for the treatment, but, its uses are limited due to side effects like skin irritation. In our study, we have formulated a novel combination nanoemulsion gel containing adapalene and resveratrol for the treatment of pustules, assuming that it won't produce much skin irritation and also expecting a synergistic activity. The spontaneous emulsification method was employed for the preparation of nanoemulsion, and it was incorporated into carbopol 934 gel. Further, various evaluation tests, including *in-vitro* and *in-vivo* studies, were performed. Cumulative percentage drug release was more for marketed formulation as compared to prepared formulation, whereas, the prepared formulation had a better efficacy as compared to marketed formulation. Thus, we can say that the formulation has exhibited its synergistic activity on combining with a polyphenol. Additionally, as an outcome of the skin irritation study, the formulation did not produce any skin irritation. Hence, we can conclude that the novel formulation containing adapalene and resveratrol served as a better candidate for the management of acne (pustules).

INTRODUCTION: Acne vulgaris is a common skin condition that affects most people with ages ranging from 12-24 [1]. Basically, acne can be categorized into blackheads, whiteheads, papules, pustules and nodules. Mechanisms involved in the evolution of acne are hyper-proliferation of follicular epithelium, excess sebum production from sebaceous gland, *Propionibacterium acne* (bacteria), and finally inflammatory mediators like interleukins, tumor necrosis factor, fatty acids, and other bacteria-derived mediators.

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Though various treatment methods are available, the most preferred one is topical medications because of the ease of administration and their direct action on the affected area, and they can be chosen either as a monotherapy or a combination therapy. The main aim of our study is to formulate a combination therapy using a polyphenol as an adjuvant. Adapalene is used as the choice of drug.

The reason for choosing adapalene is that it is a retinoid derivative, used as the first-line therapy, and moreover, compared to other retinoid derivatives like tretinoin and isotretinoin, adapalene has less skin irritation. Resveratrol is used as an adjuvant, assuming that it will produce a synergistic effect with the drug. It is produced naturally by many plants, and it is present in the skin of red grapes, blueberries, raspberries, and mulberries [2].

It has been reported that resveratrol acts against the bacteria (*p.acne*) causing acne [3]. Because of these reasons, we have opted resveratrol as an adjuvant. Apart from adapalene and resveratrol, an additional ingredient used in the formulation can act against acne (*i.e.*) tea tree oil, obtained from *Melaleuca alternifolia*. Several studies have proved that tea tree oil can act against acne. It is the terpinen-4-ol and α-terpineol found in the oil, which is responsible for action [4].

We have selected nanoemulsion gel for the topical application. The reason for choosing nano-technology is that it can easily penetrate into the skin by crossing the stratum corneum, and it can produce therapeutic action. In this work, we have induced pustules (a moderate form of acne) and compared the efficacy of the prepared formulation with that of the marketed formulation (Adapalene + benzoyl peroxide).

MATERIALS AND METHODS:

Materials: Adapalene was obtained as a gift sample from Arti industries. The marketed formulation was obtained as a gift sample from the apex, Chennai, India. Capryol 90 was obtained from Gattefose India Pvt. Ltd., Mumbai. Isopropyl myristate 98% was obtained from Avra.

Methods:

Preformulation Studies: [5]

(a) Compatibility Studies:

- **DSC (Differential Scanning Calorimetry):** [2, 6] To check the purity of the drug and compatibility between the drug and excipients, DSC were carried out.

- **FTIR (Fourier Transformer Infrared Spectroscopy):** [7, 8] Again, the compatibility between the drug and excipients was determined using FTIR.

(b) Screening of Oil, Surfactant and Co-Surfactant:

- **Selection of Oil Based on the Solubility:** [9] Solubility of drug and polyphenol was checked in different oils by shake flask method. Adapalene and resveratrol were added to different oils in a volumetric flask and kept at 37 ºC in an orbitrary shaker for 24 h to determine the solubility.

Selection of Surfactant and Co-surfactant: Kolliphor and ethanol was selected as surfactant and co-surfactant for the preparation of nano-emulsion.

