Psoriasis is an immune-mediated, chronic skin disease. The pathogenesis of psoriasis includes abnormal keratinocyte differentiation, hyperproliferation of keratinocytes and infiltration of inflammatory elements. Keratinase (EC 3.4.99.11) from the fungus *Aspergillus parasiticus* was purified to homogeneity by conventional techniques. The silver nanoparticles were biologically synthesized from the fungus *Fusarium oxysporum*. The keratinase nanoformulation was prepared by mixing the purified enzyme with silver nanoparticles and this nanoformulation was named as “Nanozyme–K”. It was found that the silver nanoparticles did not have any inhibitory effect on the purified keratinase activity and in fact, it improved the storage stability of the nanoformulation. Psoriatic samples were collected from patients and the psoriatic proteins were examined by SDS polyacrylamide gel electrophoresis. It was confirmed by MALDI-MS analysis that all the psoriatic proteins belong to keratins of various types. The keratinase and Nanozyme–K completely digested the keratins of psoriatic scales and no keratin band was observed in SDS-PAGE analysis, after Nanozyme–K digestion. The efficiency of the Nanozyme–K was also tested out on psoriatic dermatitis, induced BALB/c mice. About 36% reduction in ear swelling was observed after Nanozyme–K application. Thus, it showed that the Nanozyme–K possessed good antipsoriatic activity. As it is a gentle, non invasive, technique it can be recommended for psoriatic patients even on long term basis without any possible side effects. Moreover, as it is a topical application, it will not have any possible adverse effect(s) like most of the drugs recommended for psoriatic patients. As there are no experimental data on the use of keratinase to digest psoriatic keratins available, it could be the first report of such a study.
INTRODUCTION:
Psoriasis is a chronic, skin disease and affects approximately 125 million people globally. It is an immune-mediated disease where the skin produces a thickened (hyperkeratotic) stratum corneum which desquamates in the form of abnormally large scales. The keratin polypeptide profile from such abnormal scale showed a different spectrum of polypeptides compared with normal stratum corneum. [1, 2, 3] According to Duvic et al., [4] the pathogenesis of psoriasis is due to anomalies in protein expression which include abnormal keratinocyte differentiation, hyperproliferation of keratinocytes and infiltration of inflammatory elements. Keratinases, are a special type of proteases which degrade keratins. These enzymes are produced by several bacteria, fungi and also by some dermatophytes. [5] It is well-known that keratinases have several applications in the field of cosmetic and pharmaceutical industries, leather processing and in chicken feather degradation. [5,6] Interestingly, they are also used to degrade the most recalcitrant prions for treatment of the dreaded mad cow disease. [7] Furthermore, they are also used in livestock feed for improving the nutritional content of feathers. [8] Immobilized enzymes have several advantages such as enhanced stability, easy separation from reaction mixture, easier prevention of microbial growth and possible modulation of the catalytic properties. [9] Nanoparticles are widely used for coupling proteins, ligands, drugs, etc. [10] In this communication, a purified fungal keratinase and its nanoformulation were studied on the digestion of psoriatic scales.

MATERIALS AND METHODS
Chemicals
Azocasein, bovine serum albumin (BSA), TLC plates and oxazolone were purchased from Sigma-Aldrich Chemical Company, U.S.A. Acrylamide, bis-acrylamide, sodium dodecyl sulphate, ethylenediaminetetraacetate were purchased from Sisco Research Laboratories Private limited, Mumbai, India. Keratin was obtained from Himedia Labs, Mumbai. All the other chemicals used were of analytical grade manufactured in India.

Preparation of silver nanoparticles
The silver nanoparticles (SNPs) were synthesized using the fungus, F. oxysporum (MTCC 284) according to Anitha and Palanivelu. [10] The fungal biomass after the nanoparticle production was allowed to settle at the bottom of the flask for about 30 min. The suspension above the biomass was collected and centrifuged at 6000 rpm for 10 min at room temperature. The clear supernatant was discarded and the pellet was resuspended in 500 μl of sterile MilliQ water. The nanoparticles were repeatedly washed with sterile MilliQ water and finally filtered through 0.2 μM sterile membrane filter and stored at 4°C.

