



## EFFICACY OF CENTELLA ASIATICA EXTRACT IN THE MANAGEMENT OF CRACKED FEET: *IN VITRO* AND CLINICAL EVIDENCE

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### ABSTRACT

**OBJECTIVE:** The aim of the study was to evaluate the efficacy and safety of a formulation, Centellin<sup>®</sup> CG foot care cream (CCFC) containing 2% *Centella asiatica* leaf extract, in the management of cracked heels through cell culture and human clinical studies.

**METHODS:** Human dermal fibroblast (adult) primary cell line and mouse macrophage cell line J774 were used to evaluate cell proliferation, collagen enhancement and wound healing ability of CCFC. For the clinical study, 24 female subjects suffering from cracked heels were enrolled to topically receive CCFC, twice daily for 4 weeks (28 days). Subjects underwent evaluation of efficacy parameters such as assessment of cracked heels, effect of skin moisturizing and photographs before and at the end of the treatment.

Subjects revisited the study site on day 7, 14, 21 and 28 for testing of efficacy parameters.

**RESULTS:** CCFC has a beneficial effect on cell proliferation, collagen synthesis and significantly improves wound healing in the fibroblast scratch wound model. A significant reduction in number of cracks was seen at the end of the study. There was significant increase in skin moisturizing and visually acceptable changes in the treatment photographs were recorded. Acceptability and compliance to the use of the formulation was good. No adverse events were reported during the study. **CONCLUSION:** The findings demonstrate that CCFC helps in healing of cracks, reducing scaling and pain. None of the volunteers

experienced any hypersensitivity reactions. Cell culture data demonstrates that CCFC facilitates wound healing process by stimulating cell proliferation and collagen synthesis. Therefore, it may be concluded that the CCFC is safe and efficacious in management of foot cracks.

**KEY WORDS:** Cell culture, *Centella asiatica*, cracked heels, skin barrier, skin physiology, wound healing.

## INTRODUCTION

Cracked heels, also known as heel fissures, are a common foot problem characterized by yellowing of skin on the heel of the foot; callused, hard skin growth, flaky patches, cracked and peeling skin. Leading causes include standing for prolonged periods of time (specifically on hard floors), use of open-heeled shoes which cause the heel to expand and increase pressure and medical conditions like diabetes which facilitate dry skin formation. Skin conditions such as psoriasis and eczema may also cause cracked heels and feet. Obesity can also increase the chances of cracks by putting more pressure on fat pads under the heel thereby causing it to expand. Feet that are constantly in close contact with water tend to lose their natural oil and turn dry and rough. In extreme cases, cracked heels can get infected and lead to cellulitis <sup>[1]</sup>.

Herbal extracts have played a vital role in numerous cultures for the prevention and management of various health conditions. *Centella asiatica* is a perennial herbaceous creeper which belongs to the *Umbelliferae (Apiceae)* family and has extensively been mentioned in Ayurveda. The active constituents in this herb include triterpenoid saponins, flavanoids, volatile oils, amino acids, phytosterols, sugars and tannins <sup>[2]</sup>. The extract used in the current study is Centellin<sup>®</sup> CG, standardized to contain 40% triterpenoidal saponins which are made up of constituents like asiaticosides, centellosides, madecassoside, thankuniside, isothankunic acid, centellose, asiatic acid, centellic acid, madecassic acid, brahmoside, brahminoside and brahmic acid.

Research articles published in recent times have substantiated the use of *Centella asiatica* in various conditions including wound healing. *In vivo* studies report enhanced cell growth, collagen synthesis around wound sites and quicker epithelialization. A specific component of *Centella asiatica*, asiaticoside, is reported to increase the tensile strength of skin that newly forms around wounds and even stimulate angiogenesis <sup>[3]</sup>.

Considering the extensive traditional knowledge and modern scientific validation around this herb, in the current study it is proposed that Centellin<sup>®</sup> CG foot care cream (CCFC) containing 2% *Centella asiatica* leaf extract provides significant relief to patients suffering from cracked heels within 28 days of use. Additional *in vitro* data has been reported in this study to validate the mechanisms involved.

