



Acyclic monoterpene-rich essential oil of *Cymbopogon distans* mitigates skin inflammation: a chemico-pharmacological study

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Abstract

The topical application of essential oils is considered an effective treatment for skin diseases. *Cymbopogon distans* (Nees ex Steud.) Wats (Poaceae) is a promising aromatic grass widespread in the Himalayan temperate zone. Therefore, using in-vitro and in-vivo bioassays, we examined the chemical and pharmacological characteristics of essential oil hydro-distilled from *C. distans* coded as CDA-01, specifically concerning skin inflammation. Characterization using GC-FID and GC-MS provided a chemical fingerprint for CDA-01, enabling the identification of 54 compounds; amongst them, citral (34.3%), geranyl acetate (21.2%), and geraniol (16.4%) were the most abundant. To examine the anti-inflammatory potential, CDA-01 treatment on LPS-stimulated macrophage cells in addition to 12-O-tetradecanoylphorbol-13-acetate (TPA) generated cutaneous inflammatory reaction in the mouse ear was assessed through quantification of the inflammatory markers. Consequently, CDA-01 demonstrated protection against inflammation caused by LPS by lowering the pro-inflammatory cytokines (IL-6 and TNF- α) level in HaCaT cells with negligible cytotoxicity. Consistent with the in-vitro findings, CDA-01 treatment reduced pro-inflammatory mediators (TNF-, IL-6, and NO) and lipid peroxidation in an in-vivo investigation. Subcutaneous inflammation in TPA-treated mice ears was similarly decreased, as evidenced by the histological and morphological studies. As a result of our findings, it is possible that CDA-01 could be an effective treatment for skin inflammation disorders.

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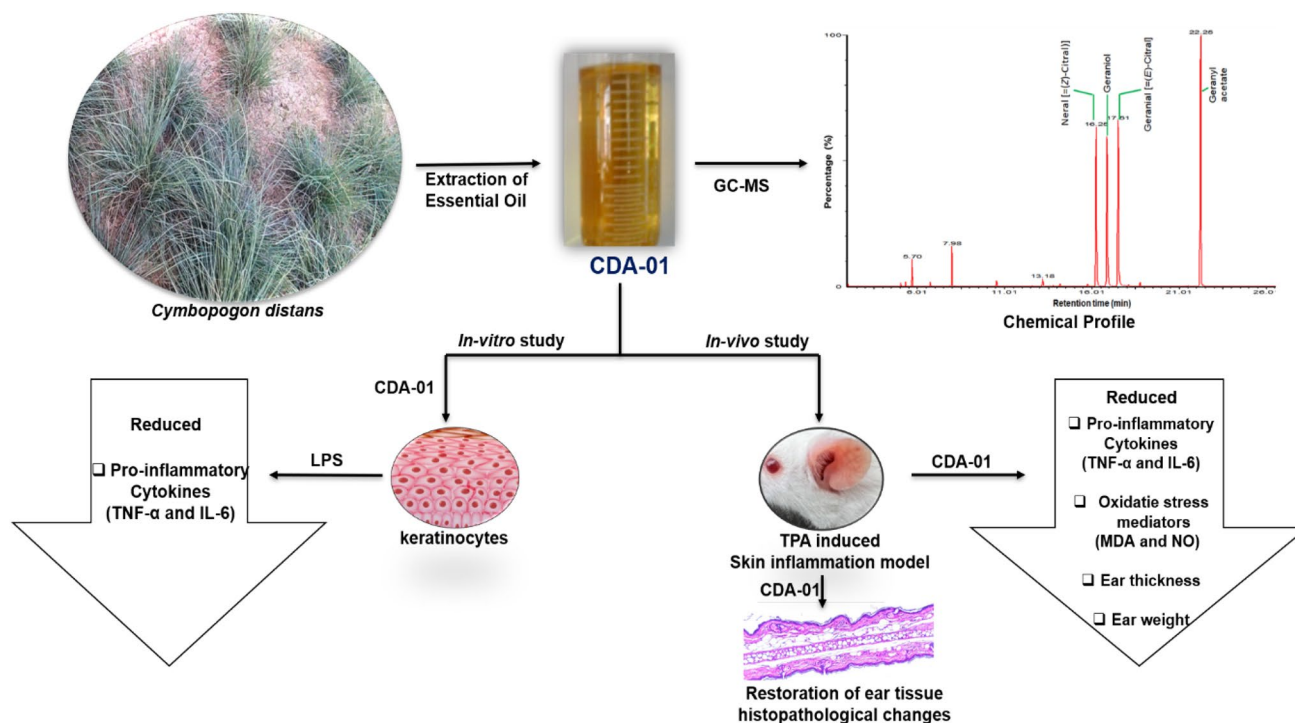
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Graphical Abstract



Keywords *Cymbopogon distans* · Citral · Geranyl Acetate · Inflammation · Skin · Mice

Introduction

The biggest organ in the body is the skin, which safeguards the body from the external environment. Inflammatory skin diseases have been associated with an improper skin immune response to infections or irritants (Salmon et al. 1994). When immune cells within skin tissue are stimulated in response to external stimuli, results in the release of inflammatory cytokines (TNF- α , IL-1, IL-2, IL-8, and IL-6), which usually marks the start of pathophysiological processes that eventually lead to skin diseases like eczema, atopic dermatitis, and psoriasis (Elias et al. 1999; Lee and Hwang 2012). To suppress this induced inflammatory response, corticosteroids are the primary class of anti-inflammatory drugs used for curing inflammation-linked skin diseases (Abraham and Roga 2014). However, it has been reported in several studies that these nonsteroidal anti-inflammatory drugs (NSAIDs) resulted in gastrointestinal ulcers, renal disorders, bleeding, skin atrophy, stretch marks, and telangiectasia. Thus, it paves the way for noninvasive therapeutics from natural sources to treat inflammatory conditions (Cashman 1996).