Formulation of Nanoemulsion: [10, 11] Spontaneous emulsification methods were employed. We have prepared 2 formulations, namely F1 (Adapalene + 5 mg resveratrol) and F2 (Adapalene +10 mg resveratrol) to check the synergistic activity of the flavonoid.

Initially, we have dissolved adapalene and resveratrol in tea tree oil and capryol 90, respectively. After dissolving, we mixed both the oils (tea tree oil and capryol 90) as well as smix together. Later the samples were titrated with water to get a translucent to transparent liquid. Further, it was subjected to thermodynamic stability studies. Further, the stable ones have incorporated into carbopol 934 gels.

Evaluation of Nanoemulsion:

Thermodynamic Stability Studies: [12, 13] Samples were subjected to thermodynamic stability studies, including heating and cooling cycle, centrifugation, and freeze-thaw cycle. Then, the stable ones were taken further.

Characterization of Nanoemulsion: Particle size, PDI (polydispersity index), and zeta potential [14] using zeta sizer (Malvern Instruments) was determined.

Preparation of Gel and Incorporation of Nanoemulsion: [15] Carbopol 934 at different concentrations including 0.4%, 0.6%, and 0.8% was dissolved in a suitable quantity of water. 0.8% concentration of carbopol was selected since it formed a clear gel. Then, the nanoemulsion was incorporated into a gel, and it was further neutralized with triethanolamine to maintain the pH between 6.8-7.4.

Characterization of Adapalene Loaded Nano-emulsion Gel:

Determination of pH: pH of the prepared formulation was checked with the help of a digital pH meter [16, 17].

Viscosity Determination: [18] Viscosity of the formulation was checked without any dilution.

It was determined by using Brookfield DV-II programmable viscometer. Spindle no 28 was allowed to rotate at 40 rpm at 19 ºC for 10 min. The initial and final values were considered.

Spreadability of the Formulation: Simple hand spreading technique was employed to determine the ease of administration.

Determination of Extrudability: [19] Formulation was filled into the lacquered aluminum collapsible tube, and weight was applied to it. The extruded quantity was weighed.

***Ex-vivo* Studies:** [20] Franz diffusion cell was used for this study. Phosphate buffer 7.4 was used as the media. The formulations were kept on the donor compartment, and later 5 ml of samples were withdrawn from the receptor compartment at different time intervals like 1, 2, 3, 4, 5, 6, 7, and 8 h and replaced with fresh buffer solution. All the samples were analyzed using UV by simultaneous determination method. Finally, the cumulative percentage of drug release was determined [21].

***In-vivo* Study:** Rabbits weighing about 2.5-4 kg of either gender were taken. All the animals were kept in the laboratory 10 days prior to the initiation of the experiment in order to get adapted to the environmental surroundings by getting permission from the Institutional animal ethics committee (IAEC) (Registration no: 118/PO/ReBi/S/1999/ CPCSEA), JSS College of Pharmacy, Udhagamandalam, Tamilnadu, India.

Skin Irritation Study: [22, 23] Skin irritation study was carried out according to "Acute Dermal Irritation/Corrosion test" as given in the OECD (404) GUIDELINE. First patch of 2 formulations (F1 and F2) and blank formulation (without drug) was applied on the skin and removed after 3min. In the same manner, it was applied for 1 h and 4 h. Since there was no skin irritation, it was observed again for 24 h, 48 h, and 72 h. And it was also kept till 14th day for the reversibility of the effect.

Induction of Pustules: [24] Hairs on the back were shaved (30 cm^2- 40 cm^2 area). Marking was carried out in such a way that in one area SLS (sodium lauryl sulphate) and in another area $HgCl_2$ (Mercuric chloride) was used for induction. $HgCl_2$ (10%, 15%) and SLS (5%) was used for the induction of pustules by dispersing in petrolatum. After the application of chemicals, the animals were kept for 24 h. After 24 h, the pustules got induced to those animals which was exposed to $HgCl_2$. SLS didn't produce any results. Thus, $HgCl_2$ was considered for the induction of pustules.

Treatment of Pustules Using Marketed and Prepared Formulation: The formulations were applied once daily and observed for efficacy. Group 1 was treated with prepared formulation (F1), group 2 was treated with 2nd prepared formulation (F2), and group 3 and 4 was treated with the marketed formulation.