Production and purification of keratinase
The keratinase was produced from the fungus Aspergillus parasiticus according to the method described by Anitha and Palanivelu. [11] The enzyme was purified to homogeneity by ammonium sulphate precipitation followed by cation-exchange chromatography and characterized. [12]

Preparation and characterization of keratinase nanoformulation
Before the preparation of keratinase nanoformulation, the effect of silver nanoparticles on the activity of the purified keratinase was studied by mixing the purified keratinase (110 units/ml) and the SNPs (OD was 0.58) in the ratio 1:1 according to Horovitz et al. [13] and incubated at 30°C for one hour. This preparation was named “Nanozyme K”. The enzyme activity and the effect of nanoparticles on the enzyme activity were assayed using azocasein according to Palanivelu. [14] The stability of keratinase in the presence of SNP was studied by incubating the nanoformulation for one week at 30°C. For control, the keratinase was mixed with equal volume of 10 mM acetate buffer, pH 5.5 (To avoid contamination, sodium azide at a concentration of 3 mM was included). After a week, both the samples were analyzed by UV–visible spectrophotometry, atomic force microscopy and enzyme assays. To study the long term stability, the purified keratinase with and without SNPs were incubated at 30°C for 30 days. Aliquots were taken at five days intervals and the
enzyme activity was determined as described by Anitha and Palanivelu. [11]

Collection of psoriatic scales

The study was approved by the Internal Research and Review Board, Ethical Clearance, Bio-
safety and Animal Welfare Committee of the University. After informed consent from patients and
as per the doctor’s directions, samples of psoriatic scales with psoriasis and other hyperkeratotic
disorders, who had received no treatment for the period of one week, were collected by scraping the
skin surface with a sterile scalpel into a sterile collection tube. Stratum corneum near the nails of
normal individuals was also collected following the same procedure. Both the normal and psoriatic scales
were stored at -20°C until further analysis.

Extraction of soluble proteins and keratins

The soluble proteins and keratins were
extracted from the normal and psoriatic scales
according to Matoltsy et al., with slight modification.
[15] About 5 mg sample of the normal stratum corneum
and psoriatic scales were taken in separate sterile
centrifuge tubes. The samples were suspended in 500
μl of 0.2 M phosphate buffer, pH 7.0 and mixed by
vortexing. The supernatant containing soluble proteins
were obtained by centrifugation at 12,000 rpm for 10
min at room temperature. The pellets were washed
twice with the buffer and then macerated well, using
sterile micropestle in the same buffer. The
supernatant, containing the keratin polypeptides were
used for further analysis. Keratin from Himedia,
prepared in the same way, was used as control.

Analysis of protein profiles from normal and
psoriatic scales by SDS –PAGE

The protein profiles of the normal and two
different psoriatic skin samples were analysed in 12%
Sodium dodecyl sulphate polyacrylamide gel
electrophoresis (SDS-PAGE) according to Laemmli.
[16] Forty μl of the supernatant containing about 15 μg
of protein from each sample was mixed with the 40 μl
of 2X loading dye, incubated in a boiling water bath
for 3 min and subjected to SDS-PAGE. The gels were
stained by Coomassie brilliant blue. The relative
molecular masses of the proteins were determined
with known standard protein molecular weight
markers.

MALDI–MS analysis and protein identification
from psoriatic scales

The major proteins of the psoriatic scales,
separated by the SDS-PAGE were excised and
analyzed by Matrix–Assisted Laser Desorption
Ionization Mass Spectrometry (MALDI-MS).
Determination of molecular weight and identification
of the proteins were done using MALDI-MS analysis.
Fragment spectra were searched against the human
subset from the National Center for Biotechnology
Information (NCBI) non-redundant protein sequence
database (NCBInr) and SWISS-PROT database.
Database searches were carried out using the
MASCOT search engine.