## MATERIAL METHODS

### Formulation Details

CCFC has the following constituents (%w/w): Purified Water-83.30; Disodium EDTA-0.05; Carbomer-0.20; Glyceryl stearate & PEG100 stearate-3.00; Cetyl alcohol-2.00; Isopropyl Palmitate and Pentaerthirityl Tetraisostearate-2.00; Nonionic Emulsifying Wax-3.00; Caprylic Capric triglycerides-2.00; Pentaerthirityl Tetra-di-t-butyl hydroxyhydrocinnamate-0.20; Centellin<sup>®</sup> CG-2.00; Amino Methyl Propanol-0.10; Cyclopentasiloxane, Dimethiconol, Dimethicone Crosspolymer and Phenyltrimethicone Blend-0.75; Tocopheryl Acetate-0.20; Phenoxy Ethanol & Ethyl hexyl Glycerin-0.80; Fragrance-0.40.

### *In vitro*

#### *Sample preparation*

Stock solutions of CCFC were prepared in dimethyl sulfoxide (DMSO) at 2 mg/ml. Serial dilutions of the stock was prepared to obtain the desired final concentrations.

#### *Cell culture*

Human dermal fibroblast (adult) primary cell line (HDFa) and mouse macrophage cell line J774 were purchased from American Type Culture Collection (Rockville, MD). The cells were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/mL of penicillin and streptomycin in 5% CO<sub>2</sub> at 37°C.

#### *Cell viability/proliferation*

Cell viability was determined by Sulforhodamine B (SRB) assay<sup>[4]</sup>. Cells were seeded at a density of  $5 \times 10^3$  cells/well in DMEM with 10% FBS in 96 well plates and allowed to form a monolayer in a humidified incubator at 37°C and 5% CO<sub>2</sub> atmosphere for 24 hours. Cells were treated with different concentrations of CCFC for 72 hours. Cell monolayers were fixed with 10% (w/v) trichloroacetic acid and stained for 30 min with SRB, after which the excess dye was removed by washing repeatedly with 1% (v/v) acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution for OD determination at 510 nm using a

microplate reader (TECAN Ltd, Männedorf, Switzerland). Cells in DMEM with 0.1% DMSO were used as positive control while media without cells were used as negative control. The results were expressed as percentages of control.

#### ***Collagen enhancement assay***

HDFa cells were cultured as monolayer at  $5 \times 10^3$  cells/well in DMEM with 2% FBS in 96 well plate and treated with non toxic concentrations of CCFC for 72 hours. The culture supernatant was collected to estimate collagen concentration. Cell layers were extensively washed with phosphate-buffered saline (PBS) three times and then fixed with Bouin's fluid for 1 h at room temperature (RT). Collagen fibers were stained with 1% Picrosirius red in saturated aqueous solution of picric acid at RT for 1 h. <sup>[5,6]</sup> The stained cell layers were extensively rinsed three times with 0.01 N HCl to remove non-bound dye. Cells were lysed with 0.1 N NaOH solutions and placed on a microplate shaker for 30min at RT. The optical density at 540 nm was measured with a microplate (TECAN Ltd, Männedorf, Switzerland) to quantify the collagen <sup>[7]</sup>. The percentage collagen content (%) was calculated as  $100 \times (\text{absorbance of treated sample} / \text{absorbance of control})$ .

#### ***Wound healing assay***

HDFa cells were seeded at a density of  $5 \times 10^4$  cells/well in DMEM 2% FBS in 24 well plate to form a monolayer of fibroblasts <sup>[8]</sup>. When the cells were confluent, artificial wounds were created in the monolayer by making a linear scratch in the centre of each well using the tip of a sterile 200  $\mu$ L plastic pipette tip. The cellular debris created from the scratch was removed by gently washing the wells with PBS. The wounded cells were treated with the test substances at different concentrations. Complete media and media supplemented with 0.5% DMSO were used as control. Photomicrographs were taken after 24 hours using phase contrast microscope to record wound closure. The culture supernatant was collected to estimate cytokine concentration. The cells were then fixed in 4% formaldehyde and stained with 1% crystal violet. The images were captured and area of wound closure was quantified using Image-J software.

