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Original Article

Assessing the Cytotoxicity and Wound Healing Potential of Pranic Healing Colours: An *in vitro* Study on HaCaT Cell Line

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ABSTRACT

Background: Pranic Healing is a complementary therapy that uses specific colours to enhance the healing of wounds. **Objectives:** Our research sought to investigate the impacts of integrating various Pranic Healing (PH) colours on wound healing. **Materials and Methods:** HaCaT cells were subjected to cytotoxicity and scratch assays. After wound formation, Pranic colours were projected to the cells individually or in combination. The data was collected at the 24th, 48th and 72nd hr. **Results:** Cytotoxicity tests revealed PH colours were safe. Cell viability did not differ significantly between groups, according to the Kruskal-Wallis test. However, there were substantial variations in the impact that PH colours had. In contrast, a combination of colours proved more effective than single colours in promoting wound healing. **Conclusion:** The study underscores the potential impact of PH colours on enhancing cell health and promoting wound healing.

Keywords: Biofield, Complementary therapy, Prana, Wound healing, Yoga.

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INTRODUCTION

Wound healing refers to the organised process of skin recovery, which involves intricate cellular, humoral and molecular mechanisms.[1] The wound healing assay, or scratch assay, is a standard *in vitro* technique used in a variety of disciplines to investigate the migration of cells. The assay involves the formation of a monolayer wound, obtaining images during wound closure and comparing cell-migrated areas at initial and final hr.[2,3] Wound dressings are traditionally applied to protect the wound from external contamination and along with that, some therapeutic drugs can also be added to make the wound heal faster.[4] Topical application of the hormone 'insulin' on skin excision wounds can enhance the quality of wound healing and decrease the time required for gap closure.[5] Wound dressings with embedded antibiotics are valuable in treating local infections, but in some cases, high levels of antibiotics can lead to systemic toxicity. Scientists are seeking more effective ways to treat chronic and acute wound infections.

Biophoton emission, the faint light naturally emitted by all living organisms, plays a significant role in the process of wound healing and overall cellular communication.[6] These biophotons, which are light particles emitted by cells, are thought to be involved in various biological processes, including cell growth and differentiation. In the context of healing, intentional manipulation of biophoton emission, such as through the healer's intention, has been shown to influence cellular activities.[7,8] This phenomenon is leveraged in pranic healing, a modality that uses prana, or subtle energy, to promote healing. Pranic healing operates on the principle that the body's energy field, or aura, can be influenced and balanced to facilitate physical and emotional healing. Practitioners of pranic healing use different colours of prana, which have specific healing properties and can be projected onto the body to target specific ailments. PH techniques involve focusing on specific targets while projecting different colours through visualisation, known as the projection of colour prana. Advanced Pranic Healing with coloured prana is more effective and faster, with specific colours having specific effects on the body's energy centres, or chakras. For instance, green prana is used to soothe inflammation, while blue prana is used to calm and reduce pain.[9]

Pranic Healing (PH) colours have been proven effective in clinical trials for treating various conditions. The use of light red and electric violet colours in PH therapy had a significant impact on decreasing depression symptoms and enhancing mood and emotional well-being.[10] The colors green, orange and red were

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found to enhance respiratory function and quality of life in COPD patients, leading to better breathing.[11] Colour pranas were found to accelerate wound closure, lower infection rates and improve healing in diabetic foot ulcer trials.[12] Pranic healing colours have been used to increase agriculture yield.[13] and reduce anxiety in zebrafish.[14] PH colours have proven to help support different health conditions when used alongside conventional treatments. This study aims to investigate the potential effects of different PH colours on cytotoxic and wound healing using skin-based cell culture studies. Cell culture studies are essential for understanding and validating the role of complementary therapies like pranic healing in healthcare. They provide a controlled environment to investigate these therapies' cellular and molecular effects, ensuring their safety, efficacy and integration into evidence-based medical practice.

