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A new perspective for psoriasis: Dithranol nanosponge loaded hydrogels

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ABSTRACT

We developed dithranol nanosponge integrated Carbopol hydrogel (DTHNS-HG) as a novel perspective for psoriasis management. Dithranol was chosen as antipsoriatic moiety for this system. In this study, our objective was to fabricate and evaluate DTHNS-HG for *in vivo* anti-psoriatic activity using mouse tail model. Nanosponges (NSs) were synthesized using diphenyl carbonate and β-cyclodextrin employing melt method and appropriately characterized. The selected nanosponges were then embedded into Carbopol hydrogels and assessed for viscosity, spreadability and texture analysis. The developed formulations were further evaluated in vivo for antipsoriatic potential (% drug activity,% orthokeratosis and relative changes in epidermal thickness). Additionally, oxidative stress markers were also checked in mouse tail samples. DTH loaded cyclodextrin nanosponges were successfully fabricated and displayed acceptable mean particle size (274.6 ± 43.54 nm with 0.545 PDI) and zeta potential (-28.3 ± 6.34 mV). Further, DTHNS amalgamated hydrogel showed satisfactory results in terms of spreadability, viscosity and texture profiles. The degree of orthokeratosis was significantly ($p < 0.05$) increased with DTHNS-HG (0.5 and 1.0% w/v) and marketed ointment, as compared to the untreated group (control). Similarly, the drug activity and changes in epidermal thickness were found significant. Our findings from present investigations suggested a new cargo for delivery of DTH in topical medicinal strategies for psoriasis. Hence, this novel hydrogel showed promising outcomes for psoriasis.

1. Introduction

Dermal disorders represent global health problems, which are associated with an extensive financial burden on patients suffering from them. Among them, psoriasis is a chronic, autoimmune, inflammatory dermal disorder, which is prevalent in a considerable percentage of population. It is manifested by red scaly erythematous plaques, swelling, pain, skin flaking and itching, which occur due to enhanced keratinocyte proliferation in epidermal layer with dilation of dermal vasculature, loss of differentiation controlled by inflammatory infiltrate in skin strata and irregular skin growth [1,2]. Sometimes, it is associated with hereditary transmission. This disorder is exacerbated and triggered either through environmental factors or streptococcal infection [3]. Psoriasis is commonly occurring at cutaneous surfaces of the elbow, sacral areas, knees and scalp [4,5]. Mostly, it occurs at the age before 35 years and becomes life-long ailment. At present, it is not curable and leads to psychosocial disability, finally resulting in depression [3]. Thus, it is necessary to find effective and safe active molecule, which has superior patient acceptability and potential therapeutic efficacy to treat psoriasis [6].

One of the oldest yet, dithranol (DTH) is a highly effective antipsoriatic agent having a direct inhibitory effect on proliferation of keratinocytes *via* accumulating in the cellular mitochondria [7,8]. DTH inhibits PMN function [9], modulates the arachidonic acid metabolism [10] and is found able to modulate surface receptors of epidermal cells [11]. Lately, it has been revealed that it exerts its pharmacological effect *via* induction of keratinocyte apoptosis [12].

Despite being safe and effective against psoriasis, DTH administration is associated with numerous side effects that discourage its widespread use. For instance, topical application of DTH results in skin irritation, stinging, burning, redness and staining of the skin and clothes [13,14]. The literature shows several attempts to overcome these shortcomings while maintaining drug efficacy. For instance, shorter application time has been recommended followed by washing the skin with special cleansing agents [15,16]. Aqueous cream and wax-ester-based preparations of DTH have also been recommended [17,18]. However, DTH clinical utility remains limited despite these modifications. Therefore, efficient drug delivery systems that is able to

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minimize DTH side effects and maximize its efficacy remain yet to be developed [19].

Nanoscience is thoroughly explored for drug development in favor of dermatological applications. Interestingly, numerous cross-linked and chemically modified cyclodextrin-based polymers have been fabricated and assessed to enhance the efficacy of active molecules [20].

Cyclodextrin nanosponges (NSs) have emerged as predominant cargo for delivery of poorly soluble active molecules [21]. Particularly, we focused on cyclodextrin nanosponges, fabricated *via* crosslinking of β-cyclodextrin (β-CD) with diphenyl carbonate (DPC). Besides maintaining the active molecule stability, this delivery system is biocompatible without haemolytic activity and cytotoxicity, easily sterilized and thermally stable [21,22]. Amid nanocolloidal drug delivery systems, NSs offer a number of merits over other cargos in terms of high solubilization capacity, high pay load, efficient drug targeting, ease of scale up and sustained release [23]. Moreover, NSs are interesting alternatives as novel carriers for poorly water-soluble active moieties [24]. In this regards, it is of particular interest to our study.

To prepare a topical formulation possessing required consistency, DTH nanosponges can be amalgamated into commonly used dermal cargos such as hydrogels, lotions, ointments and creams. Among these, hydrogels are usually selected because of their good tissue compatibility, easy manipulation of swelling level, controlled release feature and superior targeting to the viable epidermis [25]. Due to solid powder state, the direct topical application is not convenient for the patients; so, the amalgamation of DTH nanosponges into a hydrogel system is preferred.

In light of this, the present work deals with the development and characterization of DTH-loaded nanosponges and incorporation of them into hydrogel to improve topical delivery of DTH and minimize related unfavorable side effects. The proposed hydrogel system will hydrate the skin and improve the performance of the nanosponge-mediated delivery of entrapped active molecule. The results of in vivo anti-psoriatic studies indicate that the DTH-loaded NS formulation may be a promising cargo for dermal delivery of DTH.

2. Materials and methods

2.1. Reagents and chemicals

Dithranol was purchased from Hi-media Laboratories, India. β-cyclodextrin was kindly supplied by Roquette Ridhi Siddhi Pvt. Ltd, India. DPC was procured from Sigma-Aldrich, Milan (Italy). Carbopol 934 was purchased from Loba Chemie Pvt. Ltd., Mumbai (India). Sodium chloride and triethanolamine were obtained from S D Fine Chem. Ltd., Mumbai (India). Potassium dihydrogen phosphate was procured from HPLC, Mumbai (India). All other chemicals and reagents used were of analytical grade.