#### ***Enzyme-linked immunosorbent assay (ELISA)***

The culture supernatant from the wound healing assay was used to estimate the levels of transforming growth factor (TGF)- $\beta$  and fibroblast growth factor as per instructions by the manufacturer.

### ***Statistical analysis***

Statistical analysis data are expressed as mean  $\pm$  standard deviation. Differences in measured variables between experimental and control group were assessed by two-tailed t-test. Results were considered statistically significant at  $p < 0.05$ . Graph pad prism software was used for statistical analysis (Graph-Pad, La Jolla, CA, USA).

### **Clinical study**

#### ***Volunteer enrollment***

Female volunteers were enrolled for this open-label study after signing informed consents. Participants were informed about the cream composition and its role in treating cracked heels prior to commencement of the study. Volunteers presenting cracks/fissures in feet, especially in the heel region, associated with dryness, pain and roughness of the sole were included in the study. Volunteers were included in the study if a “YES” was indicated to all the inclusion criteria and “NO” to all of the exclusion criteria. Inclusion criteria: healthy female volunteers, 18 years of age or older with cracks in both feet. Exclusion criteria: a) History of known dermatological diseases such as Psoriasis, eczema, ichthyosis vulgaris b) History of peripheral vascular disease c) Use of topical steroids or moisturizers in the previous 2 weeks d) Known hypersensitivity to topical preparations.

#### ***Study design***

In this split controlled study, 12 participants received treatment on their right feet and 12 on the left feet, with the untreated foot of each subject serving as a control. Volunteers suffering from cracked heels visited on screening day. Subjects underwent physical examination and assessment of heels. Screening visit was followed by baseline visit (Visit 1-Day 0) wherein the subjects, based on the inclusion-exclusion criteria, were enrolled in the study. Subjects were dispensed with study medication and were instructed to wash feet properly prior to application of the cream twice daily. Subjects were informed to apply fingertip unit of CCFC twice daily in the morning and night for a period of 28 days.

Subjects underwent assessment of efficacy parameters such as examination of cracked heels and changes in moisturising the feet. Subjects returned to the study site on Visit 2 (Day 7), Visit 3 (Day 14), Visit 4 (Day 21) and Visit 5 (Day 28). On Visit 2, Visit 3 & Visit 4 subjects were dispensed with study medication. Efficacy assessments such as decrease in the number of cracks, effect of skin moisturizing, visually acceptable changes in the treatment photographs were conducted on Visit 5 (Day 28). All subjects were followed up at weekly

intervals for a period of 4 weeks, and the symptom score evaluation was done during each follow up visit. Pain assessment was conducted at each visit. After fourth week, subjects independently rated the overall improvement on the following scale: 1-No changes, 2-Average, 3-Good, 4-Excellent, 5-Extremely Excellent.

The primary efficacy endpoint was a decrease in the number of cracks, scaling, pain and laxity of skin. The secondary safety endpoints were short and long-term safety, as assessed by the incidence of adverse events.

## RESULTS

### Cell culture data

#### *Viability and proliferation of human dermal fibroblasts*

The viability of human fibroblast cells were assessed at different concentrations, of CCFC ranging from 50 µg/ml to 0.19 µg/ml. At a concentration of 6 µg/ml the cream showed 41.8% increase in proliferation (P=0.02) while at 3 µg/ml, the increase was 38.2% (P=0.03). Significant increase in proliferative effect was observed up to 0.78 µg/ml (P<0.05). At higher concentrations minor reduction in cell viability was observed (Fig.1).

#### *Collagen synthesis in dermal fibroblasts*

Wound healing is aided by the fibroblasts migrating towards the injured area which produce collagen to increase tissue permeability. To understand the effect of CCFC on collagen synthesis, treated dermal fibroblast cells were analyzed for their capacity to synthesize collagen. The cream was found to significantly increase the collagen synthesis by 21% (p=0.02) at 6 µg/ml, while it reduced to 3.48 % at 3 µg/ml and was negligible at 1.5 µg/ml (Fig.2).