MATERIALS AND METHODS

Cell culture and reagents

The HaCaT cell line (Human Epidermal Keratinocyte Cells) was procured from the National Centre for Cell Sciences (NCCS), Pune, India. Methyl Thiazolyldiphenyl Tetrazolium bromide (MTT) and Phosphate-Buffered Saline (PBS) were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Insulin (Monocomponent biosynthetic r-DNA insulin, Torrent Pharmaceutical Ltd.) was procured from the marketplace. The HaCaT cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Himedia) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS, Gibco) and a 1% antibiotic cocktail consisting of Penicillin (100 U/mL), Streptomycin (100 µg/mL) and Amphotericin B (2.5 µg/mL). The cells were maintained in 25 cm^2 tissue culture fla16sks and incubated in a cell culture incubator set at 37°C with 5% CO_2 and controlled humidity. The entire experiment was conducted concurrently in four wells.

Cytotoxicity assay

The cytotoxic effect of the test component on the viability of the HaCaT cells was determined by MTT assay. The experimental groups consisted of the control group (media-treated), standard group (2.5 and 5 µg/mL insulin-treated) and test group (PH-treated). HaCaT cells were seeded at a density of 2×10^4 cells per well in a 96-well plate and incubated for 24 hr. The cells were then treated with different concentrations of insulin and various coloured *pranas* for PH. The following advanced PH protocol was followed.[9]

- Invoke and scan before, during and after treatment.
- Localised sweeping.
- Project energy colours to the wells.
- Stabilise.

The plate was observed at 24, 48 and 72 hr intervals following the PH treatment. MTT solution (2 mg/mL) was added after 72 hr and incubated at 37°C for 3 hr, followed by the addition of Dimethyl Sulfoxide (DMSO) to the cell culture. The MTT solution stains viable cells, while DMSO solubilizes the formed formazan. The measurement of the plate's absorbance was taken at a wavelength of 540 nm. Data were used to determine % cytotoxicity [% growth inhibition: 100 (Mean OD of individual peptide-treated group/ mean OD of control groupX100)]and % cell viability [100-(% cytotoxicity)].[15]

In vitro evaluation of wound healing activity by Scratch wound assay

HaCaT cells ($5x10^4$ cells/well) were seeded in 24-well plates and incubated at 37°C with 5% CO_2 in an incubator. After the formation of the monolayer cells, scratches were made in each well using sterile plastic tips. Any detached or debris cells were removed by washing with PBS and replaced with 1 mL of fresh medium in the absence or presence of insulin. The experimental groups comprised (a) control group, (b) standard group (Insulin, 5 µg/mL), (c) test sample group (with PH only), (d) PH+Pre-treated Insulin (5 µg/mL).

- The following Advanced PH protocol was adhered during treatment.[9]
- Invoke and scan before, during and after treatment.
- Localised thorough sweeping with Light Whitish Green (LWG).
- Project energy colours into the wells.
- Stabilise with Light Whitish Blue (LWB) and release projected energy.

The colour prana involved in healing as a single Colour were White, Light Whitish Red (LWR), Light Whitish Violet (LWV) and Electric Violet (EV). The Multi Colour were Light Whitish Orange-Light Whitish Yellow (LWO-LWY), LWR-LWY, LWO-LWR and LWG-LWO-LWR. Three healers performed PH sessions.

Following the PH treatment, the plate was incubated at 37°C with 5% CO_2. Photographs were taken on days 0 (0 hr), 24 hr, 48 hr and 72 hr. The images were analysed using Image J software.[16] The percentage of closed area was measured and compared with the value obtained before treatment (0 hr). Similarly, images were taken until the complete closure of the wound. An increase in the percentage of closed areas indicated the migration of cells.[15] Data were used to calculate wound closure (%) [% wound healing closure=(A-B)x100/A, where A is wound area at 0 hr time, B is wound area at 24 or 48 hr].

Statistical analysis

The Shapiro-Wilk test assessed normality, while the homogeneity of treatment variables was examined. SPSS-16 was utilised for non-parametric analysis, employing the Kruskal-Wallis test followed by *post hoc* Dunn's test. Additionally, *t*-tests and Mann-Whitney U tests were employed to compare PH for Wound Healing between single colours with a white shade and multiple colour prana.