Taking into consideration the light sensitive issues of DTH, all the formulations and physicochemical characterization studies were conducted in dark.

2.2. Fabrication and characterization of DTH loaded nanosponges

The blank cyclodextrin nanosponges (hyper cross-linked polymer structures) were fabricated using β-CD and DPC (1:6 molar ratio) employing the melt method, as previously reported [26]. Briefly, anhydrous polymer (4.548 g) and cross-linker (5.126 g, finally homogenized) were gradually heated (90 to 100 ^{o}C) under a magnetic stirrer for 6 h and cooled at ambient temperature. The obtained solid was washed using double distilled water and acetone [26,27]. The DTH was loaded in blank cyclodextrin nanosponges via freeze-drying [27]. The fabricated DTH loaded nanosponges were characterized in terms of particle size and surface charge using a Malvern Zetasizer Nano (Malvern Instruments Ltd., Worcestershire, UK) [27].

The application of the solid powder (nanosponges) onto skin is not possible owing to its non-adhesive and particulate nature, and it also leads to an imperceptible drug amount to the target site. Hence, it was necessary to incorporate DTH nanosponges into hydrogels for topical application.

2.3. Formulation and evaluation of nanosponge based topical hydrogel

Carbopol 934 was selected for hydrogel preparation to solve the issue of topical delivery for DTH nanosponges [28]. For this, the specified amount (1 g) of Carbopol 934 powder was slowly added to distilled water (100 ml) and soaked for 24 h. Further, the gelling agent is dispersed uniformly to obtain smooth dispersion by agitation using a magnetic stirrer (500 rpm). The obtained dispersion was allowed to stand for 15 min to expel the entrapped air. At this stage, appropriate amount of DTH nanosponges [27] and DTH were incorporated in the gel base with continuous stirring to obtain, homogeneous DTH nanosponge hydrogel (DTHNS-HG) and DTH hydrogel (DTH-HG), respectively. Thereafter, triethanolamine (plasticizer) was added to the viscous solution with constant stirring [29].

Hydrogels were visually examined for their consistency, homogeneity and color. The pH of fabricated hydrogels was determined using a pH meter (Eutech Digital pH meter, Malaysia).

2.3.1. Determination of viscosity

The rheological properties of plain hydrogel (P-HG), DTH-HG and DTHNS-HG were studied by Brookfield viscometer (model DV-E) with Spindle (No-6) at 25 ^{o}C. The spindle was dropped down vertically into engineered HG by taking precaution that spindle does not come in direct contact with beaker. The spindle was rotated at chronological speed (0.5 to 100 rpm), followed by viscometer measurements (time interval: 1 min) when HG gets stabilized. Each sample was equilibrated at 25 ^{o}C prior to every measurement. Results were presented as plots of viscosities versus shear rate for all samples.

2.3.2. Determination of spreadability

Spreadability of prepared P-HG, DTH-HG and DTHNS-HG, was assessed by parallel plate method (placing samples (0.5 g each) within 1 cm circle over glass plates). Different weights i.e. 15, 20, 30, 50, 70, 100, 150 and 200 (gms) were allowed to rest on the respective formulations, for 1 min, resulting in spreading of hydrogels [30].

2.3.3. Determination of texture profile

Texture analysis provides information regarding hardness, springiness, cohesiveness and adhesiveness of prepared HGs. The analysis depends on the penetration of a probe from texture analyzer to HG at a predefined velocity, depth and force. In the present investigation, formulations viz. P-HG, DTH-HG and DTHNS-HG were evaluated for texture analysis using TA-XT2i Texture analyzer [30].

2.4. In vivo studies of nanosponge hydrogel formulations

2.4.1. Animals and ethical compliance

The research protocol adhered the guidelines for care and use of laboratory animals, all animal studies were carried out as per the protocol agreed by the Animal Ethics Committee, Guru Jambheshwar University of Science and Technology, duly accepted for the use of supervision and control of experiments on animals (Swiss albino mice) by Government of India (Ref. letter no. IAEC/2017/44–52).

Adult male Swiss albino mice (weight 20–30 g) were chosen for animal studies in the current investigation. The experiments were carried out after acclimatizing the animals for 10 days, to the laboratory conditions. The Swiss mice were kept at controlled temperature (25° ± 1 °C), in a 12 h dark/light cycle and maintained with water (*ad libitum*) and standard animal food.

2.4.2. Experimental design and treatment

In the present study, the Swiss mice were grouped ($n = 6$) as represented in Table 1. The anti-psoriatic effect of the fabricated hydrogels was investigated *via* mouse tail model, as per designed protocol [30]. All the prepared hydrogel formulations were applied to the mouse tail (once daily) with the help of paint brush for two weeks (Table 1). After 24 h of last application, the mice were euthanized and their tails were collected, and consequently stored in a deep freezer (−80 ^{O}C), until further use.

For histopathological evaluation, the representative tail samples were cut and fixed in neutral buffered formalin (10%). After fixation, tissue samples of tail were decalcified using 10% EDTA solution. Following this, the samples were processed for histopathological examination as per routine procedure. Tissue samples were washed under the running tap water overnight to remove formalin. Subsequently, samples were processed employing ascending grades of ethanol (50, 70, 80, 90 and 100%) for 1 hour each, cleared in benzene (2 changes of 30 min each) and infiltrated with paraffin. After that, paraffin embedded tissues were cut into 4 μm thick transverse sections using rotary microtome (Yorco Millennium, YSI062) and stained with haematoxylin and eosin (H. & E.) [31]. The stained sections were visualized under light microscope and epidermal changes viz. orthokeratosis and parakeratosis were recorded in samples. For this, each section was visualized through ten sequential high-power fields (40× objective) from each skin section.

2.4.3. Measurement of oxidative stress markers

Tail samples from all groups of mice (stored in a deep freezer at −80 ^{O}C) were processed further for biochemical assessment. Firstly, tail was weighed and appropriately diluted with phosphate buffer saline (PBS; pH 7.4; 100 mmol/L). Subsequently, the tissues were homogenized for one minute using high speed rotor stator homogenizer (Rachna Enterprises, Faridabad, India). The obtained homogenate was then centrifuged (at 10,000 rpm) for fifteen minutes. The supernatant was used for biochemical assessment [30].