#### *Wound healing in fibroblast scratch wound model*

To ensure optimum activity of fibroblasts in wound closure, CCFC at 6 and 3 µg/ml was used in the scratch wound healing assay. The ability of the cream treated human dermal fibroblasts to cover the scratch wound compared to negative control is shown in Fig. 3. Significant increase in wound closure ( $57.3\% \pm 7.9\%$ , P=0.009 and  $35.6 \pm 6.9\%$ , P=0.015) was observed at 6 and 3 µg/ml respectively compared to untreated control. Fibroblast migration toward the wound could be clearly observed in the treated wells. The concentration of TGF-β produced in untreated control was below the detection limit. Treatment with 3 µg/mL of CCFC showed

4 pg/mL of TGF- $\beta$ , while the concentration increased to 79 pg/mL with 6  $\mu$ g/mL of the cream.

### Clinical study

A total of 24 patients were screened and enrolled into the study. No participants were excluded during screening. Out of 24 subjects between the age group of 18 to 40, 22 subjects completed the study, two subjects dropped out citing personal reasons. Subject demographics have been provided in Table 1.

There were no adverse events reported during the study and compliance to the use of the formulation was good. Nature of the skin showed profound dryness at the screening visit (Fig.4).

Significant reduction in the number of cracks and increase in the moisturization of the heel was observed at the end of the study (Fig. 5).

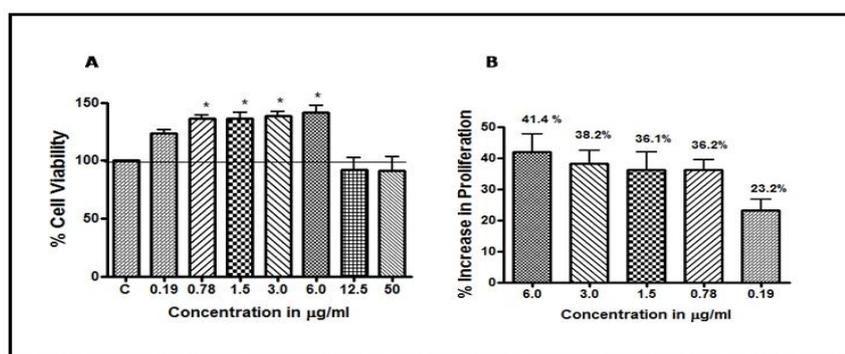
Drastic visual change was noted in the photographs taken after 4-weeks treatment (Fig.6).

Overall product rating also showed good acceptance among the study subjects (Fig.7).

**Table 1: Subject demographics**

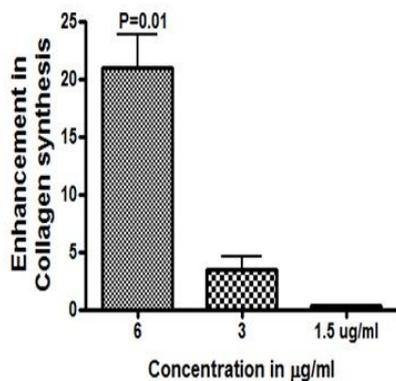
Demographics	% of Respondents
<b>Age</b>	
18-24	0
25-34	77.27
35-40	22.73

