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IN VITRO AND *IN VIVO* EVALUATION OF ARSENIC TRIOXIDE-LOADED NANOCREAM FOR PSORIATIC TREATMENT

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ABSTRACT

Psoriasis is a chronic inflammatory skin disorder characterized by hyperproliferation and abnormal differentiation of keratinocytes, often managed with corticosteroids, which pose long-term side effects. Arsenic Trioxide, known for its antitumor and immunomodulatory properties, has shown promise in dermatological applications when formulated as a nanocream. This study aimed to formulate and evaluate Arsenic Trioxide-loaded nanocreams for their antioxidant, physicochemical, and anti-psoriatic efficacy using *in vitro* and *in vivo* models. Nanocreams were formulated with varying concentrations of Arsenic Trioxide and evaluated for physical stability, pH, viscosity, emulsion type, homogeneity, and particle size over 12 weeks. Antioxidant activity was assessed via the DPPH method. *In vivo* anti-psoriatic potential was tested using the mouse-tail model and UV-B-induced photodermatitis model in rats, compared against Clobetasol propionate 0.05% cream. Skin irritation tests were conducted on healthy volunteers.The 10% Arsenic Trioxide nanocream exhibited superior antioxidant activity with an IC_{50} of 1.456 µg/mL, outperforming vitamin E. Stability tests confirmed consistent physicochemical properties over time. Particle size remained within the nanometric range (≤500 nm) for optimized formulations. No irritation was observed in human volunteers. Histopathological evaluation of skin in both mice and rats demonstrated a significant reduction in epidermal thickness and inflammatory features in groups treated with the Arsenic Trioxide nanocream, comparable to standard therapy. The Arsenic Trioxide nanocream demonstrates excellent stability, antioxidant capacity, skin compatibility, and anti-psoriatic activity, suggesting it as a promising alternative to conventional treatments for psoriasis.

Key words: Arsenic Trioxide, Nanocream, Psoriasis, Antioxidant, DPPH, Mouse-tail model, UV-B dermatitis, Histopatholo.

1 INTRODUCTION

Psoriasis is a persistent, immune-mediated skin disorder that affects nearly 2–3% of the global population. It manifests as erythematous plaques with silvery scales, primarily due to excessive keratinocyte proliferation and inflammatory cell infiltration.[1] Although corticosteroids remain the mainstay of therapy, long-term use is associated with systemic side effects, skin thinning, and tachyphylaxis, thereby necessitating the development of safer alternatives.[2]

Arsenic Trioxide (ATO), a well-established agent for the treatment of acute promyelocytic leukemia, has recently gained attention for its dermatological applications. Studies have shown that ATO modulates keratinocyte proliferation and possesses immunosuppressive properties that may benefit psoriatic skin. However, its topical use is limited by formulation challenges, skin irritation, and instability.[3,4]

To overcome these limitations, nano-based drug delivery systems offer a promising approach. Nanocream formulations enhance drug penetration, stability, and therapeutic index while minimizing adverse effects. In this study, Arsenic Trioxide was incorporated into nanocreams using

high-shear emulsification techniques.[5-7] The formulations were evaluated for their physicochemical stability, antioxidant activity, safety in human volunteers, and anti-psoriatic potential using both the mouse-tail model and UV-B-induced dermatitis model in rats. The findings aim to establish the feasibility of Arsenic Trioxide nanocreams as a novel, topical therapeutic option for psoriasis management.[8]

2 MATERIALS AND METHODS

2.1 Materials

The drug Arsenic Trioxide was purchased from HempCann Solutions Pvt Ltd. Polyvinayl alcohol, Sodium lauryl sulphate, ethanol were purchased from Sigma-Aldrich, Mumbai.

2.2 Method

2.2.1 Formulation of arsenic trioxide nanocream

Arsenic Trioxide nanocream was prepared using a high-energy emulsification method involving high-shear stirring with a mixer. The process began by mixing cetyl alcohol with Arsenic Trioxide and stirring the mixture at 350 rpm on a hotplate stirrer set to 55°C for 30 minutes. Concurrently, methyl paraben and propyl paraben were dissolved in distilled water and heated on a hotplate until fully dissolved, then allowed to cool. Tween 80 and propylene glycol were added to the cooled paraben solution and stirred with a magnetic stirrer at 350 rpm for 30 minutes. This water phase was gradually poured into the oil phase, and the resulting mixture was stirred at 2000-3000 rpm for 8 hours to form a thick emulsion. The emulsion was then homogenized with a mixer for 30 minutes. Finally, a few drops of rose-scented perfume were added, and the mixture was blended thoroughly with a mixer to achieve a homogeneous cream mass.