Effect of keratinase on the digestion of psoriatic
scales

The digestion of keratins obtained from the
normal and psoriatic scales by the purified keratinase
and its nanoformulation were analyzed by SDS-
PAGE.

Sample preparation and experimental setup

Keratins from the normal and psoriatic scales
were prepared as described elsewhere. After the
removal of soluble proteins from the scales by
centrifugation at 12,000 rpm for 10 min, the residue
was resuspended and macerated well with sterile
micropestle in 0.2 M phosphate buffer, pH 7.0. Then,
the purified keratinase of about 200 μl containing 11
μg (22 units) of keratinase was added and incubated at
37°C for 12 h. The control was maintained without the
addition of keratinase and incubated along with the
test samples. Both the samples were separated by a
regular 12% SDS-PAGE and analyzed.

Effect of keratinase and its nanoformulation on the
digestion of psoriatic scales

The keratinase activity of the free keratinase
and its nanoformulation were determined according to
Gradisar et al. [17] using standard keratin and psoriatic
scales as substrates. In all the experiments, 5 mg of the
substrate was used. Purified keratinase without the
SNPs was used as control. The protein concentrations,
before and after digestion of the psoriatic keratin, was
determined by Bradford’s method. [18] The SDS–
PAGE analysis was carried out to determine the extent
of digestion of the keratins from the psoriatic scales with the purified keratinase and its nanoformulation with respect to the control keratin.

**Preparation of Nanozyme –K lotion and its effect on dermatitis induced mice model**

The keratinase based nanoformulation, the Nanozyme–K lotion was prepared as described elsewhere and the lotion was packed in a sterile container.

**Oxazolone-induced dermatitis in mice**

To assess the effect of Nanozyme–K lotion on dermatitis was investigated. Dermatitis in experimental rats was induced by oxazolone. In this study, BALB/c mice (males) were used. All animals were housed under constant environmental conditions. Animals were divided into six groups of two animals each: Group 1- control (without any treatment), Group 2- treatment with oxazolone, Group 3 – treatment with Topisal lotion (clobetasol propionate and salicylic acid lotion, Clobetasol is the drug approved by FDA for the treatment of psoriasis was used as reference), Group 4 – treatment with purified keratinase, Group 5 – treatment with silver nanoparticles and Group 6- treatment with keratinase nanoformulation.

Psoriasis was induced in mice according to the method of Fuji et al. \[19\] Mice were initially sensitized by the application of 1.5% oxazolone in ethanol on the abdomen. Then, both the sides of both the ears were challenged with 20 μl of 1% oxazolone in a mixture of acetone and olive oil (4:1) every 3 days starting from 7th day after the initial sensitization. Different test and reference solutions in the same volume of 20 μl were applied to both sides of the ear 3 h after each application of oxazolone. Ear thickness, an index of inflammation, was measured using a screw gauze. A sample of mouse ears were excised on 22nd day (72 h after final application of oxazolone) and fixed in formalin. The samples were subjected to biopsy (histopathological study), stained with hematoxylin-eosin and then observed under light microscope.

**RESULTS**

**Effect of silver nanoparticles on the purified keratinase**

The silver nanoparticles (SNPs) were added to the purified keratinase in the ratio 1:1. After 60 minutes, the azocasein assay was performed to determine the effect of SNP on the enzyme activity. It was found that the silver nanoparticles slightly enhanced the enzyme activity (Fig. 1). Thus, it is clear that the silver nanoparticles at least did not have any inhibitory effect on the purified keratinase for making nanoformulations.

![Fig. 1 Effect of SNPs on the purified keratinase](image-url)
Preparation and characterization of keratinase SNP-formulation

Keratinase SNP-formulation was prepared by mixing purified keratinase and SNPs in the ratio 1:1 and incubated at 30°C for a week which facilitates the binding of nanoparticles to the enzyme. After one week of incubation, it was observed that the keratinase and SNP-mixture settled at the bottom, whereas SNPs alone did not settle. The settled particles were resuspended in 10 mM acetate buffer, pH 5.5. The change in colour from initial yellowish brown colour to pale brown in the keratinase and SNP-mixture was observed. The settled particles contained 59% of the enzyme activity and ~40% of the enzyme activity was found in the supernatant. This experiment suggested that ~ 60% of keratinase is bound to the SNPs.