RESULTS

Cytotoxic study

Different PH groups (W, LWR, LWV, EV, LWO-Y, LWR-LWY, LWO-R, LWG-LWO-LWR) and standard drug insulin (2.5 µg/mL and 5 µg/mL) were tested for toxicity on HaCaT cells at 24 hr and 48 hr (Figure 1). No toxicity was observed. The viability percentage of all treatments was more than 88.40% during the 24th and 48th hr. Among healing colours, the viability percentage of LWR at the 48th hr (99.92%) was found to be more compared to LWV, EV, LWO- Y, LWR- LWY, LWO- R, LWG- LWO- LWR.

Wound Healing

The effect of colour prana on wound healing at the 24th and 48th hr was studied. There was a significant difference (X^2=26.579, *p*=.003) found between White (Mean Rank=21.25), LWR (Mean Rank=14.25), LWV (Mean Rank=19.00), EV (Mean Rank=10.25), LWO-Y (Mean Rank=8.25), LWR-LWY (Mean Rank=14.25), LWO-R (Mean Rank=25.25), LWG- LWO- LWR (Mean Rank=31.25), Pre-treated Insulin (Mean Rank=37.25), Standard Insulin (Mean Rank=38.75) and Control treatments (Mean Rank=27.75) at 24 hr. Dunn's test showed LWO-Y was significantly less effective compared to LWG-LWO-LWR (z=2.53, *p*=.011), pre-treated EV (z=3.36, *p*=.001), standard insulin (z=3.19, *p*=.001) and control(z=2.15, *p*=.032) at 24 hr. Complimentary application of EV along with Pre-treated insulin found more healing closure than the control and LWG- LWO-LWR (Figure 2).

At the 48th hr, wound closure exhibited significant differences (X^2=27.57, *p*=.002) with various PH colors, EV pre-treated with insulin, standard insulin and the control. Significant differences were noted between EV (z=3.183, *p*=.002), LWR (z=2.30, *p*=.021) and LWO-LWY respectively with control (z=2.59, *p*=.01). These treated PH colors demonstrated lower wound closure compared to the control. 100% healing was found among LWO-R, LWG–LWO-LWR, Standard Insulin and Control treatments. Wound healing closure of control, standard and different colours of prana is provided in Figure 3.

For each group, we analysed 16 wells using either single colours or multiple colours. This was done to see how the choice of colours affected the percentage of wound closure. At 24th hr, the effect of the colour difference in PH on wound closure was not statistically significant (t=-.781, *p*=.441). However, at the 48th hr, the use of multiple colours in healing was significantly more effective (U=68, *p*=.018) in filling the wound gap. The mean wound closure percentage at the 48th hr for the multiple colours used was 96.69%, compared to 86.54% for the single colour used (Figure 4).