The formulation of Arsenic Trioxide Nanocream was developed by preparing five different formulations, designated as F1 through F5, each containing varying concentrations of Arsenic Trioxide while maintaining constant amounts of other excipients. Formulation F1 served as the control and did not contain Arsenic Trioxide, whereas formulations F2, F3, F4, and F5 contained 2 mg, 4 mg, 6 mg, and 8 mg of Arsenic Trioxide, respectively. Each formulation included 30% Tween 80 as the emulsifying agent, 5% propylene glycol as a humectant, and 0.5% cetyl alcohol as the emulsifying wax. To ensure microbial stability, 0.1% methylparaben and 0.05% propylparaben were added as preservatives. The total volume of each formulation was adjusted to 100 mL using distilled water. This series of formulations was designed to assess the effect of increasing concentrations of Arsenic Trioxide on the physicochemical properties and therapeutic potential of the nanocream.

2.2.2 Arsenic trioxide cream

Cream preparations containing 10% canola oil were made using a cream base with the addition of 10% Arsenic Trioxide. The resulting product is a white cream that is slightly solid and has a distinctive smell. The results of the cream formulation with a 10% concentration of Arsenic Trioxide are shown in Figure 1.

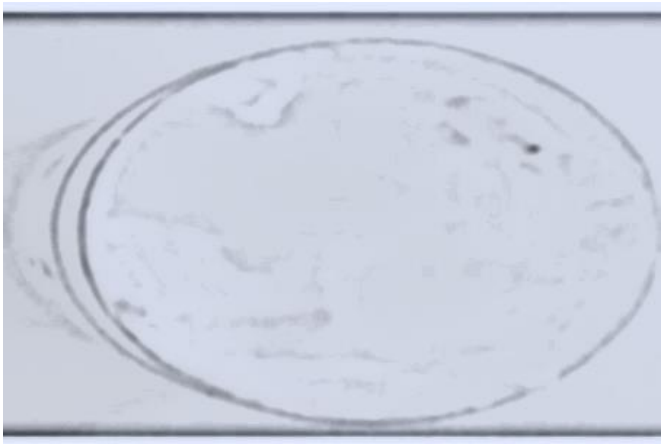

Figure 1: Cream with 10% concentration of Arsenic Trioxide

2.3 Antioxidant Activity Testing

To determine antioxidant activity using the DPPH method, begin by mixing 0.1 mL of the sample with 3 mL of a 0.004% DPPH radical solution in 95% ethanol. Vortex the solution to ensure homogeneity. Incubate the mixture in a dark room at room temperature for 30 minutes. After incubation, measure the absorbance at a wavelength of 520 nm. Use 96% ethanol as the standard. Calculate the antioxidant activity by reducing the absorbance of the control by the absorbance of the sample, then divide this difference by the control absorbance and multiply by 100%.[9]

2.4 Observation of Physical Stability of Preparations

Each Arsenic Trioxide nanocream and cream preparation was placed in a glass container and stored separately at two different temperatures. Specifically, the samples were stored in a climatic chamber at 40°C ± 2°C and 75% ± 5% relative humidity for four weeks and at room temperature for 12 weeks. Each formula was subjected to visual observations of color, odor, shape, and phase separation once a week.

2.5 Cycling Test

Samples were stored at 4°C for 24 hours, then transferred to an oven at 40°C ± 2°C for 24 hours (one cycle). This test was conducted for six cycles, with observations for any physical changes, such as separation, after each cycle.

2.6 Homogeneity Examination

A specific amount of each preparation was applied to a piece of glass or another suitable transparent material. The preparation must show a homogeneous composition with no visible coarse grains.

2.7 Determination of The Type of Preparation Emulsion

To determine the type of emulsion, methylene blue was gradually added to the preparation. If the dye dissolves when stirred, the emulsion is identified as oil-in-water.

2.8 Preparation pH Measurement

The pH of the preparations was determined using a pH meter. pH measurements were taken immediately after manufacturing and then weekly for four weeks at room temperature.

2.9 Viscosity Determination

Viscosity measurements were conducted by placing the preparation in a 100 ml beaker and selecting the appropriate spindle number. This measurement was performed in triplicate using a Brookfield DV-E viscometer. Viscosity of the Arsenic Trioxide nanocream preparations was measured before and after storage at room temperature for 0, 1, 2, 3, and 4 weeks.[10,11]

2.10 Centrifugation Test

The centrifugation test was performed immediately after the preparation was made by measuring it once. The preparation was placed in a centrifugation tube and centrifuged at 3750 rpm for 5 hours.