The UV–visible spectra recorded for free SNP and SNPs bound to the enzyme is shown in Fig. 2. The strong surface plasmon resonance centered at ~435 nm was observed for SNPs whereas a broad peak was seen in SNPs bound to the enzyme sample.

By Atomic Force Microscopy (AFM) analysis, it was found that the sizes of the silver nanoparticles were in the ranges of 25 – 60 nm (Fig. 3A). The three-dimensional view has clearly shown that most of the nanoparticles were round in shape and only very few were irregularly shaped (Fig. 3B). The AFM image of free keratinase showed that the enzyme was not in regular shape, but existed as small particles of above 800 nm (Fig. 3C). In the case of keratinase and SNP-mixture, the nanoparticles were found closer to the keratinase enzyme (Fig. 3D) and possibly bound to the enzyme (Fig. 3E). The sizes of the enzyme-bound SNPs were also found to be much larger (Fig. 3F).
Long term stability of keratinase and keratinase SNP formulation

In the long term stability study, 83% of the keratinase activity was retained after five days of incubation at 30°C. After 20 days of incubation, less than 40% of the activity was retained. In contrast, in the presence of silver nanoparticles, more than 90% of the enzyme activity was retained after 15 days of incubation (Fig. 4). At the end of 30 days, more than 80% of the activity was retained. These results suggested that the storage stability of the keratinase was improved significantly in the presence of silver nanoparticles as compared to the free enzyme.

SDS-PAGE analysis of psoriatic proteins

The keratins from the normal and psoriatic scales were extracted and resolved in 12% SDS-PAGE. It was found that, both the psoriatic samples contained three major protein bands with the molecular masses of ~ 69 kDa, 60 kDa and 52 kDa, respectively (Fig. 5). In the psoriatic sample 1, poorly defined protein bands with molecular masses of ~ 48, 42, 35 and 30 kDa were also observed. In the psoriatic sample 2, an additional protein band with an approximate molecular mass of 50 kDa was observed in addition to the three major proteins. Psoriatic scale extracts were slightly distinguishable from that of the normal stratum corneum. In the psoriatic skin samples, it was found that the 69 kDa protein levels decreased in relative amount (Fig. 5, lanes 2 and 3) as compared to the polypeptide chain composition of normal stratum corneum. The other proteins, viz. 60 and 52 kDa were found in high concentrations in the psoriatic scale extracts. In addition to these three major proteins, proteins of lower molecular masses of ~ 14.5 and 15 kDa were also found in the psoriatic scale extracts and not in the normal skin extracts. The keratin from Himedia showed a protein band with a molecular mass of ~ 10 kDa.
**MALDI –MS analysis of proteins from psoriatic scales**

The 69 kDa, 60 kDa and 52 kDa protein bands obtained from the psoriatic scale extracts were excised from the gel and subjected to MALDI-MS analysis. Two databases, viz. NCBInr and SWISS-PROT were used in the MASCOT search to look for proteins that matched the peptide spectra of these three proteins. Interestingly, all the three proteins were identified as keratins from both the NCBInr and SWISS-PROT protein sequence databases.

In the NCBInr database, the 69 kDa protein showed hits to keratin 1 and keratin, type II cytoskeletal 1 whereas, in the SWISS-PROT database analysis, it showed hits to the keratins, type II cytoskeletal 1 and type II cytoskeletal 2.

The 60 kDa psoriatic protein showed hits to the proteins such as keratin 6A, keratin 6C, keratin type II, keratin type II cytoskeletal 6C, keratin type II cytoskeletal 6A with molecular masses of 60, 59.96, 59.91, 59.98 and 60 kDa, respectively in NCBInr database. The protein molecular mass was found to be very similar to the keratin type II cytoskeletal 6A and keratin type II cytoskeletal 6C with molecular masses of 60 and 59.98 kDa, respectively, in SWISS-PROT analysis.