For detection of any alteration in antioxidant balance in psoriatic mice tail, measurement of MDA (malondialdehyde), a lipid peroxidation end product and NO (nitrite) levels were carried out using mouse tail homogenate [30,32]. The soluble proteins in this homogenate were assessed *via* Biuret method, taking bovine serum albumin as the standard [33].

MDA present in homogenate samples was determined by an assay, in which reaction of MDA with thiobarbituric acid results in formation of 1:2 adduct, which was assessed by spectrophotometer (at 532 nm) [34].

Since nitrite accumulation confirms NO production and its accumulation was determined using Greiss reagent, as reported previously [35]. Herein, to estimate nitrite levels, equal amounts of supernatant (0.5 ml) and Greiss reagent (0.5 ml) were mixed at ambient temperature and incubated for ten minutes (in dark). The samples were appropriately analyzed through UV–Visible spectrophotometer (at 540 nm). The concentration of nitrite in the samples was measured from a sodium nitrite standard plot.

2.5. Statistical analysis

The obtained data [mean ± standard error of the mean (SEM) / mean ± standard deviation (SD)] were statistically investigated by One-way / Two way ANOVA tests followed by Tukey's post-test / Bonferroni post-tests for multiple comparisons wherever applicable, using the software GraphPad Prism (version 5.01, GraphPad Software Inc. San Diego, California, USA). A worth of $p < 0.05$ was set as the level of significance.

Table 1
Experimental groups in animal study: The Swiss mice were divided into 4 groups ($n = 6$) and treatment was started for each group as follow.

Groups	Treatment
Group I	Swiss mice treated with plain Carbopol hydrogel, daily.
Group II	Swiss mice treated with compound dithranol ointment (marketed), daily.
Group III	Swiss mice treated with DTHNS-HG (equivalent to DTH 0.5% w/v), daily.
Group IV	Swiss mice treated with DTHNS-HG (equivalent to DTH 1.0% w/v), daily.

3. Results and discussion

Direct topical application of DTH may cause folliculitis [36]. Further, owing to the increased vascularity of psoriatic skin, DTH rapidly penetrates in psoriatic lesions and results in skin damage with respect to normal healthy skin [37].

From a dermatologist's point of view, there is plenty of hopes from nanoscience. Therefore, in the current research, we fabricated nanosponge based hydrogel, that was recognized for augmentation of dermal delivery of dithranol as an active moiety [27]. DTH loaded nanosponge based hydrogel was compared with commercial DTH formulation, to investigate its effectiveness in the management of psoriasis. The preclinical findings determined after 2 weeks of experiment, described that encapsulation of DTH in nanosponges and engrossment in hydrogel would step up the treatment response, that is usually encountered treatment during time course for this disorder. DTH nanosponge hydrogel fabricated from Carbopol 934 was found potential in managing psoriasis manifestations without any adverse effects, as described in Fig. 1. Hence, this research work describes above mentioned targeted cargos for topical treatment of psoriasis, which has not been yet explored, in spite of the well-known strength of DTH moiety.

3.1. Fabrication and characterization of DTH loaded nanosponges

The biocompatibility and acceptability of β-cyclodextrin for topical administration have been well documented and therefore, it was employed for fabrication of nanosponges with DPC, the additional cavities of these nanostructures facilitated by an additional peripheral interaction, can play a vital role in enhanced drug encapsulation and stabilization, as described earlier [30]. The hydrogen bond, hydrophobic and van der Waals interactions are responsible for cyclodextrin (CD) complex formation [38].

The cyclodextrin nanosponges fabricated *via* the melt method (β-CD: DPC; molar ratio 1:6) displayed satisfactory particle size (274.6 ± 43.54 nm), PDI (0.545) and zeta potential (−28.3 ± 6.34 mV).

3.2. Formulation and evaluation of nanosponge based topical hydrogel

Carbopols are known to form microgel architecture cross-linking named as hydrogels and act as a suitable vehicle for topical delivery [6]. Hydrogels represent a three-dimensional viscoelastic polymeric network of hydrophilic polymers formed *via* hydrogen bonding and ionic interactions. Further, owing to suitable biocompatibility, hydrogels are able to sense variations in temperature, heat, pH and other stimulations. HG have capacity to absorb water upto 10 to 20 folds to their molecular weight and modulate drug release [39,40]. Hydrogels also facilitate embedding of nanosponges and make them suitable for management of dermatological disorders [6].

Hydrogels loaded with nanosponges were found semi-transparent, homogeneous and smooth. No particulate matter was observed in HG, evidencing no grittiness. All HGs showed pH in the range 5.5 to 5.8, which is well closer to the pH of skin, and hence, should not provoke any skin irritation [6].

3.2.1. Determination of viscosity

Rheological activities of dermal products have a pioneer impact on their spreading, contact time and retention in skin strata [41,42]. In order to prolong skin retention, HG loaded DTH cyclodextrin nanosponges have been crafted in the current study [43]. Carbopol based