2.11 Determination of Nanocream Particle Size

The particle size of the nanocream was determined using a FRITSCH Analyzer 2.2 particle size analyzer. The working principle of the tool is based on Laser Diffraction (LAS), where particles passing through a laser beam scatter light at various angles. A computer analyzes the scattered intensity distribution to provide a particle size distribution.

2.12 Volunteer Irritation Test

Cosmetics were applied behind the ear and left for 24 hours. Observations were made for any changes, such as redness, itching, or roughness of the skin.

2.13 Anti-Psoriasis Activity

2.13.1 Mouse-tail model for psoriasis

All procedures of the study were conducted in accordance with the guidelines set by the CPCSEA and an approved IAEC protocol number (IAEC-17-019). Fifteen male Swiss albino mice were allowed to acclimatize for 5 days and were randomly assigned to three groups: six mice in Group I (standard group, treated with Clobetasol propionate 0.05% cream), six mice in Group II (Arsenic Trioxide NLC-loaded cream), and six mice in Group III (placebo control).

The samples were applied locally to the tails at a rate of 2-5 mg per animal, uniformly to the proximal part of the tail. A plastic cylinder was placed over the tail and fixed with adhesive tape to ensure a contact time of 2 hours. After this period, the cylinders were removed, and the tails were wiped with cotton. The mice were treated once daily for 2 weeks.

Two hours after the last treatment, the animals were sacrificed, and the tails were fixed in 10% buffered formalin and processed for histopathology. Longitudinal sections of about 5 µm thickness were prepared, stained with hematoxylin-eosin, and permanent slides were prepared for evaluation. The sections were examined under a light microscope to observe alterations in epidermal thickness, elongation of ridges, and orthokeratosis. The animals were also observed for mortality, clinical signs, and changes in body weight.[12-13]

2.13.2. Rat ultraviolet ray B photo dermatitis model for psoriasis

Healthy male Sprague Dawley rats weighing 150-200 grams were kept in a 12-hour light/12-hour dark cycle at a temperature of 20.4 to 23.8 °C and a relative humidity of 36 to 61%. The rats were provided with food and reverse osmosis water treated with ultraviolet light ad libitum. All procedures were conducted in accordance with the guidelines set by the CPCSEA and an approved IAEC protocol number (IAEC-17-009).

The study design included three groups: Group I (6 animals) received 2-5 mg/kg of Clobetasol propionate 0.05% cream as the standard treatment, Group II (6 animals) received 2-5 mg/kg of Arsenic Trioxide loaded cream, and Group III (3 animals) received 2-5 mg/kg of placebo cream. The hair on the

dorsal skin of the rats was clipped and carefully shaved. An area of 1.5 x 2.5 cm on one side of the flank was irradiated for 15 minutes (1.5 J/cm²) at a vertical distance of 20 cm with UV-B lamps, resulting in biphasic erythema.

After 72 hours, the test anti-psoriatic cream, standard cream, and placebo cream were applied topically at 2-5 mg/rat on the irradiated site once daily. The irradiated rats were sacrificed on day 11 after UV-B irradiation using CO2 anesthesia. Skin biopsies were immediately taken, fixed in 10% formalin, and embedded in paraffin. Tissue sections (4 µm thick) were stained with hematoxylin and eosin and examined under a light microscope to observe alterations in epidermal thickness, elongation of ridges, and orthokeratosis. The study also evaluated mortality, clinical signs, and body weight changes. Data were analyzed using one-way ANOVA followed by Dunnett's Multiple Comparison test.[14-16]

3 RESULT AND DISCUSSION

3.1. Antioxidant Activity of Arsenic Trioxide Nanocream

Testing the antioxidant activity of Arsenic Trioxide nanocream at a wavelength of 516.5 nm using the DPPH method resulted in an IC50 of 1.456 µg/mL. In comparison, vitamin E, according to the literature, has an IC50 of 2.146 µg/mL. A compound with an IC50 value of less than 50 µg/mL is categorized as a very strong antioxidant.

3.2 Physical Stability of Preparation

Nanocream and cream stored in a climatic chamber at 40°C ± 2°C and 75% ± 5% RH for four weeks, and at room temperature for 12 weeks, showed stable physical properties for the nanocream. This stability was evidenced by the absence of phase separation, color changes, or odor changes. In contrast, the cream preparations exhibited color changes (turning yellow) and odor changes (turning rancid), as shown in Table 1. After 6 cycles, the cycling test results showed no changes in the shape, color, or odor of the nanocream. Similarly, the cream preparations did not exhibit any changes in shape, color, or odor. Neither the nanocream nor the cream showed any phase separation during this test.