**Figure 1: Effect of CCFC on viability and proliferation of human dermal fibroblasts**



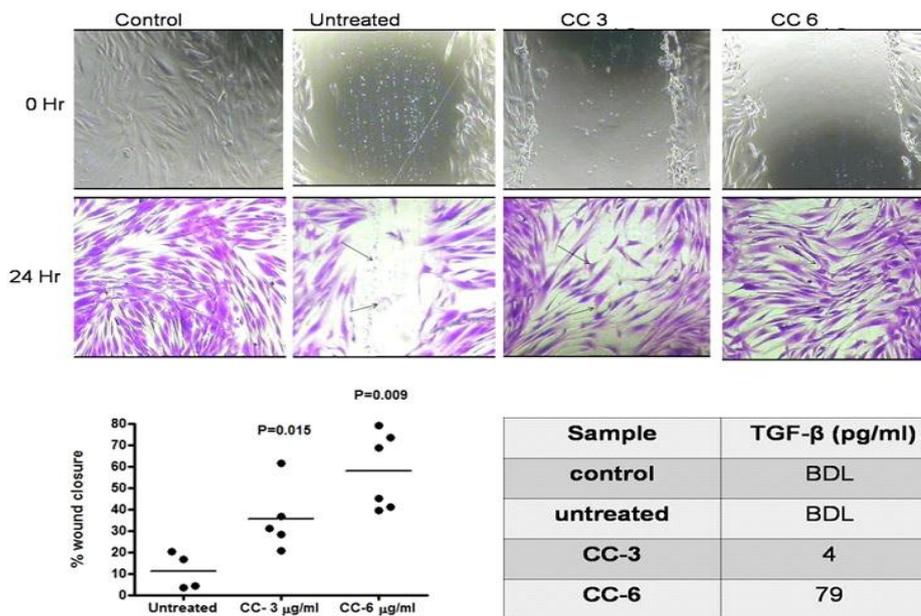
The viability (A) and proliferation (B) of human dermal fibroblast cell line (HDF-a), cells treated with CCFC (0.19-50  $\mu$ g/mL) were evaluated over the course of three days. Mild reduction in cell viability was observed at 12.5 and 50  $\mu$ g/mL. Cell viability is given as a percentage of control cells. C: Media control, standard growth medium treated cells, \* indicates comparison with control, \* $p < 0.05$ .

Figure 2: CCFC enhances collagen synthesis in dermal fibroblasts



Enhancement in collagen synthesis was determined in comparison to untreated media control over a period of 72 hours. Collagen fibers were stained with 1% Picrosirius red, and stained collagen was estimated at 540nm following cell lysis. The increase in collagen synthesis was significant (P=0.01) at 6 µg/mL

Figure 3: CCFC significantly improves wound healing in fibroblast scratch wound model



**Increased migration of fibroblasts cells treated with CCFC in scratch assay.** A) wound closure for 0h, and 24h. E: plant extract treated group (200µg/ml) wound closure for 0h, 12h and 24h. B) Quantification of Wound closure, level of significance calculated compared to untreated control. Arrows show the migrating cells towards to the scratch area. C)

Quantification of TGF-β levels in the culture supernatant

C: Control (healthy cells growing in medium), untreated: wounded cells in growth medium, CC-3: wounded cells treated with 3µg/mL CCFC, CC-6: wounded cells treated with 6µg/mL CCFC