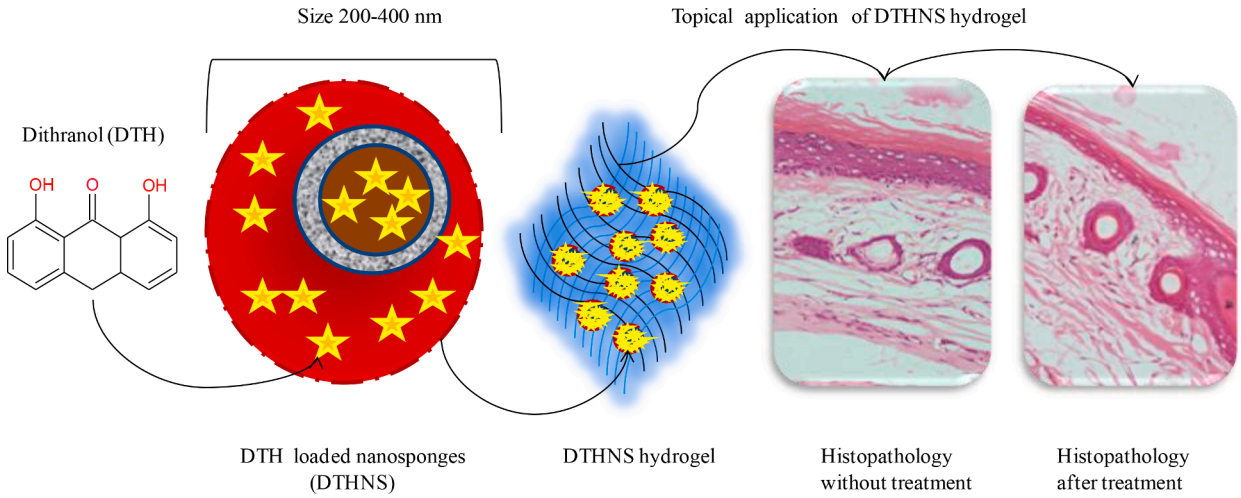

Fig. 1. Schematic representation of the DTHNS based hydrogel for psoriasis management.

hydrogels are well known for their compatibility with numerous active drugs. Additionally, HGs possess higher viscosity at a lower concentration, good stability and leads to adequate patient compliance [44,45]. Further, the viscosity of prepared HGs is found to affect drug release as well as its retention at the site of application. In this regard, the viscosity of P-HG, NS-HG, DTH-HG and DTHNS-HG was evaluated in the present investigation. A plot between the shear rate and the measured viscosity of all the engineered hydrogels showed a reduction in viscosity, with the increase in shear rates (Fig. 2), indicating shear-thinning (pseudoplastic) behavior of the HG formulations [46]. Furthermore, it was observed that the viscosity of the DTH-HG was higher in comparison to DTHNS-HG. As anticipated on entrapment of DTH into nanosponges, the viscosity of the nanosponge hydrogel (DTHNS-HG) was dropped and found nearly equal to the P-HG, because of nanosize of embedded formulation [47]. As shearing rate was increased, the viscosity of nanosponge hydrogel was subsequently decreased. The obtained rheograms represented shearing behavior on long-chain Carbopol molecules (Fig. 2). Similar results were obtained in a previous research investigation by our group for babchi oil (BO) nanosponge based hydrogel [30]. As per literature reports, in case of topical delivery, the HG should neither flow immediately after its application on the skin surface, nor it resisted application at the optimum viscosity [43]. In a similar fashion, DTHNS-HG exhibited this behavior at appropriate viscosity laying the acceptable limits for topical application. Thus, this feature provided additional merit of prolonged retention to the nanosponge HG for psoriatic patients, resulting in improved patient acceptability.

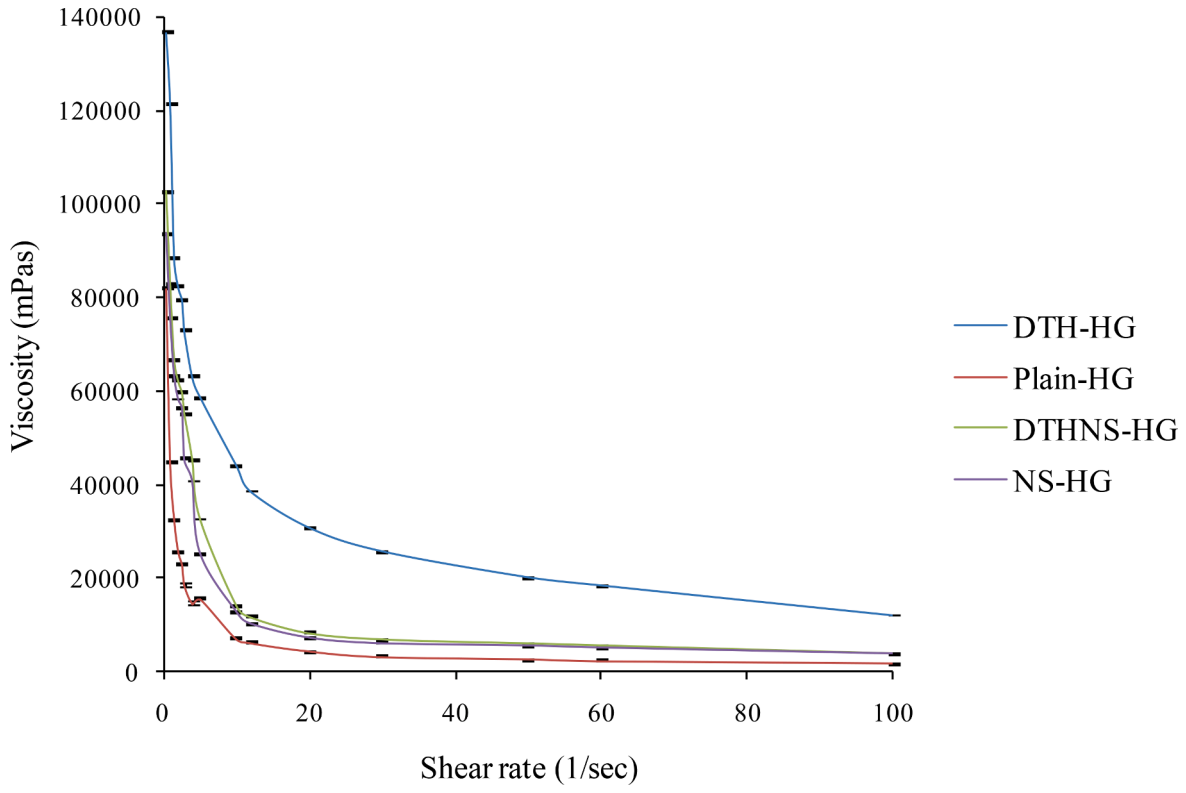

Fig. 2. Viscosity studies of the fabricated hydrogels.