The psoriatic protein of 52 kDa was found to be similar to keratin 14 (51.62 kDa), keratin, type I cytoskeletal 14 (51.59 kDa), keratin 14, (isoform CRA_b) (44.69 kDa), keratin (51.63 kDa), keratin, type I cytoskeletal 16 (51.24 kDa) and type 1 keratin 16 (51.21 kDa) in NCBInr database and keratin, type I cytoskeletal 14 (51.53 kDa) and keratin, type 1 cytoskeletal 16 (51.24 kDa) in SWISS-PROT database, respectively.

The corresponding matched peptides for all the three proteins are shown in Table 1. The MALDI–MS analysis thus, confirmed that the three major bands from psoriatic scales are keratins.

**Table 1 Identification of the proteins from psoriatic scales by MALDI-MS analysis using the NCBI (non-redundant) and SWISS-PROT protein sequence databases**

<table>
<thead>
<tr>
<th>Psoriatic proteins</th>
<th>Database</th>
<th>Protein hit</th>
<th>Matched Peptides</th>
<th>Accession number</th>
</tr>
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<td>69 kDa</td>
<td>NCBInr</td>
<td>Keratin 1 and keratin, type II cytoskeletal 1</td>
<td>SGGFSSGSA GIINYQRFSS CGGGGGSFGA GGGGGSRLN NQFASFDKFL EQQNVQLQTE KTHNLEPYFE SFINNLRRMQ DMVEDYRSLD LDSIIAEKVEY EEQLQITAGRI EISELNRQIS NLQGISDQAE QRNDLEDAL QAAKLADLDE IATYMYSGEC AHPVSYEVST HHTTISGGGS RGGGGGGYGS GSSYSGGSGG SYSGGGGGGS GRGSYSGGGS SYGSSGSGG SGGG GGGSYG GSSGGGGSYG YGGSSGGGGY RGSSGSSGGG S1GGR</td>
<td>AAG41947,AAF60327 &amp; NP_006112</td>
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<tr>
<td>Swiss-Prot</td>
<td>Keratin, type II cytoskeletal 1</td>
<td>Keratin, type II cytoskeletal 2</td>
<td>60 kDa</td>
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</tr>
<tr>
<td>Swiss-Prot</td>
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<td>NCBInr</td>
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<td>Keratin, type II cytoskeletal 6A</td>
<td>52 kDa</td>
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<td>Keratin 14, Keratin, type I cytoskeletal 14</td>
<td>Keratin 14 (isoform CRA_b), keratin</td>
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<td>NCBInr</td>
<td>Keratin 14, Keratin, type I cytoskeletal 16 and type I keratin 16</td>
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<th>Psoriatic proteins</th>
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<th>Matched Peptides</th>
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<td>52 kDa</td>
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<tr>
<td>Keratin, type 1 cytoskeletal 14</td>
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<th>Matched Peptides</th>
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<td>Keratin, type 1 cytoskeletal 16</td>
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<tr>
<td>Keratin, type 1 cytoskeletal 16</td>
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<th>Protein hit</th>
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<tr>
<td>Keratin, type 1 cytoskeletal 14</td>
<td>QFTSSSSMKA PSTYGGLVSV SSSRTMQNL NDROWQDYS FYFYKIIAAATI ENAQPIQLID NARLADDFFR QTVEADVNGL RVLDELTLAR NHEEEMLARQ QQTGGDVNVE MADAPGVDLS RILINEMRDAE TWFLSKLEQIE JATYRDLLEGE DAHLSQQQSS GQSRSQVVST NSQVQL</td>
<td>P08779</td>
</tr>
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</table>
**SDS – PAGE analysis of psoriatic keratins digestion by purified keratinase**

To find out whether the purified keratinase is able to digest the keratins from psoriatic scales, the enzyme was added to the two different psoriatic samples and also to the keratin (Himedia) and incubated for 12 h at 37°C. It was found that, in control (untreated) psoriasis samples, the proteins of ~69 kDa, 60 kDa and 52 kDa were clearly visible with high intensities (Fig. 6) whereas, in the purified keratinase treated samples, these proteins were almost completely digested. The molecular mass of the keratin from Himedia was found to be ~ 10 kDa. This protein was clearly visible in the control sample whereas in the enzyme treated sample, the keratin was digested (Fig. 6). The additional arrow indicates the purified keratinase.