Essential oils from plants could be an alternative to NSAIDs as they have long been used against inflammatory

diseases in folk medicine. There is a dearth of thorough chemical investigation and exploration of essential oils in animal models for disease studies. However, few studies have put forth the therapeutic significance of essential oils, compelling their investigation as a therapeutic agent. The topical application of Tetradececanoyl phorbol acetate (TPA) induces inflammation-associated molecules such as COX2, iNOS, and cytokines (IL-6, IL-1 β and TNF- α), which mimics the process of skin inflammation (Kundu et al. 2006; Stanley et al. 1991). *Cymbopogon* is a genus of about 55 species of tall perennial aromatic grass native to tropical regions of the World. *Cymbopogon distans* (Nees ex Steud.) Wats, an aromatic perennial grass that grows primarily in high mountains, valleys, and open grassy places in northwestern India, Nepal, Pakistan, Tibet, and southwestern China at 2000–3500 m altitude (Mathela et al. 1987; Zhang et al. 2011). *C. distans*-derived leads possess anti-microbial, anti-tumor, anti-viral, insect repellent, and anti-inflammatory activities (Herrmann et al. 2011; Zhang et al. 2011). The traditional and industrial significance of *C. distans* has led researchers to investigate its chemical composition in India and China, particularly concerning the essential oil and its chemotypes (Mathela 1990). The essential oil of *C. distans* is distinguished by the presence of a wide variety of chemical constituents

such as geraniol, sesquiterpene alcohols, α -oxobisabolene, geranyl acetate, citral, piperitone (Lohani et al. 2015; Mathela et al. 1988; Verma et al. 2013), *p*-menthenols (Lohani et al. 2015; Padalia et al. 2018), α -terpinene, piperitone, intermedeol, geranyl acetate (Xue et al. 1992), (*E*)-geraniol, (*R*)-citronellal, (+)-citronellol, α -elemol (Zhang et al. 2011), and nerol (Chen and Lu 2009). However, systematic chemical characterization of the essential oil isolated from *C. distans* chemotypes available in the Western-Himalayan region of Uttarakhand, India, and its pharmacological potential with special reference to skin inflammation was lacking to ensure the proper utilization of *C. distans*.

The results of this study concluded that acyclic monoterpene-rich essential oil ameliorates pro-inflammatory cytokines production in both *in-vitro* and *in-vivo* assays, and its topical application reduced the severity of skin inflammation while causing no skin irritation in experimental animals.

Material and methods

Chemicals and reagents

The cell culture requirement including Dulbecco's Modified Eagle's Medium (DMEM), antibiotic–antimycotic solution, Lipopolysaccharide (LPS) was obtained from Sigma (St. Louis, USA), along with media supplement Fetal bovine serum of GIBCO (Grand Island, NY, USA). The reagent used in different tests including 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium (MTT), sulfanilamide, phosphoric acid, trichloroacetic acid, thiobarbituric acid, from Himedia (Mumbai, India). N-1-Naphthylethylenediamine dihydrochloride (S.D. Fine Chem. Ltd, Mumbai, India). Tetramethylbenzidine, Mouse and Human-specific TNF- α , IL-6 ELISA kits (BD Biosciences, USA), Acetone, sodium sulfate, sodium acetate, and hydrogen peroxide (Merck Ltd, Mumbai, India).

Plant collection and oil extraction

The *C. distans* were collected from the Western-Himalayan region of Uttarakhand, India and captive cultivated in the experimental field at CSIR-CIMAP Lucknow, India. The fresh aerial parts of *C. distans* were hydro-distilled in Clevenger's-type apparatus for three hours to extract the essential oil. The pale yellow-coloured essential oil with the code CDA-01 was obtained after filtering and drying the essential oil over anhydrous sodium sulphate for further study. Dr. Amit Chauhan, a Plant Taxonomist from the CSIR-CIMAP Research Centre in Pantnagar, verified the authenticity of the plant specimens (Voucher No CIMPANT1007).

Chemical profile of CDA-01

GC-FID and GC-MS were used to develop a chemical profile of the CDA-01. The analysis was performed as per the analytical conditions reported previously (Singh et al. 2022). The linear retention index (LRI) was computed with reference to a homologous series of *n*-alkanes (C_7 – C_{30} saturated alkanes mixture, Supelco). Based on the findings of experimental LRI and mass spectral data, the components of CDA-01 were identified (Adams 2007). Without correcting response factors, the relative percentage of the components was calculated from the areas of GC peaks.

In-vitro study

Culture of keratinocytes

Normal human keratinocyte cell lines (HaCaT) were supplied by National Centre for Cell Sciences, Pune, India. The cells were cultured in a DMEM medium supplemented with 10% FBS in a CO₂ incubator with 5% CO₂ and humidified environment at 37 °C. The cells were further stabilized with a 1X concentration of antibiotic–antimycotic solution.

Anti-inflammatory profile assessment

The *in-vitro* anti-inflammatory effect of the CDA-01 was investigated in the HaCaT cells, which were seeded (1×10^6 live cells/ml) in a cell culture plate (48 wells), followed by an overnight incubation. Afterwards, cells were treated with CDA-01 (0.001%, 0.01% and 0.1%) and dexamethasone (1 μ g/ml) as the positive control. After half an hour of treatment, the cells were activated with stimulant (LPS, 0.1 μ g/ml) and then further incubated at 37 °C for a period of 16 h. After incubation, the supernatants were collected as an antigen source for the Enzyme Immuno Assay (EIA) study and stored frozen until analysis. The amount of TNF- α and IL-6 production was quantified through EIA and expressed as picograms per millilitre (pg/ml).

Cell viability assay

The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium) dye-based assay was performed to contemplate the suitable dose range by examining the viability of the cells in that range. For the same, the cells (HaCaT) were seeded (0.5×10^6 live cells/ml) in 96-well cell culture-tested plates and incubated in the aforementioned cell culture conditions. After an overnight incubation, cells were subjected to different concentrations of CDA-01 (0.001%, 0.01%, 0.1% and 1%) and thereafter incubated for 24 h at 37 °C. Then, 20 μ l MTT (5 mg/mL) was instilled into each well, and after 4 h of incubation, the media was replaced with 100 μ l DMSO

for solubilizing the purple formazan crystals, followed by recording the absorbance at 570 nm using a spectrophotometer (Spectramax; Molecular Devices, USA).

In-vivo study

Experimental animals

The Swiss albino mice (male) weighing 25–30 g and female New Zealand white rabbits were used in this study. They were habituated in the standard environmental condition with a temperature of 22 ± 2 °C and a 12-h light/dark cycle along with clean drinking water and food ad libitum. All the experiments were conducted by following the recommendations of the animal ethical committee of the Institute (CIMAP/IAEC/2020-23/08 and CIMAP/IAEC/2020-23/09), which was approved by Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Government of India (Registration no: 400/GO/ReBi/S/01/CPCSEA).