3.2.2. Determination of spreadability

Spreadability is another key parameter for topical products, which needs to be investigated. Spreadability is also related to accurate dose transfer to the target tissue and is associated with viscosity as well as composition of the formulation. In the present study, initially, spreading area for P-HG, NS-HG and DTHNS-HG were observed as 11.64 ± 0.84, 11.25±1.11 and 12.25 ± 1.07 cm^2, respectively, however, DTH-HG exhibited initial spreading area 10.31 ± 1.87 cm^2. After applying weight (200 g), the spreading area was noted as 39.39±1.78, 35.53± 1.77, 28.71±0.96 and 34.82±1.34 cm^2 for Plain-HG, NS-HG, DTH-HG and DTHNS-HG formulations, respectively. The spreadability behavior of fabricated hydrogels has been presented in Fig. 3. The results of this evaluation demonstrated that integration of nanosponges in the Carbopol HG did not alter their spreadability profiles in comparison to P-HG. This effect could be credited to the nanosize of particles in the formulation offered ease spreading on the skin and acceptable consistency to NS integrated hydrogels. However, it was observed that incorporation of nanosponges in the Carbopol gel resulted in a slight decrease in spreadability, when compared to P-HG and NS-HG. This observed difference may be due to integration of formulations in the hydrogel base.

3.2.3. Determination of texture profile

Texture analysis is the most widely used tool for driving essential information on the semisolid dosage forms in pharmaceuticals [48]. In addition to viscosity and spreadability, topical delivery systems engineered to be applied on the skin must possess suitable mechanical features (cohesiveness, hardness, adhesiveness and springiness). Hence, considering these practical aspects, mechanical parameters of the fabricated semisolid dosage forms are vital, in terms of their technological design [49].

In the current study, the composition of semisolid products and their influence on mechanical attributes were hence focused. To assess the mechanical characteristics of prepared hydrogels (plain, DTH and DTHNS), texture analyzer was employed. Table 2 displays the texture profile analysis of P-HG, DTH-HG and DTHNS-HG. The DTH-HG exhibited profound hardness (0.37±0.183 N), followed by P-HG (0.24 ±0.060 N), while DTHNS-HG (0.23±0.022 N) sample exhibited less hardness. The force of adhesion of fabricated HGs was in line with their hardness pattern and results displayed DTHNS-HG (−1.03±0.447 N *sec*) being more adhesive than P-HG (−1.53±0.768 N *sec*), followed by DTHNS (−3.07±1.79 N *sec*). Cohesiveness portrays the structural recovery of fabricated HS upon compression. The cohesiveness of fabricated HGs was observed in the order DTH-HG (0.76±0.031) > P-HG (0.75±0.039) > DTHNS-HG (0.74±0.023). Thus, lower consistency of DTH-HG exhibited poor spreadability, whereas optimum consistency of DTHNS-HG (similar to P-HG) indicated the ease of spreadability evenly on the skin. As fabricated HGs for dermal delivery should retain low hardness

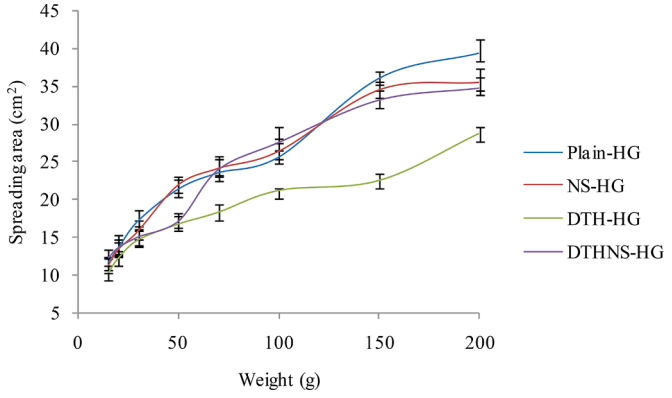

Fig. 3. Spreadability studies of the fabricated hydrogels determined by parallel plate method.

Table 2
Texture analysis of fabricated hydrogels.

Sr. No.	Texture properties	P-HG	DTH-HG	DTHNS-HG
1.	Hardness ± SD (N)	0.24±0.060	0.37 ±0.183	0.23±0.022
2.	Cohesiveness ± SD	0.75±0.039	0.76 ±0.031	0.74±0.023
3.	Adhesiveness ± SD (N *sec*)	−1.53 ±0.768	−3.07 ±1.79	−1.03 ±0.447
4.	Springiness ± SD	0.89±0.073	0.90 ±0.033	0.93±0.067

The study was carried out in triplicate (mean ±SD). P-HG: Plain hydrogel, DTH-HG: Dithranol loaded hydrogel (1.0%w/v), DTHNS-HG: Dithranol nanosponges loaded hydrogel (equivalent to DTH 1.0% w/v).

and low force of cohesion; our findings for texture examination of fabricated DTHNS-HG were found appropriate for dermal use. Moreover, one another parameter, the higher force of adhesion is desirable for prolonged retention of applied formulation over the skin. This parameter was also observed with DTHNS-HG sample (Table 2). Therefore, DTHNS-HG possessed all criterions which play pivotal role for its topical application in psoriasis.

3.3. Antipsoriatic activity (mouse tail model)

The mouse tail test is employed widely for psoriatic investigations because it is simpler model, smooth to carry out and displays good reproducibility, fine sensitivity and high correlation with the activity of oral or topical antipsoriatic therapeutics at present clinically. Additionally, this model facilitates the quantitative and histometric investigations of fabricated formulation response on epidermal differentiation [50].

In the current research, Fig. 4 illustrates the histological appearance of haematoxylin and eosin stained sections of mouse tail skin on treatment with various test samples. From control group (Group I), distinct pieces of evidence associated with parakeratosis, a higher density of nucleated keratinocytes and thinning of the granular layer were noticed. Fourteen days of treatment therapy with DTHNS-HG exhibited considerable histological changes in sections of tail skin with respect to marketed product. Throughout this time, the orthokeratotic stratum corneum provinces spread longitudinally, in the previous parakeratotic condition. Table 3 displays the influence of topical application of DTH formulations on relative epidermal thickness (%), orthokeratosis (%) and drug antipsoriatic activity (%) in the mouse tail test. The control group showed 29.80 ± 1.49% orthokeratosis, while the scaly areas presented parakeratosis. DTH ointment (marketed) showed 67.38 ± 2.09% orthokeratosis, while nanosponge hydrogel having DTH (equivalent to DTH 0.5 and 1.0%w/v) showed an almost 67.93 ± 2.05 and 71.62 ± 1.88% orthokeratotic differentiation, respectively. These findings present the potency of nanoparticles based approach to provoke normal epidermal differentiation in psoriasis [5].