RESULTS AND DISCUSSION:

Preformulation Study:

(a) Compatibility Studies:

DSC (Differential Scanning Calorimetry): Endothermic peaks were obtained for the drug as well as for the physical mixture. Since the peaks were remaining the same in all the thermograms, it indicates that there is no interaction between the drug and the excipients.

Thus, it can be concluded that drugs and excipients are compatible. DSC for adapalene, resveratrol and carbopol 934 was found to be 327.59 ºC, 268.31 ºC, and 95 ºC, respectively. While coming to the physical mixture, there was a slight peak shift for all three peaks. Thus, again the compatibility was determined using FTIR.

FTIR: There was no disappearance or appearance of additional peaks. Shifting was also not present. Thus, it indicates that the drug and excipients are compatible and doesn't produce any interactions. FTIR data are given in **Table 1**.

TABLE 1: FTIR DATA

Adapalene	Resveratrol	Carbopol 934	Adapalene and Resveratrol	Functional Group in Physical Mixture
	3202.51	3059.42	3160.37	OH group (3160.37)
1688.57		1698.69		C=O stretching (1692.71)
1601.54			1605.05	Aromatic C=C stretching (1605.18)
1425.89		1450.33	1426.48	OH bending (1444.82)
1234.27		1409.03		C-O stretching (1234.65)
	965.03		964.95	C-H out of plane bending (965.10)

Solubility Studies: Solubility data is given in **Table 2**. Based on the results, tea tree oil and capryol 90 were selected as the oil phase. The solubility of adapalene was more in tea tree oil, probably due to the affinity of the drug towards the oil whereas, the solubility of resveratrol was only 10 mg/ml in tea tree oil. Hence, it was a compulsion to opt for the other oils. 45 mg of the resveratrol solubility was achieved with 1ml of Capryol 90. The reason could be the ester group of capryol 90, which would have influenced the solubility. Therefore, tea tree oil and capryol 90 were taken as the oil phase.

TABLE 2: SOLUBILITY STUDIES

Oil	Adapalene (mg/ml) (Mean ± SD)	Resveratrol (mg/ml) (Mean ± SD)
Oleic acid	0.10 ± 0.120	2.1 ± 0.291
Soya bean oil	0.11 ± 0.126	2.3 ± 0.216
Olive oil	0.13 ± 0.131	2.5 ± 0.032
Capmul MCM	0.14 ± 0.134	2.6 ± 0.160
Labrafil	0.20 ± 0.142	2.9 ± 0.152
Labrfac	0.21 ± 0.146	3.8 ± 0.122
Isopropyl myristate	0.25 ± 0.158	5.2 ± 0.340
Castor oil	0.26 ± 0.162	6.9 ± 0.294
Capryol 90	0.38 ± 0.181	45 ± 0.375
Tea tree oil	1.00 ± 0.250	10 ± 0.264
Capryol 90+ tea tree oil	0.40 ± 0.195	12 ± 0.540

SD: Standard deviation

Screening of Surfactant and Co-surfactant: In the present study, different surfactants and co-surfactant were tested in order to check the formation of stable nanoemulsion. But, most of them were unstable and divided into two layers. The combination of kolliphor and ethanol was selected for the formulation, and different ratios were prepared in order to pick up the stable one for the final formulation. Since, the nanoemulsion is obtained by spontaneous emulsification method the addition of water makes the difference and leads to the formation of either emulsion or nanoemulsion.

Characterization of Nanoemulsion: Out of 8 samples, only 6 samples were finally selected whereas, the other 2 samples were not stable due to phase separation. Further, the following parameters were determined.

Particle Size and PDI (Poly Dispersity Index): Particle size and PDI was expected to be <100 nm and <1. Selected sample was having low particle size of 39.99 nm. They also exhibited broad size distribution.

In addition, PDI value of 0.234 was also within the range as compared to that of other samples. The maximum percentage intensity of the particle in this formulation was found to be 25%.

Zeta Potential: The normal value of zeta potential is between -30 mV and +30 mV. For the prepared formulation, it was found to be -0.665 mV. Thus, it is a stable formulation.