The homogeneity test results on nanocream and canola oil cream preparations indicated that there were no coarse grains when applied to transparent glass or other suitable materials. This confirms that the preparations have a homogeneous composition.

Determination of the emulsion type of the preparation was carried out by gradually adding methylene blue to the preparation. If the dye dissolves when stirred, the emulsion is of the oil-in-water (o/w) type.

Table 1: The stability of Arsenic Trioxide naocream and Cream

Storage	Physical Stability	
	Nanocream	**Cream**
4 weeks in Climatic chamber	Stable	Color changing into yellow Odor changing to rancid
12 weeks in room temperature	Stable	Stable

Determination of the emulsion type of the preparation was carried out by gradually adding methylene blue to the preparation. If the dye dissolves when stirred, the emulsion is of the oil-in-water (o/w) type. If water is the outer phase, the dye will dissolve and diffuse evenly throughout the water. If the emulsion is of the water-in-oil (w/o) type, the dye particles will remain clustered on the surface. The results indicated that the emulsion type for canola oil nanocream preparations was o/w.

The pH of nanocreams and creams ranged from 5.7 to 6.3. Over four weeks, the pH of the preparations decreased slightly but remained within the skin's pH range of 4.5-6.5, ensuring they are safe to use and do not cause skin irritation.

Viscosity, a measure of a liquid's resistance to flow, indicates that higher viscosity values correspond to greater resistance. The viscosity test data for canola oil and nanocream creams are presented in Table 2.

Table 2: Viscosity of arsenic trioxide nanocream and cream

Formula	Viscosity (cP)				
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**
F1	350	350	400	450	480
F2	2200	2250	2250	2300	2350
F3	6400	6400	6450	6500	6600
F4	9500	9550	9600	9680	9700
Cream	16500	16250	1900	15750	15500

The viscosity of the nanocreams increased over four weeks of storage at room temperature, indicating that the nanocream preparations became thicker over time. Conversely, the cream preparations experienced a decrease in viscosity, meaning that the cream became thinner over time.

3.3 Particle Size of Arsenic Trioxide Nanocream

The measurement of nanocream particles aims to determine

The measurement of nanocream particles aims to determine the particle size of each Arsenic Trioxide nanocream formula over 12 weeks of storage at room temperature. Particle measurements were carried out using the FRITSCH Analyzer 2.2 Nanotech. The particle size data for canola oil nanocreams with concentrations of 2.5%, 5%, 7.5%, and 10% can be seen in Table 3.

Table 3: Mean particle size of arsenic trioxide nanocream

Storage Time (Week)	Mean of Particle Size (nm)			
0	**F1**	**F2**	**F3**	**F4**
1	5882.17	348.47	321.16	318.16
2	7708.81	364.27	344.79	321.16
4	11856.11	397.25	377.56	338.36
8	13416.30	491.57	394.70	339.86
12	15561	518.23	485.40	391.89

Based on Table 6.3, nanocreams with Arsenic Trioxide concentrations of 2.5%, 5%, 7.5%, and 10% show an increase in the average particle size during 12 weeks of storage at room temperature. However, the increase in average particle size for F2, F3, and F4 remains within the nanocream requirement range of 20-500 nm. The nanocreams were prepared using a high-energy emulsification method, specifically high-shear stirring with a mixer. The decrease in mean particle size is attributed to the intensity of stirring.

The particle measurements indicate that higher canola oil concentrations result in smaller particle sizes and vice versa. This occurs because a higher oil concentration leads to more particles colliding during high-energy stirring. Thus, the amount of canola oil inversely affects particle size. Additionally, the optimal amount of surfactants also impacts particle size.

3.4 Results of The Irritation Test on Volunteers

The irritation test for nanocream and cream preparations was conducted on 12 volunteers according to specified requirements. The preparations were applied to the back of the volunteers' ears and left for 24 hours. The volunteers were divided into two groups: 6 volunteers received nanocream with a 10% canola oil concentration, and the other 6 received cream with a 10% canola oil concentration. Neither the nanocream nor the cream showed any signs of primary or secondary irritation, such as redness, itching, or skin roughening, after 24 hours. Thus, it can be concluded that both the nanocream and cream preparations are safe to use.

3.5 Anti-Psoriasis Activity

3.5.1 Mousetail model for psoriasis

Advanced anti-psoriatic studies were conducted on animal models to evaluate and compare the safety and efficacy of a nanoparticulate anti-psoriatic cream loaded with AE, PE, and CE cream versus Clobetasol propionate 0.05% cream. These studies utilized the mouse tail method for topical applications in psoriasis.