The results also indicated that marketed product, DTHNS-HG (0.5% w/v) and DTHNS-HG (1.0%w/v) possessed significantly enhanced ($p<0.001$) anti-psoriatic effect in comparison to plain HG (Fig. 4). Fig. 4 indicated ahistology of untreated mouse tail skin (treated with plain hydrogel) presenting parakeratotic differentiation. Fig. 4b displayed the marketed ointment treatment and frequent keratosis (orthokeratosis). Fig. 4c and d demonstrated DTHNS-HG (equivalent to DTH 0.5 and 1.0% w/v) treatment for fourteen days and it presented orthokeratosis clearly [51]. Although, there was no significant difference between DTHNS-HG (both concentrations) and derobin ointment (a marketed product), widely used to treat psoriasis, evidencing the potential of DTHNS-HG. Potential anti-psoriatic action of DTHNS-HG might be accredited to the encapsulation of DTH in cyclodextrin nanocarriers, which resulted in its dose reduction, when compared to marketed product (having 1.15%

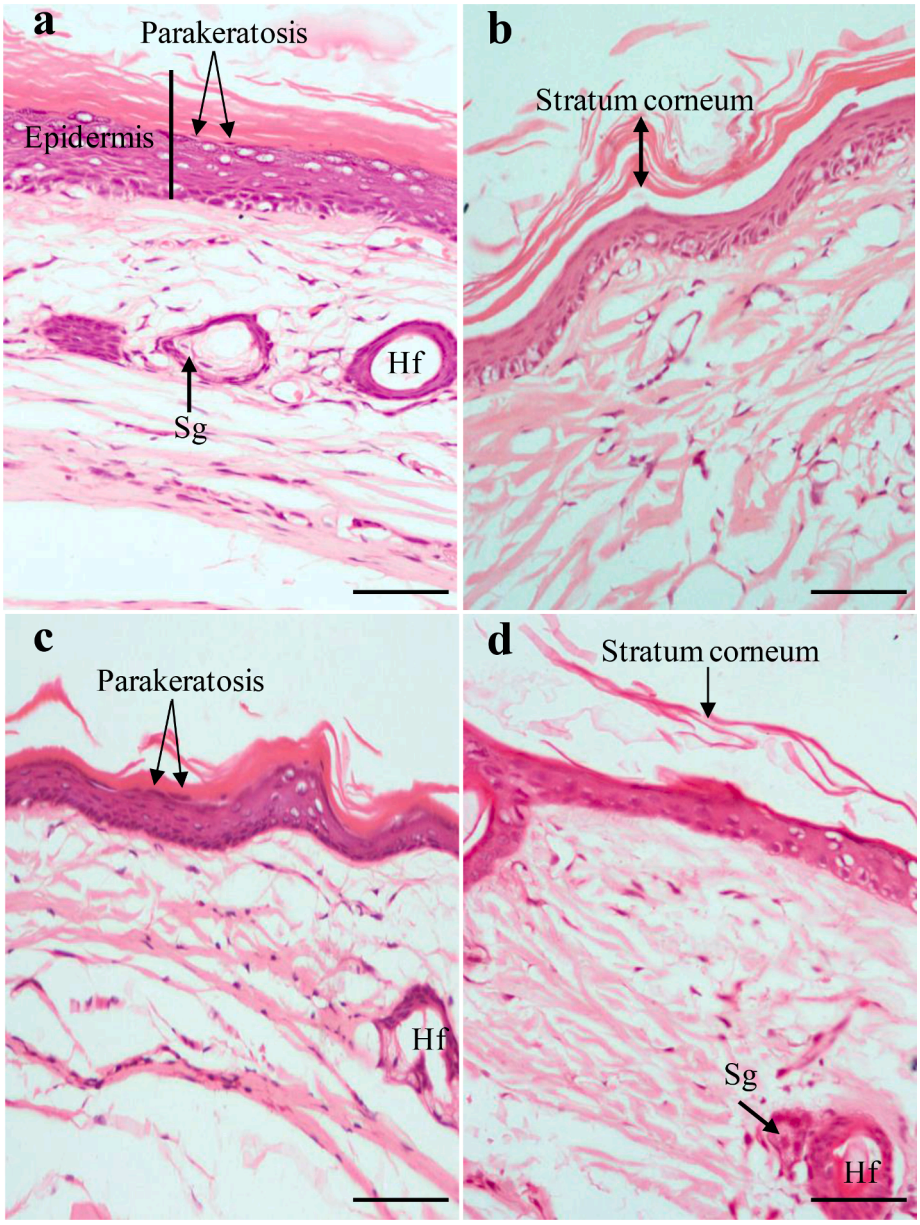

Fig. 4. Histopathological evaluation of mice tail skin after various treatments. (a) Control, (b) Marketed ointment, (c) DTHNS-HG (equivalent to DTH 0.5% w/v) and (d) DTHNS-HG (equivalent to DTH 1.0% w/v). Calibration bar = 100 μm. H.&E.×400. (**Sg:** Sebaceous gland, **Hf:** Hair follicle).

Table 3
Anti psoriatic potential of fabricated hydrogels using mouse tail model.

Sr. No.	Formulation	Relative epidermal thickness (%)	% Orthokeratosis	Drug activity (%)
1.	Plain hydrogel	100.00±0.00	29.80±1.49	0.00±0.00
2.	Marketed ointment	43.85±1.33[a]	67.38±2.09[a]	52.51±3.36[a]
3.	DTHNS-HG 0.5%w/v	44.08±1.62[a,]	67.93±2.05[a]	53.64±3.26[a]
4.	DTHNS-HG 1.0%w/v	40.91±1.72[a,]	71.62±1.88[a]	58.85±2.87[a]

The data is represented in mean±SEM, $N = 6$ per group, Statistical data analysis from the one-way ANOVA followed by Tukey's test for multiple comparisons. (Relative epidermal thickness: a $p<0.001$ vs. P-HG; % Orthokeratosis: a $p<0.001$ vs. P-HG; Drug activity: a $p<0.001$ vs. P-HG. P-HG: Plain hydrogel, DTH: Dithranol hydrogel, DTHNS-HG: Dithranol nanosponges loaded hydrogel.

w/w DTH, salicylic acid 1.15%w/w and coal tar 5.3%w/w). Furthermore, the improved activity of DTH nanosponge hydrogel may be due to the penetration of DTH loaded nanosponges and their targeting to the epidermal skin layers.