Selection of Gelling Agent: Compared to 0.4% and 0.6%, 0.8% concentration was not having a free-flowing capacity until and unless external stress was applied, and it also had good viscosity. Thus, 0.8% gel was prepared, and nanoemulsion was incorporated.

Characterization of Nanoemulgel:

Determination of pH: Formulation pH was found to be 6.4-6.5, which was equivalent to skin pH. Hence, it can be said that the formulation is compatible and may not produce skin irritation.

Viscosity Determination: The consistency of the formulations F1 and F2 were 79350 cp and 68650 cp, respectively, which are normally considered as good for gels.

Spreadability: Ease of application was taken into consideration.

Extrudability:

***Ex-vivo* Drug Release Study:** Simultaneous determination method was employed. Release of drug from the prepared formulation as well as the marketed formulation is given in **Fig. 1-3**. The release of adapalene, resveratrol, and benzoyl peroxide was determined at 282 nm, 306 nm, and 236 nm, respectively. From the graph, it was clear

that even though the prepared formulation was a nanoemulsion, the marketed formulation was having more drug release, which may be due to a higher dose of drug (5 mg) in marketed formulation as compared to that of the prepared formulation (1 mg).

TABLE 3: REPRESENTS THE EXTRUDABILITY OF THE FORMULATION WITH INCREASING WEIGHT

Initial Amount of Sample Filled in the Tube	Weight Applied	Amount of Sample Extruded	Total Amount of Sample Extruded
14.14 grams	Initial weight= 300 g	0.064 g	9.63 g
	2 kg	0.3263 g	
	4 kg	0.7047 g	
	6 kg	0.795 g	
	8 kg	1.504 g	
	10 kg	3.895 g	
	12 kg	2.341 g	