Skin irritation test

Safety evaluation in the New Zealand white rabbits ($n=4$) was carried out by following the method described by (Kumar et al. 2018). Twenty-four hours ahead of the dermal test, the New Zealand white rabbits ($n=4$) body surfaces were shaved (1-inch area) and washed with sterile water. To the shaved area, 1% CDA-01 oil was applied. It was compared to the skin area treated with acetone (reference or vehicle control). After treatment with CDA-01, rabbits were examined at 4, 24, 48, and 72 h for any signs of oedema and erythema. Upon examining the changes in skin reaction at the different time points, scoring was done to get the primary irritation index (PII) based on a grading scale (0–4) (Table S1). The cumulative irritation index was determined by finding the difference between the sum of the scores of oedema and erythema on the test side and the sum of the score of oedema and erythema on the control site divided by the number of observations.

Skin inflammation model

The inflammatory response of the skin in the right pinna of the mice was induced on both the external and internal surfaces of the ears by instilling 2.5 µg/ear TPA prepared in acetone (20 µl), which instigated the inflammatory response, generally marked by oedema formation. After 30 min of the TPA application, the treatment of the different doses of the CDA-01 (0.1, 0.3, and 1.0%) and the positive control at 0.1 mg/ear (dexamethasone) was instilled topically in the right ears of the mice of the treatment groups. The TPA-only

group served as the vehicle control, while the acetone-only group was designated as normal.

Evaluation of the macroscopic score

To determine a macroscopic score of ear inflammation, the ears of the vehicle and treatment groups of mice were examined for any visible signs of severity. All mice ears were assigned a macroscopic score ranging from 0 to 7. A macroscopic score of 0–1 indicates no skin inflammation, while a 2–3 score indicates mild inflammation, an increase up to 4–5 is a sign of moderate inflammation, and 6–7 is a sign of severity.

Assessment of the changes in ear thickness and weight

A digital screw gauge (Aerospace Instruments) was used to measure the inflammation-induced oedema formation by measuring the thickness across the medial edge of the ear and the changes in the ear thickness were expressed in µm. Before inducing inflammation, the thickness of the ears was measured, and then again measured 6 h after inflammation induction. Mice were euthanized under ether anaesthesia after 6 h of the treatment by cervical dislocation when rubor and tumor could be seen as an early indicator of peak inflammation. The ear tissues with a 1.0 cm diameter were punched to measure the wet weight, immediately transferred to the buffer (Tris–HCL, pH-7.4), and further homogenized by tissue homogenizer (Pro Scientific Inc, USA) to prepare the 10% homogenate for the biochemical assays and inflammatory cytokine estimation.

Quantification of pro-inflammatory cytokines level

After obtaining the 10% homogenate of ear tissues, they were spun in a centrifuge for 30 min at 12,000 g at 4°C. Thus, the supernatants obtained after centrifugation were used as a source of protein for the measurement of the level of cytokines. IL-6 and TNF-α (pro-inflammatory cytokines) were quantified through mouse-specific Enzyme Immuno Assay (EIA) kits from BD Biosciences, USA, and the procedure was based on the method of the manufacturer.

Quantification of malondialdehyde (MDA)

Malondialdehyde (MDA), endproduct from Lipid Peroxidation was measured by the method of Ohkawa et al., (1979). The ear tissues were first homogenized in the solution of 10% trichloroacetic acid (TCA), and 0.2 ml of the supernatants obtained were mixed with the solution containing 8.1% sodium dodecyl sulphate (0.2 mL). After incubation for a few minutes at room temperature, 1.5 ml of the 20% acetic acid (pH 3.5) was added to each reaction mixture. In the

end, 0.8% of 1.5 ml thiobarbituric acid (TBA) was added, followed by a 1-h incubation in the boiling water bath. After immediately cooling the reaction mixture, the supernatants were collected and the absorbance at 532 nm was quantified and expressed as μM MDA/ml.

Determination of nitric oxide (NO) content

The nitrite content of the ear tissues was estimated in the 10% solution of ear tissue homogenates prepared in Tris-Buffer using the Griess reagent. The Griess reagent was prepared by mixing sulfanilamide solution (1% in 5% of ortho-phosphoric acid), and N-1-naphthyl-ethylenediamine dihydrochloride (NED) solution (0.1%). The test samples were mixed with the prepared Griess reagent and incubated at 37 °C for 30 min. In the end, absorbance was recorded at 548 nm, and nitrite concentration in each sample was quantified as μM /ml using the sodium nitrite standard curve.

Histopathology

The histological changes induced by inflammatory reactions in the ear tissues were also assessed. The ear biopsies were collected from each group and then fixed immediately in a 10% buffered formaldehyde solution in PBS (pH-7.4). After rinsing in water, the fixed tissues were dehydrated through varying concentrations of alcohol, fixed in paraffin wax, and then subsequently sectioned into 4–5 μm sections and stained using hematoxylin and eosin. From the complete section, an area was selected as a representative of the infiltration of inflammatory cells, and images were captured in the light microscope at 100 \times magnifications for the qualitative evaluation.

Statistical analysis

The results of the three separate experiments were given as the mean plus the standard error of the mean (SEM). One-way analysis of variance (ANOVA) was employed to compare the groups, and then Tukey's test was used to find the significant difference, where $p < 0.05$ was considered statistically significant using Graph Pad Prism 5 software.

Results

Chemical composition of CDA-01

The fresh aerial parts of *C. distans* were hydrodistilled and obtained 0.4% essential oil (CDA-01). The CDA-01 was analyzed by GC-FID and GC-MS to develop its chemical profile. The components identified in the CDA-01 were described in Tables 1 and 2. The GC-MS chromatogram of

CDA-01 was shown in Fig. 1. Fifty-four compounds, representing up to 89.9% of the CDA-01, were identified. The CDA-01 was characterized by higher amounts of oxygenated monoterpenoids (80.3%), which were mainly represented by monoterpene aldehydes (35.2%), monoterpene alcohols (20.9%), and monoterpene esters (22.8%). The characteristic components of the CDA-01 were geranyl acetate (21.2%), geranial (19.7%), geraniol (16.4%), and neral (14.6%).

Cell viability effect of CDA-01

The viability of HaCaT cells was checked upon CDA-01 treatment (0.001%, 0.01%, 0.1% and 1%) to find its safe dose range. It was found to be safe up to a concentration of 0.1% as no change in % cell viability was perceived, while the higher concentration (1%) showed significant toxicity (Table S2). Therefore, concentrations below 1% were used for the assays prospecting the activity of CDA-01.