![Fig. 6 Protein profiles of undigested and digested keratin (Himedia) and psoriasis samples](image)

M - Molecular weight markers (14.3 – 97.4 kDa), Lane 1 - Keratin (Himedia) (Control), Lane 2 - Keratin (Himedia) digested with purified keratinase, Lane 3 - Psoriatic sample 1 (Control), Lane 4 - Psoriatic sample 1 digested with purified keratinase, Lane 5 - Psoriatic sample 2 (Control) and Lane 6 - Psoriatic sample 2 sample digested with purified keratinase.

**Digestion of psoriatic keratins by keratinase and keratinase SNP-formulation**

Psoriatic keratins were found to be digested in both the control (free keratinase) and test (SNP-keratinase mixture). There was no significant difference in the activity of the enzyme with and without the nanoparticles. But, the activity was found more for psoriatic sample 2 followed by psoriatic sample 1 and commercial keratin; 419 U/ml > 221 U/ml > 128 U/ml > 422 U/ml > 226 U/ml > 126 U/ml for control enzyme and 422 U/ml > 226 U/ml > 126 U/ml for test sample, respectively (Fig.7). There was a decrease in protein concentrations after the digestion of the keratins in all the three samples both by the free enzyme and with nanoparticles. In psoriatic sample 2, more than 70% of the protein got digested by the free enzyme and the enzyme with nanoparticles, whereas 50% of the proteins got digested in the other two samples (Fig. 8).
Fig. 7 Digestion of psoriatic keratins by free keratinase and keratinase with SNPs

The SDS-PAGE analysis of the undigested and digested keratins showed that the psoriatic keratins of ~69, 60 and 52 kDa in the psoriatic samples 1 and 2 were digested by the free enzyme and with SNPs. In all the digested samples, only the keratinase protein band of ~36 kDa was visible (Fig. 9). The keratinase assay and the electrophoretic analysis have clearly shown that the free keratinase and keratinase with SNPs could degrade the keratins and thus, can be used to treat psoriasis by a non-invasive topical application.
Effect of Nanozyme-K lotion on dermatitis induced mouse model

The dermatitis was induced in BALB/c mice using oxazolone. The treated ears showed erythema (reddening of the skin) and abrasion. The control mice were maintained without the application of oxazolone and found normal without the above symptoms (Fig. 10). The ear thickness was measured after 72 h of final application of oxazolone. The ear with repeated oxazolone application swelled and the ear thickness was found to be 3.0-fold (0.67 ± 0.02 mm) compared with that of the normal control mice, 0.24 ± 0.02 mm (Fig. 11). The Topisal lotion (used as the reference solution) had showed ~60% suppression in ear thickness (0.27 ± 0.01 mm). In the case of Nanozyme-K lotion treated mice, ~36% suppression in ear thickness was found (0.43 ± 0.04 mm). SNPs and purified keratinase alone showed ~8% and 30% suppression in ear thickness (0.62 ± 0.02 mm and 0.47 ± 0.04 mm), respectively.

**Fig. 10 Oxazolone induced allergic contact dermatitis**

Mice without oxazolone treatment (A) (Control) and after oxazolone treatment (B)
The erythematous condition was observed in the treated ears (B).