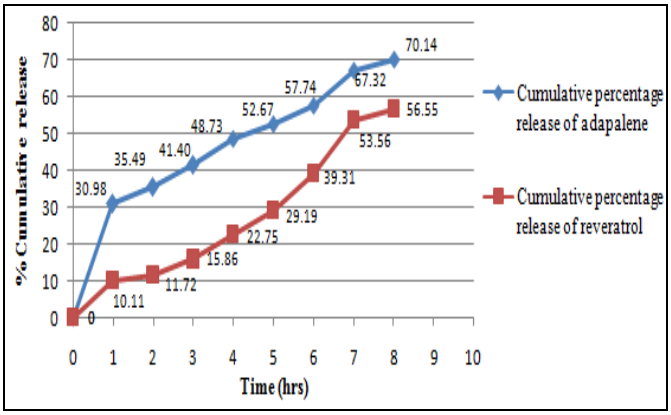


FIG. 1: CUMULATIVE DRUG AND FLAVONOID RELEASE FROM F1 FORMULATION

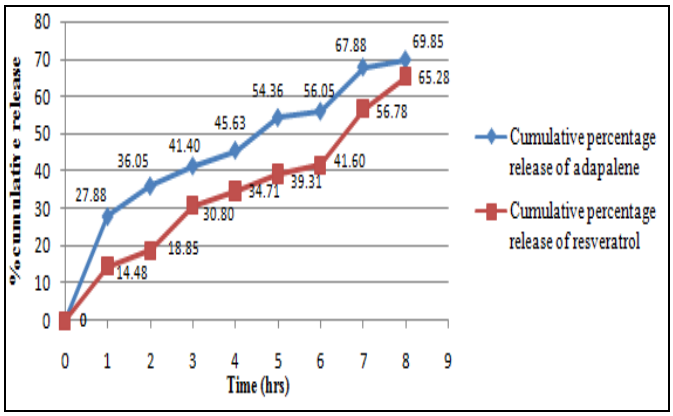


FIG. 2: CUMULATIVE DRUG AND FLAVONOID RELEASE FROM F2 FORMULATION

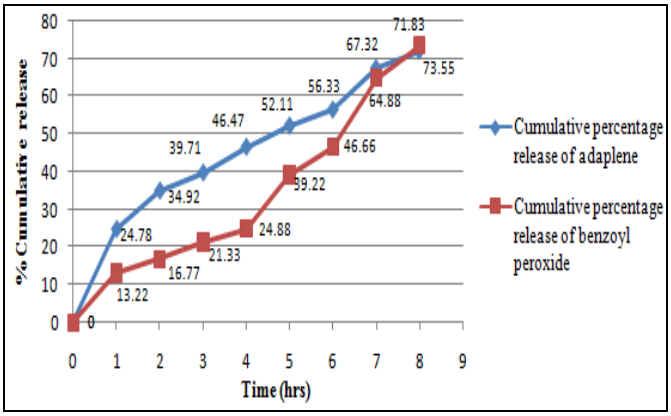


FIG. 3: CUMULATIVE DRUG RELEASE FROM MARKETED FORMULATION

***In-vivo* studies:**

Skin Irritation Study: There was no skin irritation, redness, or erythema observed during this study.

Hence, we can say that the formulation is safe for topical application. **Table 4** represents the visual observation of the skin irritation study.

Treatment of Pustules and Comparison of the Efficacy: Group 1 and 2, which were treated with prepared formulation, showed better efficacy as compared to that of the marketed formulation.

Table 5 represents the animals before and after the treatment. The mechanism of action of the prepared formulation may be as given in **Fig. 4** since the prepared formulation is a nanoemulsion and it had a capacity to penetrate easily into the stratum corneum as compared to the marketed formulation, thus producing a better efficacy.

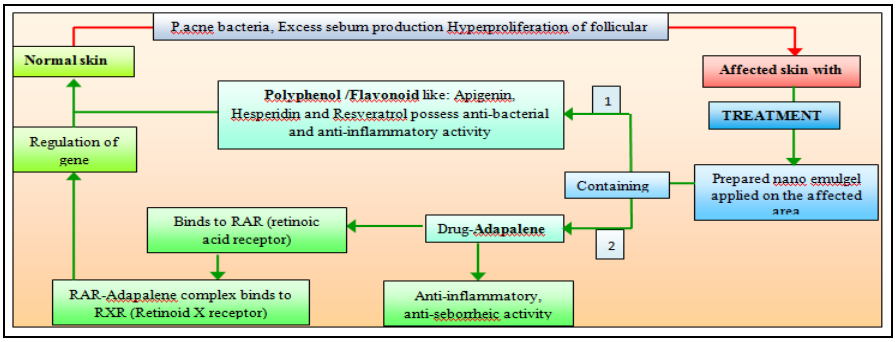


FIG. 4: MECHANISM OF ACTION OF THE PREPARED FORMULATION ON THE ACNE

In addition, tea tree oil would have also exhibited its action. Moreover, resveratrol would have shown its synergistic activity by acting as an adjuvant.

From **Table 5**, it is clearly visible that the prepared formulation had more efficacies as compared to that of the marketed formulation.

TABLE 4: REPRESENTS THE SKIN IRRITATION STUDY

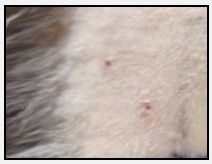
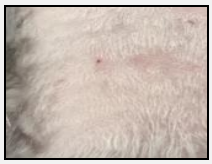
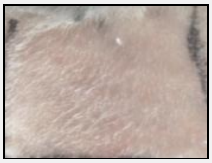
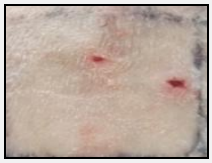
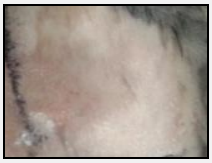
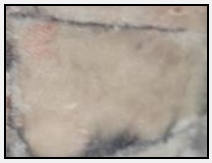
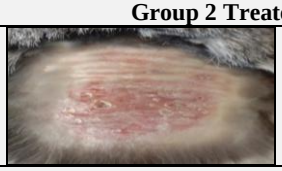
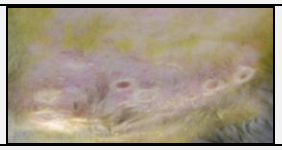
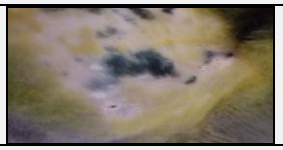
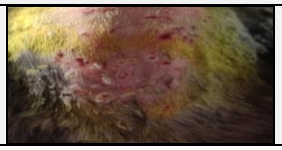
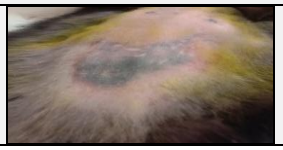
Sample	Before Application	After 1 min	After 1 h	After 4 h	At 14th Day
Blank					
F1					
F2					