CDA-01 inhibitory effect on LPS-induced pro-inflammatory cytokines level

CDA-01 was checked for its potential against LPS-induced inflammation in HaCaT cells by examining the level of cytokines (pro-inflammatory). The treatment of CDA-01 was found to have a protective role by decreasing the IL-6 and TNF- α cytokines levels, following a concentration-dependent effect. The highest concentration (0.1%) was found to be most effective, with $51.78 \pm 2.81\%$ and $53.93 \pm 4.44\%$ suppression of IL-6 and TNF- α , respectively (Fig. 2).

Acute dermal irritation study of CDA-01

For the curative and safe topical application in human skin, the safety of CDA-01 was also inspected. Henceforth, CDA-01 was applied in the one-inch area of the rabbit skin, and the probable signs of any change in the skin were observed at different time periods (4, 24, 48, and 72 h) and determined as PII scores (Table 3). There was no significant erythema and oedema formation upon CDA-01 treatment compared with the vehicle group (acetone). Following the Federal Hazardous Substances Act regulation, which stated that the material will be non-irritant if PII is less than 5.00, CDA-01, thus was not found to be irritant to the rabbit skin with PII less than 5.00 and hence considered safe for topical applications.

Ear inflammation assessment through the macroscopic score

The ears of mice treated with TPA alone (vehicle) had a macroscopic appearance that was visibly distinct and altered. The erythema score of normal mice was 0.67 ± 0.22 , which rose to 5.25 ± 0.23 upon TPA treatment for 6 h. The CDA-01

Table 1 Chemical composition of the essential oil of *Cymbopogon distans* (CDA-01)

LRI ^a	LRI ^b	Compound	Content (%)	Compound class
900	889	2-Heptanone	t	Aliphatic ketone
922	921	Tricyclene	0.3	Cyclic monoterpene hydrocarbon
926	924	α -Thujene	t	Cyclic monoterpene hydrocarbon
933	932	α -Pinene	0.4	Cyclic monoterpene hydrocarbon
947	946	Camphene	1.9	Cyclic monoterpene hydrocarbon
972	969	Sabinene	t	Cyclic monoterpene hydrocarbon
976	974	β -Pinene	t	Cyclic monoterpene hydrocarbon
986	981	6-Methyl-5-hepten-2-one	0.9	Aliphatic ketone
991	988	Myrcene	0.1	Acyclic monoterpene hydrocarbon
1002	1001	δ -2-Carene	t	Cyclic monoterpene hydrocarbon
1024	1020	<i>p</i> -Cymene	0.1	Cyclic monoterpene hydrocarbon (aromatic)
1028	1026	Limonene	3.2	Cyclic monoterpene hydrocarbon
1032	1028	1,8-Cineole	0.1	Cyclic monoterpene oxide
1036	1032	(<i>Z</i>)- β -Ocimene	t	Acyclic monoterpene hydrocarbon
1046	1044	(<i>E</i>)- β -Ocimene	t	Acyclic monoterpene hydrocarbon
1057	1054	γ -Terpinene	t	Cyclic monoterpene hydrocarbon
1070	1067	(<i>Z</i>)-Linalool oxide (furanoid)	0.1	Cyclic monoterpene oxide
1088	1086	Terpinolene	0.1	Cyclic monoterpene hydrocarbon
1102	1095	Linalool	0.7	Acyclic monoterpene alcohol
1139	1136	(<i>E</i>)- <i>p</i> -Menth-2-en-1-ol	0.4	Cyclic monoterpene alcohol
1152	1148	Citronellal	0.4	Acyclic monoterpene aldehyde
1165	1166	Borneol	1.6	Cyclic monoterpene alcohol
1170	1167	Octanoic acid	0.1	Aliphatic acid
1175	1174	Terpinen-4-ol	0.2	Cyclic monoterpene alcohol
1185	1177	(<i>E</i>)-Isocitral	0.5	Acyclic monoterpene aldehyde
1189	1186	α -Terpineol	0.4	Cyclic monoterpene alcohol
1228	1223	Citronellol	1.2	Acyclic monoterpene alcohol
1240	1235	Neral [= (<i>Z</i>)-Citral]	14.6	Acyclic monoterpene aldehyde
1255	1249	Geraniol	16.4	Acyclic monoterpene alcohol
1270	1264	Geranial [= (<i>E</i>)-Citral]	19.7	Acyclic monoterpene aldehyde
1285	1284	Bornyl acetate	t	Cyclic monoterpene ester
1303	1298	Geranyl formate	0.3	Acyclic monoterpene ester
1324	1322	Methyl geranate	0.6	Acyclic monoterpene ester
1354	1350	Citronellyl acetate	t	Acyclic monoterpene ester
1359	1355	Geranic acid	1.2	Acyclic monoterpene acid
1364	1369	Cyclosativene	0.1	Cyclic sesquiterpene hydrocarbon
1385	1379	Geranyl acetate	21.2	Acyclic monoterpene ester
1390	1389	β -Elemene	0.1	Cyclic sesquiterpene hydrocarbon
1416	1417	β -Caryophyllene	0.2	Cyclic sesquiterpene hydrocarbon
1451	1452	α -Humulene	t	Cyclic sesquiterpene hydrocarbon
1475	1475	γ -Gurjunene	0.1	Cyclic sesquiterpene hydrocarbon
1490	1489	β -Selinene	0.2	Cyclic sesquiterpene hydrocarbon
1498	1500	α -Murolene	0.1	Cyclic sesquiterpene hydrocarbon
1502	1504	Cuparene	t	Cyclic sesquiterpene hydrocarbon
1512	1513	γ -Cadinene	0.4	Cyclic sesquiterpene hydrocarbon
1517	1521	(<i>E</i>)-Calamenene	t	Cyclic sesquiterpene hydrocarbon (aromatic)
1522	1522	δ -Cadinene	0.2	Cyclic sesquiterpene hydrocarbon
1551	1548	Elemol	0.1	Cyclic sesquiterpene alcohol
1564	1562	Geranyl butanoate	0.3	Acyclic monoterpene ester
1580	1582	Caryophyllene oxide	0.5	Cyclic sesquiterpene oxide
1612	1608	Humulene epoxide II	t	Cyclic sesquiterpene oxide
1653	1652	α -Cadinol	0.5	Cyclic sesquiterpene alcohol
1757	1755	Geranyl hexanoate	0.3	Acyclic monoterpene ester
1953	1953	Geranyl octanoate	0.1	Acyclic monoterpene ester

^aLRI: experimental linear retention index, determined on HP-5 fused silica capillary column with reference to homologous series of *n*-alkanes

^bLRI; linear retention index from literature, t: trace (<0.05%)