Figure 4: Representation of skin types of subjects in the study

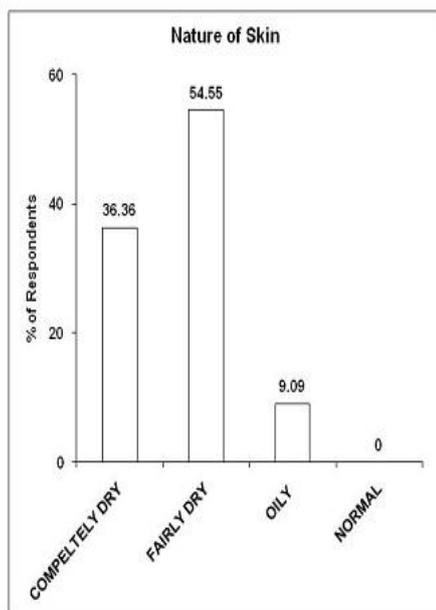


Figure 5: Effect of CCFC on the number of cracks and its moisturizing benefits

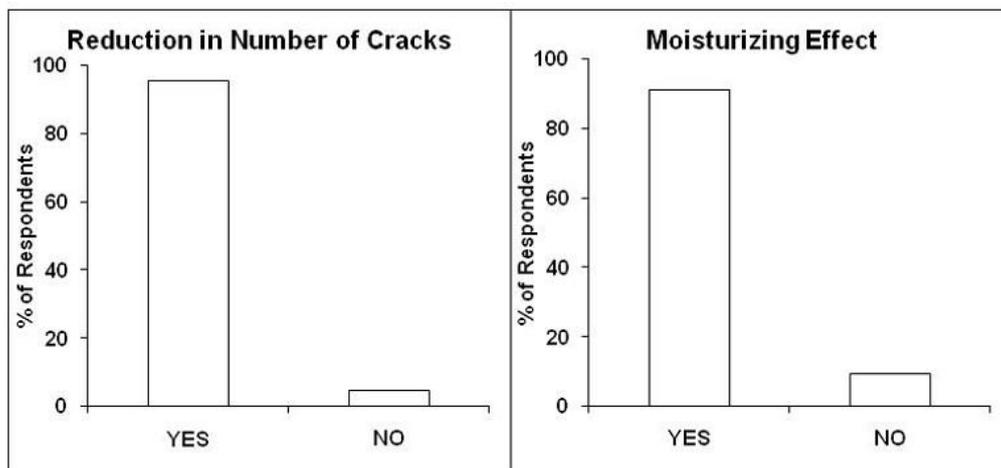
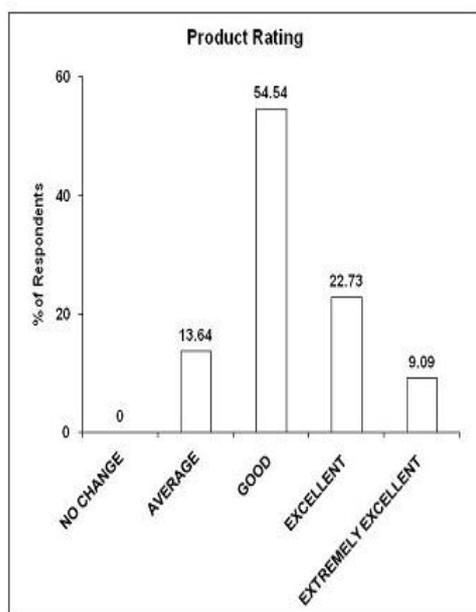


Figure 6: Comparison of the treated and untreated feet at day 28 of the study



Figure 7: Product rating by subjects



## DISCUSSION

Wound healing is a relatively complex process which can be divided into the following stages: a) coagulation and haemostasis b) inflammation c) proliferation and d) wound remodelling coupled with formation of scar tissue. Results from this study indicate that CCFC has a beneficial effect on cell proliferation and collagen synthesis in dermal fibroblasts. Collagen is one of the most abundant (25%) proteins in the body and is produced by fibroblasts. In close contact with other proteins like elastin, collagen forms a matrix to support components like sebaceous glands, blood vessels and dermal cells. Stimulation of collagen synthesis during wound healing is vital to ensure cell multiplication and new tissue formation<sup>[9]</sup>. Few other studies have also underscored the potential of this herb and demonstrated that in addition to improving cell proliferation, it enhanced DNA, protein and collagen content of granulation tissues in rats. Greater cross linking of collagen was also reported<sup>[10]</sup>. Similar results were obtained when used in a gel formulation to treat open wounds in rats<sup>[11]</sup>. A report by Shetty *et al.* indicates that *Centella asiatica* can also counter the inhibiting action that dexamethasone has on wound healing in rats<sup>[12]</sup>.

A positive effect on wound healing obtained from cell culture studies was validated by conducting an in-house clinical study. For most of the population, cracked heels are a cosmetic problem, but when they deepen, the condition can turn painful. If left untreated, in due course of time the pain can intensify and cause the cracks to bleed post which the skin is

left highly susceptible to bacterial attack. Therefore, the Centellin<sup>®</sup> CG foot care cream can have moisturizing, analgesic and collagen boosting activity which helps in healing cracks and reducing pain associated with it.

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**Conflict of interest:** None declared

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