Compared to the normal control group, the mice treated with the standard drug (Clobetasol propionate 0.05% cream) and the test drug (Arsenic Trioxide based nanocream) showed a significant reduction in epidermal thickness of the tail skin. Based on these results, it can be concluded that both the standard and test drug formulations may possess potential anti-psoriatic activity. (Figure 2)

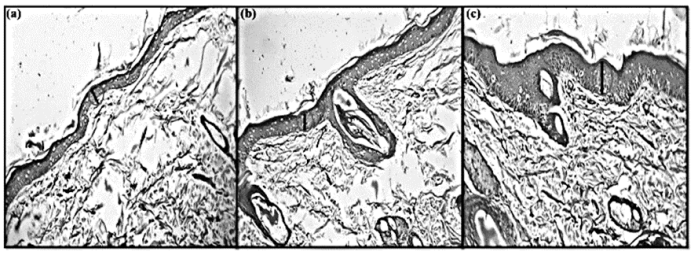

Figure 2: Histopathology of mice tail skin for, a) Standard Group I, b) Test group II, c) Placebo group III

3.6 Rat Ultraviolet Ray-B Photodermatitis Model for Psoriasis

The Rat Ultraviolet Ray-B photodermatitis model was used to evaluate the antipsoriatic potential of a anti-psoriatic cream compared to Clobetasol propionate 0.05% cream. Compared to the normal control group, the rats treated with the standard drug (Clobetasol propionate 0.05% cream) and the test drug (Arsenic Trioxide loaded nanocream) exhibited a significant reduction in epidermal thickness and inflammatory changes. Based on these results, it can be concluded that both the standard and test drug formulations have potential anti-psoriatic activity. (Figure 3)

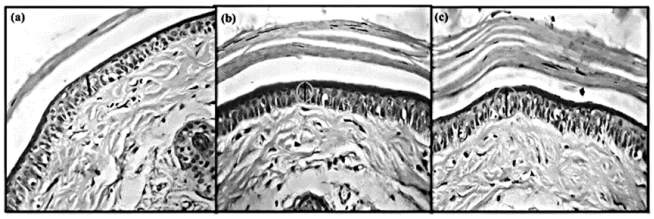

Figure 3: Histopathology of Rat Skin for a) Standard group I, b) Test group II, c) Placebo group III

4 CONCLUSION

This study successfully developed and evaluated Arsenic Trioxide nanocreams with promising therapeutic potential for the treatment of psoriasis. The optimized formulation demonstrated excellent antioxidant activity, high physical and chemical stability, and favorable skin compatibility. Notably, both the mouse-tail and UV-B-induced rat models confirmed significant anti-psoriatic efficacy, comparable to standard Clobetasol propionate treatment. The nanocream maintained particle size within acceptable nanometric limits and showed no signs of irritation in human volunteers, further validating its suitability for topical application. Overall, the findings suggest that Arsenic Trioxide nanocream could serve as an effective and safer alternative to corticosteroids in the management of psoriasis. Future studies involving clinical trials are warranted to confirm these results and establish long-term safety.

REFERENCES

1. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker JNWN. Psoriasis. Lancet. 2021;397(10281):1301–15.
2. Mrowietz U, Kragballe K, Reich K, Spuls P, Griffiths CEM, Nast A. Definition of treatment goals for moderate to severe psoriasis: A European consensus. Arch Dermatol Res. 2011;303(1):1–10.
3. Liu Q, Jin Y, Zhang R, Liu Y, He X. Arsenic trioxide: a potential anti-psoriasis agent. Exp Dermatol. 2019;28(7):751–754.
4. Guo Q, Zhou Y, Wu Z, Liu Y. Topical drug delivery with nanosystems: recent advances and future perspectives. Pharmaceutics. 2021;13(3):437.
5. Jain S, Patel N, Madan P, Lin S. Quality-by-design based development of topical nanocarrier system for psoriasis: optimization and mechanistic evaluation. Int J Pharm. 2020;579:119-174.
6. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25–30.
7. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199–200.
8. Puglia C, Bonina F. Lipid nanoparticles as novel delivery systems for cosmetics and dermal pharmaceuticals. Expert Opin Drug Deliv. 2008;5(6):745–55.
9. Bairagee D, Verma P, Jain N. Fabrication and *in vitro* characterization of Niosomal formulations for controlled delivery of ranitidine HCl. Lat Am J Pharm. 2022;41(1):85-91.
10. Rieger MM, Rhein LD. Surfactants in Cosmetics. 2nd ed. New York: CRC Press; 1997.https://doi.org/10.1201/9780203737743.
11. OECD, Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. OECD Guidelines for the Testing of Chemicals, OECD Publishing; Paris 2008.