Table 2 Class composition of terpenoids of CDA-01 essential oil

Class	Cyclic Terpenoid	Acyclic Terpenoid	Total
Monoterpenoids			
Monoterpene hydrocarbons	6.0	0.1	6.1
Monoterpene oxides	0.2	–	0.2
Monoterpene aldehydes	–	35.2	35.2
Monoterpene alcohols	2.6	18.3	20.9
Monoterpene esters	–	22.8	22.8
Monoterpene acids	–	1.2	1.2
Sesquiterpenoids			
Sesquiterpene hydrocarbons	1.4	–	1.4
Sesquiterpene oxides	0.5	–	0.5
Sesquiterpene alcohols	0.6	–	0.6
Others			
Total identified	11.3	77.6	89.9

treatment at 1%, 0.3% and 0.1% significantly reduced the erythema to 2.60 ± 0.27 , 3.00 ± 0.20 , and 4.17 ± 0.11 , respectively. The dexamethasone resulted in a macroscopic score of 1.58 ± 0.20 . The severity of inflammation assessed through the macroscopic score is also depicted in Fig. 3.

Effect of CDA-01 on TPA-induced oedema in mice ears

To find out whether CDA-01 mitigate the skin inflammation induced in mice ears by TPA, the ears were monitored in a time-dependent manner and observed up to the time acute inflammation reached its peak, recorded by the changes in oedema. The thickness measurement was done before TPA application and after 6 h (peak inflammation). The treatment of CDA-01 has shown a dose-dependent decrease compared to TPA treated vehicle group, with $48.15 \pm 4.68\%$ inhibition at the highest dose (1%). The ear weight was also found to

be correlated with the thickness; a dose-dependent decrease in weight was monitored in the CDA-01 treated group compared to the vehicle group with a maximum inhibition of $46.66 \pm 3.30\%$ at a 1% dose of CDA-01 (Table 4).

Quantification of pro-inflammatory cytokines

The measurement of cytokines, specifically pro-inflammatory mediators, is one of the primary markers of estimating acute skin inflammation. Thus, we quantified pro-inflammatory cytokines (TNF- α and IL-6) and found that CDA-01 dose-dependently decreased the production of cytokines as compared to TPA treated vehicle group (Fig. 4). The % suppression of TNF- α and IL-6 at the highest dose of 1% CDA-01 was $46.00 \pm 5.60\%$ and $74.8 \pm 3.17\%$, respectively.

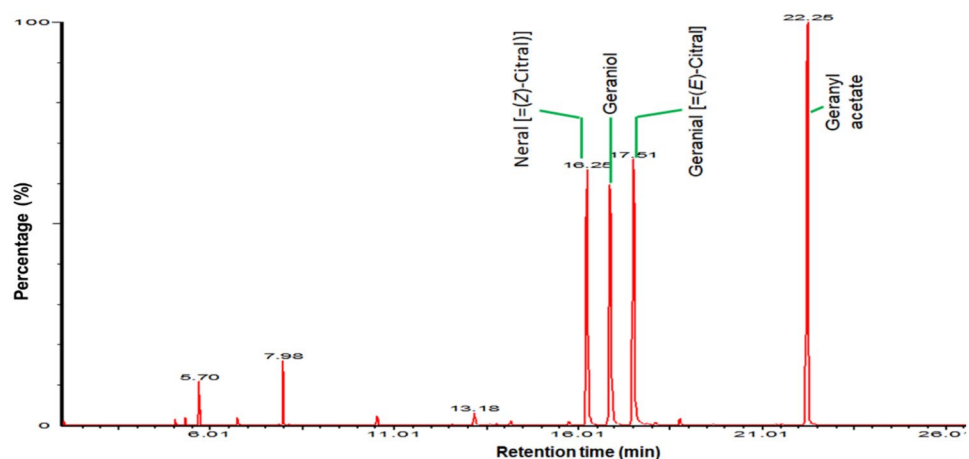
Effect of CDA-01 on the production of malondialdehyde and release of NO

The malondialdehyde production was significantly increased in the vehicle group (TPA alone), while in the CDA-01 treated groups, a significant decrease was observed in a dose-dependent manner, owing to $76.62 \pm 2.25\%$, $71.6 \pm 2.09\%$ and $44.6 \pm 2.26\%$ chemo-suppression at doses of 1%, 0.3%, and 0.1%, respectively. Similarly, the NO level was also raised significantly in the vehicle group, and a significant decline was found in the CDA-01 treated group with $70.11 \pm 3.16\%$, $50.31 \pm 3.61\%$ and $42.93 \pm 4.24\%$ chemo-suppression, respectively (Table 5).

Histological analysis of CDA-01

To further substantiate the anti-inflammatory potential of the CDA-01, histopathological changes were observed in

Fig. 1 GC–MS chromatogram (TIC) of CDA-01 essential oil



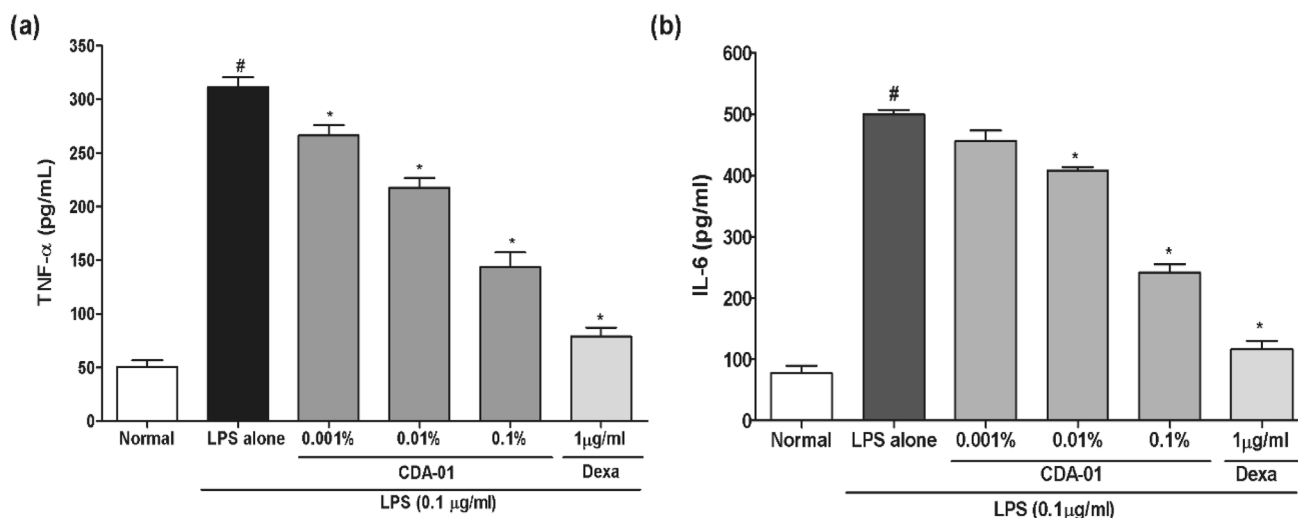


Fig. 2 Effect of CDA-01 on LPS-induced inflammation in HaCaT cells. Data are expressed as mean \pm SEM; $n=3$. *LPS versus treatment, #normal versus LPS (ANOVA; Tukey test), $P < 0.05$