**Fig. 11 Effects of keratinase and SNP on the ear thickness of mice after sensitization by oxazolone**
For histopathological analysis, the ears lobes were excised and subjected to biopsy. It was found that the oxazolone treated ear swelled so dramatically that the entire section could not be shown. Intra-epidermal inflammatory cell infiltration by polymorphonuclear cells and lymphocytes in the superficial dermis and epidermal hyperplasia were observed in the oxazolone treated ear (Fig. 12). Topisal lotion (used as the reference solution) had significantly suppressed the ear swelling. The Nanozyme–K lotion and the purified keratinase treated ears also showed suppression of ear thickness and improvement on the inflammation of the ear (Fig. 12 G and H). However, SNP treatment did not show any significant inhibition, but the cellular infiltrates were observed (Fig. 12 E and F). Thus, these results suggest that the keratinase and SNP formulation (Nanozyme – K lotion) exhibit good anti-psoriatic activity.

**Fig. 12 Histopathological images of mice ear, after application of oxazolone and after treatment with various agents**

A – Control, B – After application of oxazolone, C and D – magnified views of B, E and F - Treatment with SNP, G – Treatment with Nanozyme – K lotion, H – Treatment with purified keratinase, I – Treatment with Topisal lotion.

The images A, B, E, G, H and I are taken with 40X magnification and C, D and F are with 100X magnification.

**DISCUSSION**

Industrially important enzymes are immobilized onto nanomaterials for improved performance that would pave the way to myriad of applications and commercialization. Nanoparticles having high surface to volume ratio favours high binding capacity and hence high catalytic efficiency and stability of the conjugated enzymes. In the present work, we mixed biologically synthesized SNPs with the purified keratinase and observed that the enzyme was stable in the presence of SNPs for about 20 days without much loss in activity at 30°C. Konwarh et al. [20] found that immobilized keratinase on to polymer – assisted iron oxide magnetic nanoparticles, had resulted in an almost fourfold increase in the keratinase activity and exhibited a significant thermostability and storage stability over the free enzyme. It was also observed that the activity and stability of cholesterol oxidase was found increased after attachment to the magnetic nanoparticles. [21]

In the present study, UV-visible spectrophotometry analysis showed a broad absorption band for the keratinase mixed with silver nanoparticles. Shifting (red shift) of peak from higher energy levels (435 nm, low λ) to lower energy levels (477 nm, high λ) was observed. This suggests possible interactions between the amino acid side chains of the enzyme and the nanoparticles. Such bindings pave
way for the coupling of biomolecular entities for enhanced stability. [20] Similar shifting in the UV–visible absorption spectra was observed by Horovitz et al. [13] when they mixed the gold nanoparticles with the major aleurone protein of barley. For the enhancement of glucose oxidase activity, Pandey et al. [22] have used the thiolated gold nanoparticles. Red shift from 525 nm to 533 nm with broad absorption band and increase in size of the gold nanoparticles with agglomeration were observed by them in the case of glucose oxidase immobilized gold nanoparticles. Similarly, agglomeration of SNPs with the keratinase was also observed in the present study.

Several investigators have studied keratin polypeptides of psoriatic scales in an effort to identify defective chains in abnormal keratin. Each study has provided different data about the number, molecular mass and amount of keratin polypeptides in normal and psoriatic conditions. [15] In our study, we found that the keratin polypeptides with the molecular masses of ~69, 60, 52, 48, 42, 35, 30 kDa in psoriatic sample 1, 69, 60, 52, 50 and 35 kDa in psoriatic sample 2 and 69, 60 and 52 kDa in normal stratum corneum. Matoltsy et al. [15] found 7 prominent polypeptides (67, 59, 57, 50, 48 and 40 kDa) from psoriatic scale keratin and three major protein bands of 67, 59 and 57 kDa in the normal horny cell keratin.

The smaller molecular weight proteins of 15.5, 14.5, 13 and 11 kDa were also observed by Bowden et al. [23] in psoriatic scales. In our study also, we observed a low molecular weight protein of ~15 kDa in psoriatic scales. Variations in the relative amount of high molecular weight polypeptides (57 – 67 kDa) and the presence of low molecular weight polypeptides (40 – 50 kDa) is the characteristic feature of psoriatic scale proteins.