The nanoformulations act as efficacious alternatives that significantly construct micro reservoirs in skin annexes, to circumvent the hyperkeratinization, a major concern in psoriasis. In a similar trend, DTHNS-HG (both concentrations) showed an evident reduction in relative epidermal thickness while augmentation in% orthokeratosis and drug activity *vis-a-vis* plain hydrogel. Collectively, all prepared nanosponge hydrogels improved the antipsoriatic efficacy of dithranol with respect to its commercial product, evidently due to augmented interaction of DTH nanosponge with the skin annexes.

The nonexistence of granular layer augmented the possibility of wounds in psoriatic patients, and it is essential to defend the dermal tissues from ultraviolet radiation, chemicals and microbes [50]. Recently, some antipsoriatic molecules and their formulations like tazarotene proniosomal gel [52] babchi oil and clobetasol propionate nanosponge based hydrogel [24,30], thymoquinone loaded ethosomal

vesicles [53], zedoary turmeric oil and tretinoin loaded liposomal gel [54] induced orthokeratosis, when tested *via* mouse tail model and this was investigated on the basis of epidermal thickness after application of test samples.

The visual observations were taken into consideration with histopathology to assess the biocompatibility and safety of prepared hydrogels. Visual inspection presented no sign of skin irritation (swelling and erythema) after application of hydrogels. Furthermore, no variation in tail skin samples with DTHNS-HG treatment groups was observed in microphotographs with respect to the normal skin. The outcomes indicated the dermal compatibility and safety of prepared nanosponges of dithranol, owing to the barrier formed by nanosponges, as well as biodegradable and biocompatible features of Carbopol hydrogel.

3.4. Measurement of oxidative stress markers

In context with the intriguing impact of oxidative stress in psoriasis, and the imperative role of DTH in minimizing inflammation and oxidative damage, the positive effects of DTH nanosponge hydrogel in ameliorating inflammation in psoriatic skin have been speculated (Fig. 5).

Malondialdehyde levels, a known biomarker of lipid peroxidation, were found enhanced in psoriasis. ROS influences the production of MDA in psoriatic skin than normal [30,55]. An elevation in free radicals results to overproduction of MDA (the end product of polyunsaturated fatty acids). In the current investigation, MDA levels were observed notably elevated in the untreated tail of mice (control group). The topical application of prepared DTHNS-HG (equivalent to DTH 0.5 and 1%w/v) and marketed ointment exhibited a remarkable reduction in MDA levels with respect to control group, with a p value below 0.001 (Fig. 5a). The present outcomes witnessed the efficiency of DTHNS-HG, distinctively illustrating its merit in psoriasis.

Nitric oxide is generated *via* nitric oxide synthase enzymes mediating biotransformation. Higher NO levels in the skin annexes are recognized to act chiefly in the proliferation of keratinocytes in psoriasis [56]. In the present research work, NO levels were observed as: sham group (plain-HG) > DTHNS-HG (equivalent to DTH 0.5%w/v) > DTHNS-HG (equivalent to DTH 1.0%w/v) > Dithranol ointment (marketed; 1.15% w/w) (Fig. 5b). Furthermore, NO levels were noticed to be remarkably lower in DTH ointment (1.15%w/w) and DTHNS-HG (equivalent to DTH 1.0%w/v) ($p<0.001$), when compared to sham groups, whereas, in case of DTHNS-HG (equivalent to DTH 0.5%w/v), it was found significant at p value below 0.01, with respect to the sham group (Fig. 5b). The NO levels were found potentially significant ($p<0.05$) in the marketed ointment treatment group with respect to DTHNS-HG (equivalent to DTH 0.5%w/v). The findings of DTHNS-HG (equivalent to DTH 1.0% w/v) clearly advocated its superior therapeutic effectiveness. These findings are in agreement with the histopathological observations, signing complimentary indication for augmented pharmacological action of CPNS hydrogel [24]. This might have led to the neutralization of excess ROS.

In the light of findings of biochemical estimation (NO and MDA levels), merits of fabricated DTH loaded cyclodextrin nanosponge hydrogel has been evidenced over contemporary DTH hydrogel for the regulation of ROS production and associated effects in psoriatic skin (Fig. 6). These biomarkers provide a clear sign of reduced oxidative stress and distinctive initiation of orthokeratosis, as ascertained by assessing their levels on treatment with DTHNS-HG.

4. Conclusion

The present work demonstrated the development of dithranol nanosponge amalgamated hydrogel (DTHNS-HG), as an effective approach for the management of psoriasis in preclinical studies. The evaluation of developed DTHNS-HG in terms of spreadability, texture analysis and viscosity studies indicated the effectiveness of semisolid dosage form for dermal delivery. *In vivo* antipsoriatic studies on mouse tail showed significant improvement in drug activity of DTHNS-HG treated group with respect to the control group. The assessment of oxidative stress markers (MDA and NO levels) ascertained the effective role of fabricated formulation to manage psoriasis *via* controlling oxidative stress in keratinocytes. This research provides a promising model as multifunctional cyclodextrin nanosponge hydrogel that will facilitate the development of promising antipsoriatic topical approach, thereby growing the existing treatment alternatives to patients and clinicians, and preventing the risk of systemic unfavorable side effects.