Table 3 Effect of CDA-01 on primary skin irritation test in the rabbit skin

Hours after treatment	Mean score			Irritant intensity
	Control site	Treatment site	Cumulative irritation index (CII)	
4	0.50	0.75	0.25	No
24	0.00	0.00	0.00	No
48	0.00	0.00	0.00	No
72	0.00	0.00	0.00	No

Data are represented as mean \pm SEM; $n=4$; control versus treatment < 0.05

the inflamed ear tissue by H&E staining. By examining the microscopic images, striking differences in the pathology of the vehicle group were observed with an increased ear thickness in conjunction with the infiltration of leukocytes in the dermis leading to the disruptive connective tissue as evidence of inflammation. Compared to the vehicle group, CDA-01 treatment dose-dependently decreased the ear thickness and the TPA-induced pathologies (Fig. 5).

Discussion

Repeated skin exposure to infections (bacterial, viral, or fungal), allergies, autoimmune reactions, and parasites causes skin diseases (Sabat et al. 2019). They can range from minor rashes with redness to chronic illnesses such as rosacea, psoriasis, and dermatitis (Wu et al. 2019). Diseases

of the skin that are associated with inflammatory conditions are common in both developed and developing countries; these diseases not only lower people's quality of life but also place a financial burden on their country. (Fuchs et al. 2001). While non-steroidal anti-inflammatory drugs are helpful in treating acute inflammation, they have limited efficacy in treating chronic inflammation and a multitude of unwanted side effects (Moore et al. 1998). The popularity of essential oils has grown in the nutritional, pharmaceutical, and cosmetic industries due to their antimicrobial, antioxidant, anticancer, and anti-inflammatory effects (Bakkali et al. 2008). Monoterpenes are industrially significant volatile chemicals in hydro-distilled essential oils extracted from aromatic plants. To increase the utilization functional components (monoterpene) of essential oil in nutritional, cosmetic, and medicinal products, it is still necessary to comprehend their biological activities. In this research work, the essential oil hydro-distilled from *Cymbopogon distans* was coded as CDA-01. Chemical analysis of CDA-01 exhibited its main components, including oxygenated derivatives of acyclic monoterpenoids (geranyl acetate, geranial, neral, and geraniol). The observed chemical profile of the oil and the earlier report on the chemical makeup of *C. distans* were closely correlated (Mathela et al. 1988; Verma et al. 2013). Oxygenated acyclic monoterpene derivatives are used in cosmetic, pharmaceutical, flavouring, and fragrance industries (Kozioł et al. 2014). The most promising monoterpenes of industrial importance are geraniol, camphor, citral, citronellol eucalyptol, ocimene, myrcene, limonene, linalool, menthol, and pinenes (Loza-Tavera 1999). *In-vitro* pharmacological potential of CDA-01 demonstrated that the level of the pro-inflammatory cytokines (TNF- α and

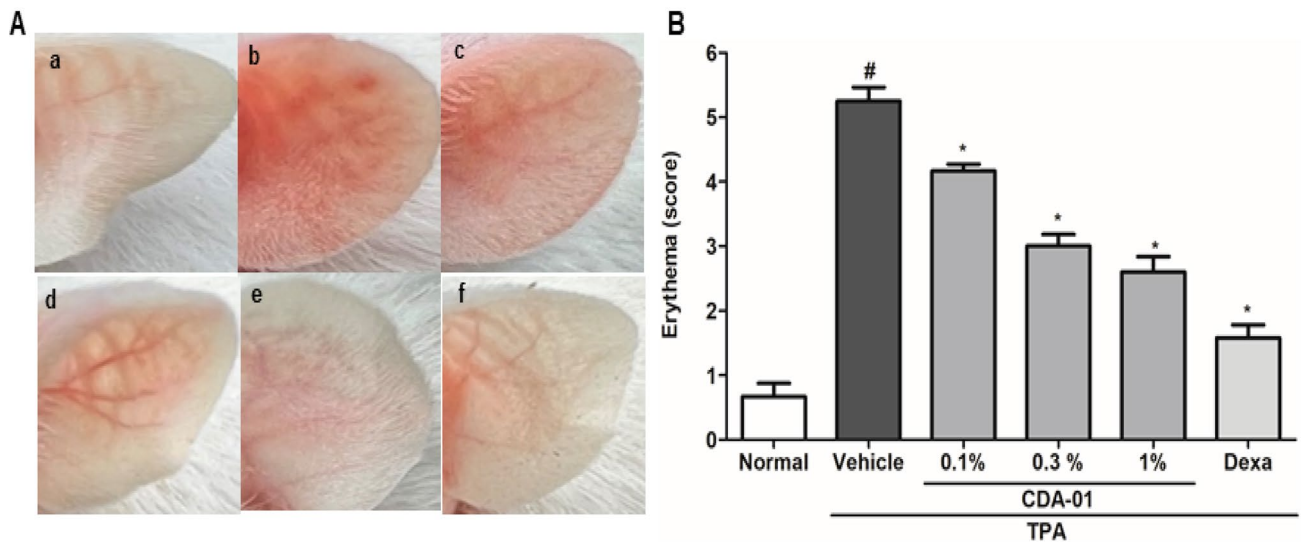


Fig. 3 The photographs indicating the severity of inflammation (A) along with the macroscopic scoring (B) of the inflamed ear treated with CDA-01 after stimulation with TPA, a) Normal (b) Vehicle (c)

CDA-01 (0.1%), (d) CDA-01 (0.3%) (e) CDA-01 (1%) and (f) Dexamethasone. Results are represented as mean ± SEM; n = 6. *TPA versus treatment, # normal versus TPA (ANOVA; Tukey test), P < 0.05

Table 4 CDA-01 effect on TPA-induced inflammation mediated ear swelling in Swiss albino mice (a) Change in ear thickness and (b) Ear weight