TABLE 5: REPRESENTS THE TREATMENT OF PUSTULES WITH THE PREPARED AND MARKETED FORMULATION

Treatment of Pustules	
Before Treatment	**After Treatment**
Group 1 Treated with Formulation F1 with Treatment Time of 12 Days	
	
Group 2 Treated with Formulation F2 with the Treatment Time of 11 Days	
	
Group 3 Treated with Marketed Formulation Containing Adapalene and Benzoyl Peroxide with the Treatment Time of 26 Days	
	
Group 4 Treated with Marketed Formulation Containing Adapalene and Benzoyl Peroxide with the Treatment Time of 19 Days	
	

CONCLUSION: Pustules are one of the moderate to severe forms of acne, in which the top layer of the skin will be covered with pus. The main aim of our study was to decrease the treatment time that is taken for the management of acne vulgaris. In addition, to decrease the scars as well as the skin irritation caused by other retinoid derivatives. Moreover, the addition of resveratrol has also been served as an adjuvant and helped in decreasing the treatment time. Since we have implemented nanotechnology in this work, the formulation can easily cross through the startum corneum and act on the affected area. In addition to these factors, we have also used tea tree oil in our formulation. Tea tree oil possesses different activities like anti-bacterial and anti-oxidant etc. And it has also been widely used for the treatment of acne. In our study, tea tree oil would have also exhibited its action on pustules. Thus, the preparation of nanoemulsion gel containing adapalene and resveratrol was useful in the treatment of pustules, and moreover because of this synergistic activity, we can also reduce the dose of the drug (adapalene). Even the treatment time for the prepared formulation was less as compared to that of the marketed formulation. The treatment time taken by marketed formulation was 19-26 days, whereas prepared formulation took only 11-12 days for the treatment. Hence, it can be said that the prepared formulation had improved efficacy in the treatment of pustules. Therefore, it can be concluded that the novel combination of adapalene and resveratrol would serve as a better combination therapy in the management of acne vulgaris (pustules).

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CONFLICTS OF INTEREST: Authors declare that there no conflicts of interest.

REFERENCES:

1. Ray C, Trivedi P and Sharma V: Acne and its treatment lines. Inter J of Res in Pharma and Biosci 2013; 3(1): 1-16.
2. Silva RC, Teixeira JA and Nunes WDG: Resveratrol: a thermo analytical study. Food Chem 2017; 237: 561-65.
3. Docherty JJ, McEwen HA and Sweet TJ: Resveratrol inhibition of propionibacterium acnes. Journal of Antimicrobial Chemotherapy. 2007; 59: 1182-84.
4. Hammer KA: Treatment of acne with tea tree oil (Melaleuca) products: a review of efficacy, tolerability and potential modes of action. International Journal of Antimicrobial Agents 2014; 1-5.
5. Shaikh NM, Swamy SV, Narsing NS and Kulkarni KB: Formulation and evaluation of nanoemulsion for topical application. J of Drug Delive and Thera 2019; 9(4): 370-5.
6. Nadal JM, dos Camargo AG, Novatski A, Macenhan WR, Dias DT, Barboza FM, Lyra A, Roik JR, de Paula JP, Somer A and Farago PV: Adapalene-loaded poly (ε-caprolactone) microparticles: physicochemical characterization and *in-vitro* penetration by photoacoustic spectroscopy. PloS One 2019; 14(3).
7. Bhalekar M, Upadhaya P and Madgulkar A: Formulation and evaluation of adapalene-loaded nanoparticulates for epidermal localization. Drug Delivery and Translational Research 2015; 5(6): 585-95.
8. Ansari KA, Vavia PR, Trotta F and Cavalli R: Cyclodextrin-based nanosponges for delivery of resveratrol: *in-vitro* characterisation, stability, cytotoxicity and permeation study. AAPS Pharm Sci Tech 2011; 12(1): 279-86.
9. Priya S, Koland M and Kumari S: Nanoemulsion components screening of quetiapine fumarate: effect of surfactant and co surfactant. Asian J Pharm Clin Res 2015; 8(6): 136-40.
10. Zeng L, Liu Y, Pan J and Liu X: Formulation and evaluation of norcanthridin nanoemulsions against the *Plutella xylostella* (Lepidotera: Plutellidae). BMC Biotechnology 2019; 19(1): 16.
11. Rai VK, Mishra N, Yadav KS and Yadav NP: Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and applications. Journal of Controlled Release 2018; 270: 203-25.
12. Najafi-Taher R, Ghaemi B and Amani A: Delivery of adapalene using a novel topical gel based on tea tree oil nanoemulsion: Permeation, antibacterial and safety assessments. Eur J Pharm Sci 2018; 120: 142-51.
13. Gurpreet K and Singh SK: Review of nanoemulsion formulation and characterization techniques. Indian J Pharm Sci 2018; 80(5): 781-89.
14. Harde H, Agrawal AK, Katariya M, Kale D and Jain S: Development of topical adapalene-solid lipid nanoparticles loaded gel with enhanced efficacy and improved skin tolerability. RSC Advances 2015; (55): 1-38.
15. Ubaid M, Ilyas S, Mir S, Khan AK, Rashid R, Khan MZ, Kanwal ZG, Nawaz A and Shah A, Murtaza G: Formulation and *in-vitro* evaluation of carbopol 934-based modified clotrimazole gel for topical application. An Acad Bras Cienc 2016; 88(4): 2303-17.
16. Prabu SL, Umamaheswari A, Rajakumar S, Bhuvaneswari1 PL and Muthupetchi S: Development and evaluation of gel incorporated with synthesized silver nanoparticle from ocimum gratissimum for the treatment of acne vulgaris. American Journal of Advanced Drug Delivery 2017; 5(3): 107-17.
17. Avinash S, Gowda DV, Suresh J, Ram AS, Srivastava A and Osmani RM: Formulation and evaluation of topical gel using eupatorium glandulosum michx. for wound healing activity. Der Pharmacia Lettre 2016; 8(8): 255-66.
18. Jangdey MS, Gupta A and Saraf S: Fabrication, *in-vitro* characterization and enhanced *in-vivo* evaluation of carbopol-based nanoemulsion gel of apigenin for UV-induced skin carcinoma. Drug Deliv 2017; 24(1): 1026-36.
19. Aiyalu R, Govindarjan A and Ramasamy A: Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Braz J Pharm Sci 2016; 52(3): 493-07.

20. Elmataeeshy ME, Sokar MS, Bahey-El-Din M and Shaker DS: Enhanced transdermal permeability of terbinafine through novel nanoemulgel formulation; development, *in-vitro* and *in-vivo* characterization. Future Journal of Pharmaceutical Sciences 2018; 4(1): 18-28.
21. Gupta A, Gulati M and Pandey NK: A validated UV spectrophotometric method for simultaneous estimation of tretinoin and benzoyl peroxide in bulk and semi solid dosage form. J Chem 2009; 2(3): 649-54.
22. Wang J, Li Z, Sun F, Tang S, Zhang S, Lv P, Li J and Cao X: Evaluation of dermal irritation and skin sensitization due to vitacoxib. Toxicology Reports 2017; 4: 287-90.
23. Mekonnen A, Tesfaye S, Christos SG, Dires K, Zenebe T, Zegeye N, Shiferaw Y and Lulekal E: Evaluation of skin irritation and acute and subacute oral toxicity of lavandula angustifolia essential oils in Rabbit and Mice. Journal of Toxicology 2019; 1-8.
24. Wahlberg JE and Maibach HI: Sterile cutaneous pustules: a manifestation of primary irritancy identification of contact pustulogens. J Investi Derma 1981; 76(5): 381-83.
25. Janani SK, Kumar RS, Karthika SSNUC and Vasanthi C: Will the polyphenol and adapalene combination be a good strategy on acne vulgaris. Medical Hypotheses 2019; 133.

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