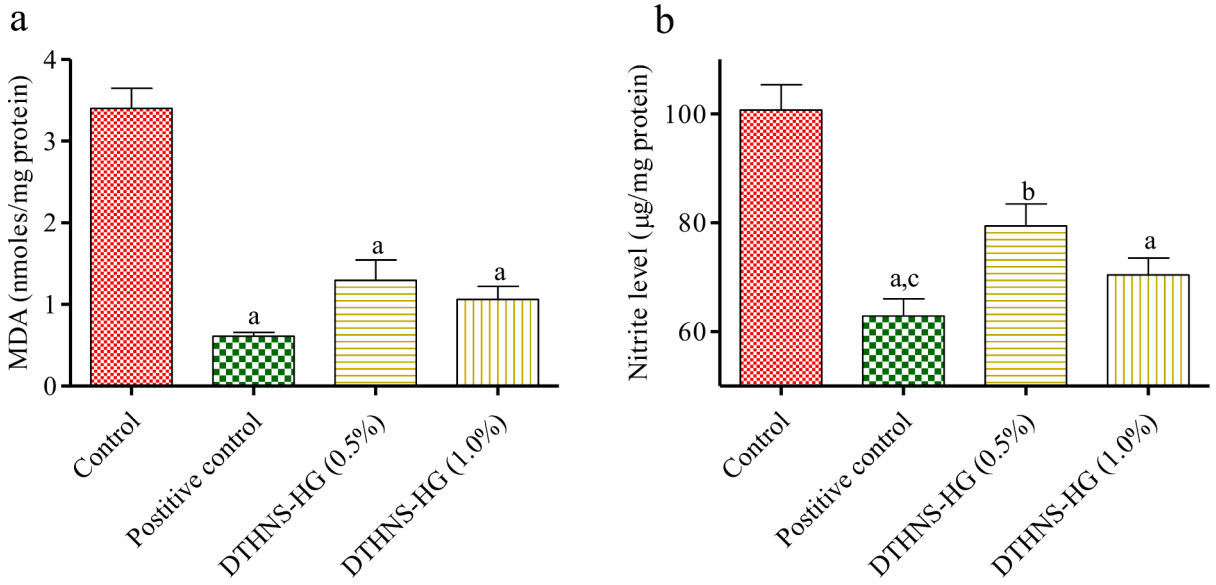

Fig. 5. The mean levels of malondialdehyde (**a**) and nitric oxide (NO) expression (**b**)in the tail tissue of mice treated topically from different experimental groups, ($N = 6$) each group. Data represents mean ± SEM. Data was analysed by one way ANOVA, followed by Tukey's test for multiple comparisons. Positive control: treatment with marketed ointment; DTHNS-HG: Dithranol-loaded nanosponge hydrogel.
(a): a $p<0.001$ vs. control.
(b): a $p<0.001$ vs. control, b $p<0.01$ vs. control, c $p<0.05$ vs. DTHNS-HG (equivalent to 0.5%w/v).

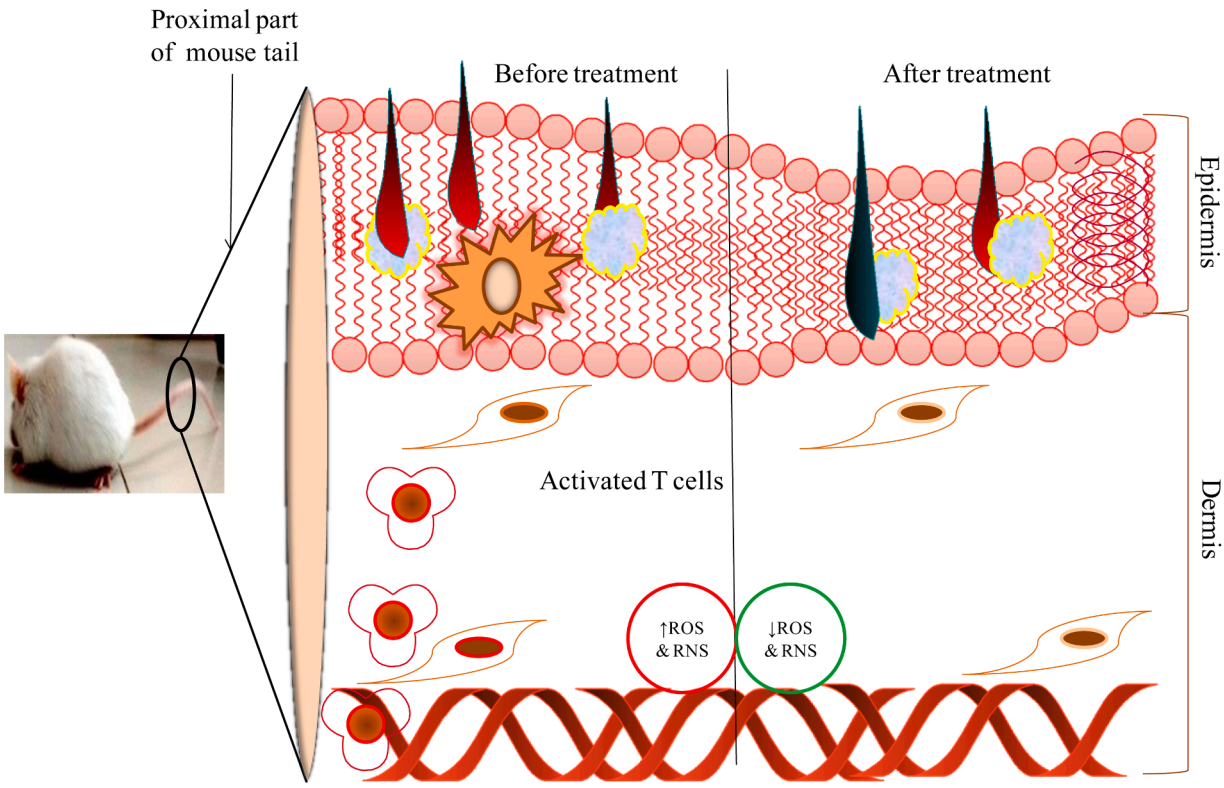

Fig. 6. Schematic representation of role of oxidative stress markers before and after application of DTHNS-HG in mouse tail. (ROS: Reactive oxygen species, RNS: Reactive nitrogen species and T cell: T lymphocyte).

Advancement in the academic area of therapeutic novel formulations may be modulated into commercial dosage forms in the near future.

Author contributions

Sunil Kumar performed all the experiments, analyzed the data, and wrote manuscript. Sunil Kumar and Dr. Rekha Rao contributed in designing of experiments. Dr. Rekha Rao performed proofreading of the manuscript. Dr. Babu Lal Jangir assisted in performing and analyzing histopathology studies.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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