Treatment	Change in ear Thickness (mm)	Inhibition (%)	Weight (mg)	Inhibition (%)
Normal	23.33 ± 6.73	NA	20.95 ± 1.16	NA
Vehicle	135.00 ± 7.35 [#]	0	57.22 ± 1.54 [#]	0
CDA-01 (0.01%)	105.00 ± 6.78 [*]	22.22 ± 4.59	38.95 ± 2.46 [*]	31.92 ± 4.71
CDA-01 (0.1%)	88.33 ± 7.16 [*]	34.57 ± 4.84	37.00 ± 3.94 [*]	35.33 ± 6.88
CDA-01 (1%)	70.00 ± 6.93 [*]	48.15 ± 4.68	30.52 ± 1.89 [*]	46.66 ± 3.30
Dexamethasone	55.00 ± 7.87 [*]	59.26 ± 5.32	25.18 ± 0.86 [*]	55.98 ± 1.50

Results are represented as mean ± SEM; n = 6. *TPA versus treatment, # normal versus TPA (ANOVA; Tukey test), P < 0.05

IL-6) from activated keratinocyte cells was significantly (P < 0.05) reduced in a concentration-dependent manner as compared to LPS-treated cells. Previous scientific studies also demonstrated that the immune response triggered by LPS can lead to the release of various pro-inflammatory molecules, which can further contribute to tissue damage (Ross et al. 2004) and essential oils and their bioactive are capable of lowering pro-inflammatory cytokines production (Kumar et al. 2018; Singh et al. 2021). The chronic overproduction of pro-inflammatory cytokines is observed in skin disorders and the regulation of the cytokine’s overproduction could promote promising therapy against chronic inflammation-associated diseases (Newton and Dixit 2012). The cytotoxicity profile of CDA-01 in HaCaT cells revealed that the effective concentration of CDA-01 was not inducing cytotoxicity in keratinocytes cells. Additionally, through a

primary skin irritation test on rabbit skin and a TPA-induced skin inflammation test on mice, we have assessed the safety and therapeutic effectiveness of CDA-01 in the *in-vivo* system. The CDA-01 treatment on rabbit skin revealed that it is not an irritant to the skin. This study is comparable to the earlier findings that some essential oils are safe when topically applied to mammalian skin (Kumar et al. 2018; Maurya et al. 2018). Therapeutic benefits of CDA-01 were observed against TPA-induced skin inflammation in mice, as evidenced by a decrease in ear oedema, ear weight, pro-inflammatory cytokines level (TNF- and IL-6), malondialdehyde, nitric oxide level, and an improvement in histopathological alterations in inflammatory skin tissue. Several pre-clinical studies on skin inflammation revealed that skin exposure to TPA (12-O-tetradecanoylphorbol-13-acetate) activates an inflammatory reaction similar to that seen in

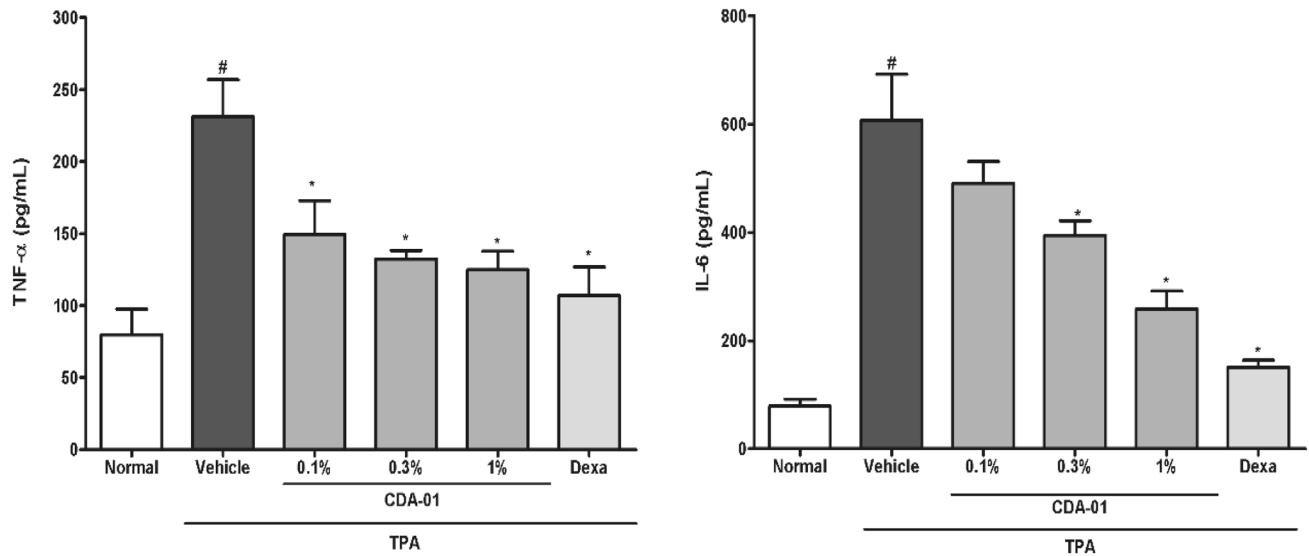


Fig. 4 Effect of CDA-01 on the pro-inflammatory markers in TPA-induced ear inflammation in Swiss albino mice **(A)** Ear homogenate TNF- α **(B)** Ear homogenate IL-6. Results are represented as

mean \pm SEM; $n=6$. *TPA versus treatment, # normal versus TPA (ANOVA; Tukey test), $P < 0.05$

Table 5 CDA-01 effect on markers of oxidative stress (a) Malondialdehyde (MDA) and (b) Nitric oxide (NO), quantified from a homogenate of ear tissue

Treatment	Nitric oxide (NO) ($\mu\text{M/ml}$)	Inhibition (%)	Malondialdehyde (MDA) ($\mu\text{M MDA/ml}$)	Inhibition (%)
Normal	1.04 \pm 0.16	NA	5.78 \pm 1.37	NA
Vehicle	35.78 \pm 1.81 [#]	0.00	71.38 \pm 7.68 [#]	0.00
CDA-01 (0.01%)	19.79 \pm 0.81*	44.68 \pm 2.26	40.73 \pm 3.03*	42.93 \pm 4.24
CDA-01 (0.1%)	10.13 \pm 0.75*	71.68 \pm 2.09	35.47 \pm 2.58*	50.31 \pm 3.61
CDA-01 (1%)	8.37 \pm 0.81*	76.62 \pm 2.25	21.33 \pm 2.25*	70.11 \pm 3.16
Dexamethasone	3.65 \pm 0.28*	89.80 \pm 0.77	14.22 \pm 0.95*	80.08 \pm 1.33