The major protein (the 69 kDa protein) was reduced in quantity in the sample 1 and 2 of psoriatic scales compared to normal stratum corneum. Similarly, the reduction or absence of 68 kDa polypeptide in the psoriatic scales was observed by Bowden et al. [23] and that of 66 kDa was observed by several workers in the psoriatic scales. [1, 2] It was suggested that the keratins from the psoriatic lesions was abnormal and appeared “pre–keratin like”. This is due to defective and variable expression of pre-keratin polypeptides and failure in the post–translational modifications during differentiation. [23]

The MALDI–MS analysis revealed that the psoriatic proteins are keratins of various forms such as keratin 1 and keratin type II cytoskeletal 1, keratin 6A, keratin 6C, keratin 14 and its isoform and keratin, type 1 cytoskeletal 16, respectively. According to Thewes et al. [24] k6 and k16, markers of abnormal hyperproliferative conditions are upregulated in psoriatic epidermis whereas k1 and k10, markers of terminal differentiation are downregulated. Similar observations were made in our study also, where the 69 kDa protein (keratin 1) was found to be quantitatively reduced in the psoriatic samples 1 and 2.

An experiment was carried out to find out whether the psoriatic keratins could be degraded by the purified fungal keratinase. SDS–PAGE analysis of undigested and digested keratins revealed that the purified keratinase could degrade the psoriatic keratins efficiently. In this study, for making nanoformulation, SNPs were used. Titanium dioxide and zinc oxide metal nanoparticles have been used in various sunscreen agents and approved in countries like Australia. In collaboration with Department of Health and Ageing and Therapeutic Goods Administration, the Australian government conducted a “Review of the scientific literature on the safety of nanoparticulate titanium dioxide or zinc oxide in sunscreens”. They reported that the adverse effects of titanium dioxide (TiO2) and zinc oxide (ZnO) nanoparticles in sunscreens depend primarily upon the ability of the nanoparticles to penetrate into viable skin cells. In fact, various evidences suggest that the titanium dioxide and zinc oxide nanoparticles do not reach viable skin cells; rather, they remain on the surface of the skin and in the outer layer of the skin that is composed of non-viable cells. [25-27] It was shown that both the free enzyme and with SNPs efficiently digested the keratins of the psoriatic scales. There are no experimental data on the use of keratinase to digest psoriatic keratins available. Therefore, this is likely to be the first report of such a study. Preliminary experiments on animal model was carried out by using oxazolone to induce psoriatic dermatitis in mice and found swelling and...
inflammatory cell infiltration in treated animals. Presence of cellular infiltrates and hyperplasia of epithelium was observed for the two variants such as [44AANA47] – CCL5 and Met- CCL5 at 0.05 mg/kg by Canavese et al. [28] Massive recruitment of leukocytes to the inflammation site is the hallmark of inflammatory skin disorders.

The anti–psoriatic effect of Nanozyme–K lotion, purified keratinase and SNPs were tested on this model by topical applications. Nanozyme–K lotion showed ~36% suppression in the ear thickness whereas, the purified keratinase without SNPs showed ~30% suppression. Shin et al. [29, 30] have studied the inhibitory effects of ginsenoside Rb1 and compound K, ginsenoside Rg5 and its metabolite ginsenoside Rh3 in similar dermatitis model and found that the components Rg5 and Rh3 at a dose of 0.05% showed suppressive rates of 26.9% and 34.1% whereas Rb1 and compound K showed 29% and 76% of suppression in ear thickness, respectively. However, better suppression of ear swelling by 52.7% and 63.2% were observed using Chunghyuldan (CHD) and metabolized CHD. [31] Thus, the present work reveals that the keratinase is also effective in suppressing psoriatic dermatitis. Moreover, better results may be obtained using various proportions of the nanoformulations and with other types of biocompatible nanoparticles.

In skin medications, keratinases are used to treat acne as well as for removing of human callus and degradation of keratinized skins. [32] It is also utilized for the production of a vaccine for dermatophytosis therapy, pharmaceutical enhancement of the nail treatment and treatment of scars and epithelium regeneration. [32-36] Thus, this enzyme may be useful to treat several other hyperkeratotic disorders as well.

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