Results are represented as mean \pm SEM; $n=6$. *TPA versus treatment, # normal versus TPA (ANOVA; Tukey test), $P < 0.05$

skin diseases (Wu et al. 2022). These experimental results are consistent with numerous earlier reports that indicated that essential oils and their molecules could lessen the severity of skin inflammation by reducing skin oedema, a measure suggestive of increased vascular permeability, dermal oedema, and epidermal keratinocyte proliferation during the inflammatory skin process. (De Vry et al. 2005; Murphy et al. 2000). As a result of the abundance of terpenes in essential oils, their better skin penetration, low skin irritation, and effectiveness against skin inflammation have paved much attention for the development of topical pharmaceutical and cosmetic formulations (Chen et al. 2016; Varman and Singh 2012).

Conclusion

This chemico-pharmacological study demonstrated that CDA-01, an essential oil isolated from *Cymbopogon distans* is rich in acyclic monoterpenoids and able to reduce skin inflammation in experimental animals without causing any harm to their skin. The results of this study provided scientific evidence for further consideration of CDA-01 in the management of inflammation-related skin disorders as a topical pharmaceutical or cosmeceutical formulation.

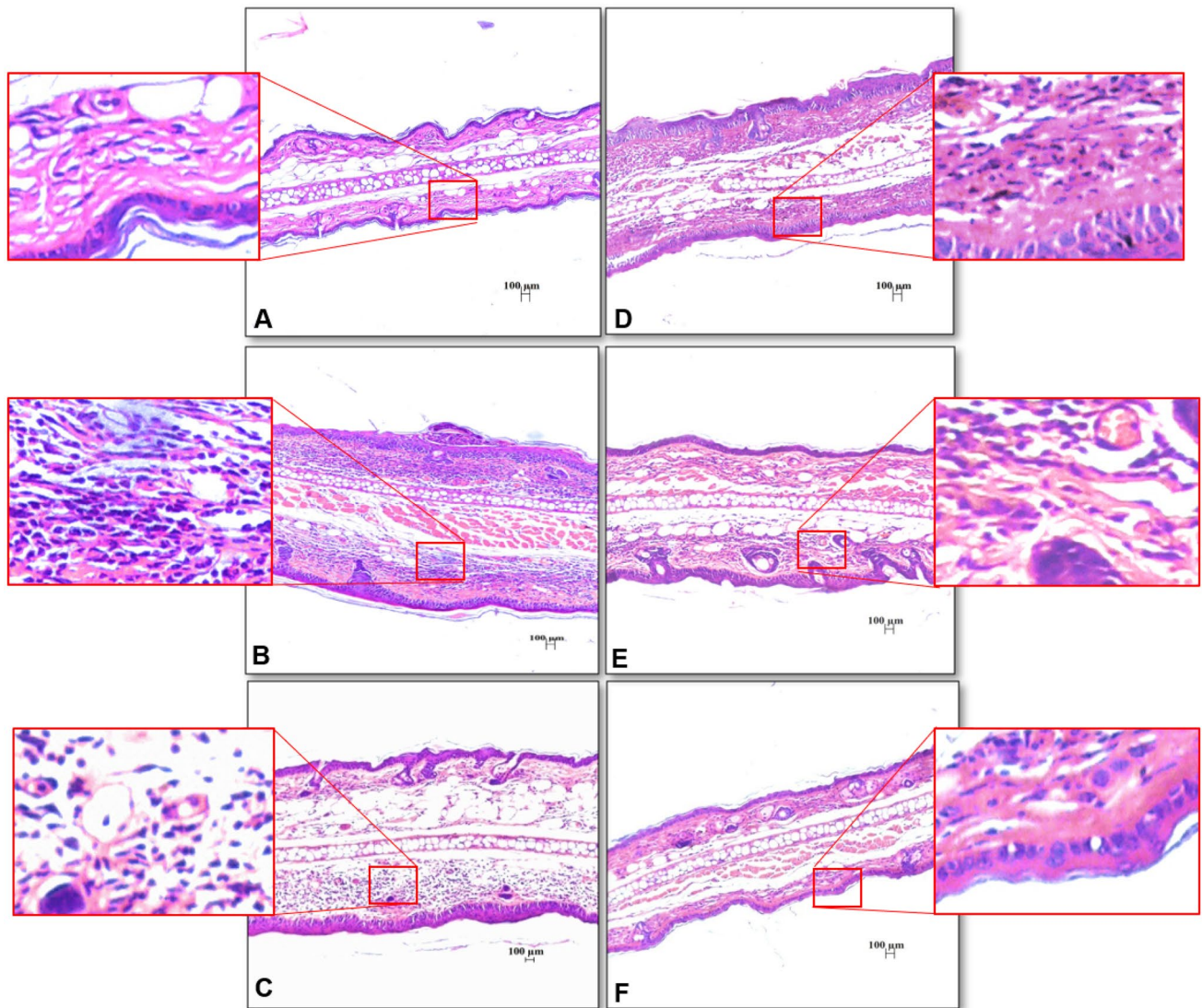


Fig. 5 The hematoxylin–eosin-stained transverse sections of the mice ears, examined under a light microscope (magnification: $\times 100$), depicting the histopathological changes upon CDA-01 treatment on TPA sensitized mice ears. Treatments: **A**; Normal, **B**; Vehicle (TPA challenged), **C**; CDA-01 (0.1%), **D**; CDA-01 (0.3%), **E**; CDA-01

(1%), **F**; Dexamethasone (0.1 mg/ear). The TPA-treated ear sections (**B**) exhibits thickening of the epidermis and infiltration of inflammatory cells, along with noticeable hyperplasia of the epithelial cells as visualised in the zoomed insets, which were found to improve upon CDA-01 treatment

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10787-023-01302-0>.

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Authors contributions DB: actual experimentation, recording of observation and manuscript preparation (biological activity). SS: actual experimentation, recording of observation, analysis and manuscript preparation (chemistry). MKS: analysis and manuscript preparation.

AKM: histopathological analysis. AC: maintenance of plant in the field and botanical authentication. RCP: extraction and analysis. RSV: planning, analysis and manuscript preparation. DUB: planning, analysis and manuscript preparation.

Availability of data and materials The data and materials for this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have claimed no conflict of interest in the content of this study.

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