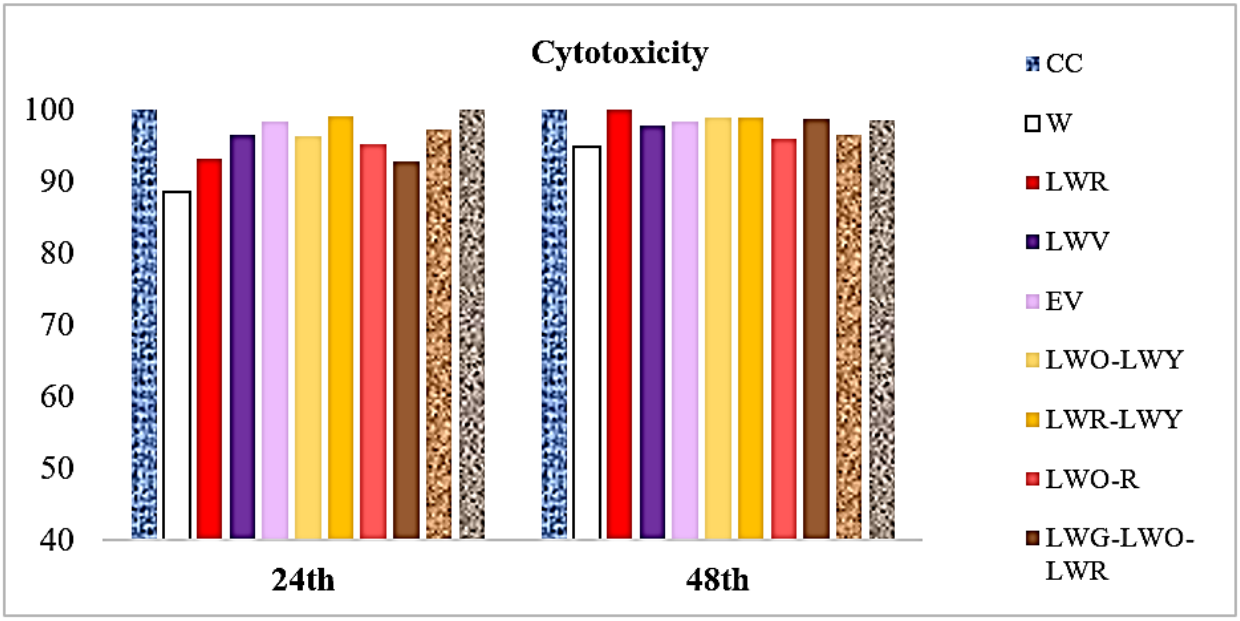

Figure 1: Results of cytotoxic effect of Pranic Healing colours on the viability of the HaCaT cells using MTT assay. CC: Control Treatment, W: White, LWR: Light whitish Red, LWV: Light whitish Violet, EV: Electric Violet, LWO-Y: Light Whitish Orange-Yellow, LWR-LWY: Light Whitish Red-Light Whitish Yellow, LWO-R: Light whitish Orange-Red, LWG-LWO-LWR: Light Whitish Green-Light Whitish Orange-Light Whitish Red, Ins5-Insulin 5 µg, Ins2.5-Insulin 2.5 µg.

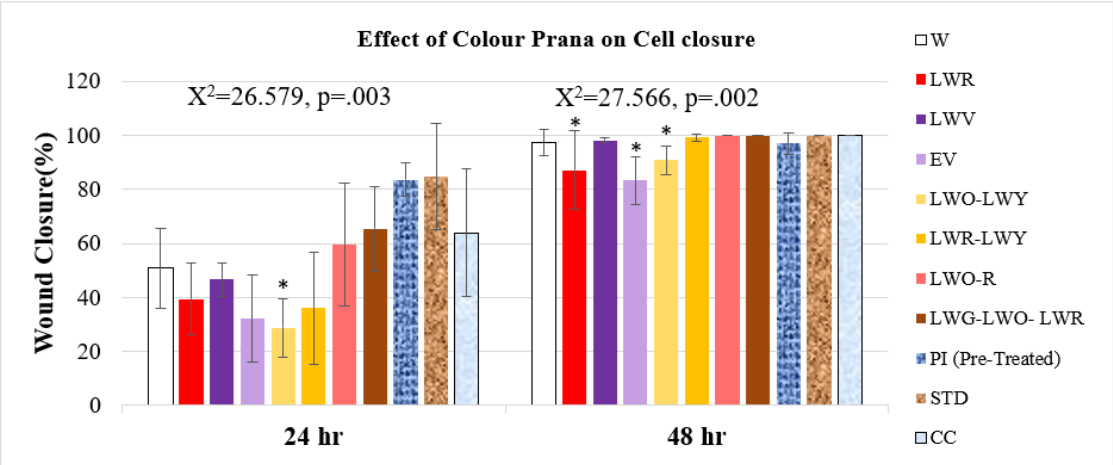

Figure 2: Colour Prana's Impact on Wound Closure using Scratch Wound Assay on HaCaT Cells. W: White, LWR: Light whitish Red, LWV: Light whitish Violet, EV: Electric Violet, LWO-Y: Light Whitish Orange - Yellow, LWR-LWY: Light Whitish Red-Light Whitish Yellow, LWO-R: Light whitish Orange-Red, LWG-LWO-LWR: Light Whitish Green- Light Whitish Orange- Light Whitish Red, PI (Pre-Treated with EV)- Insulin (5 µg/mL), STD- Standard Insulin (5 µg/mL), CC- Control Treatment.

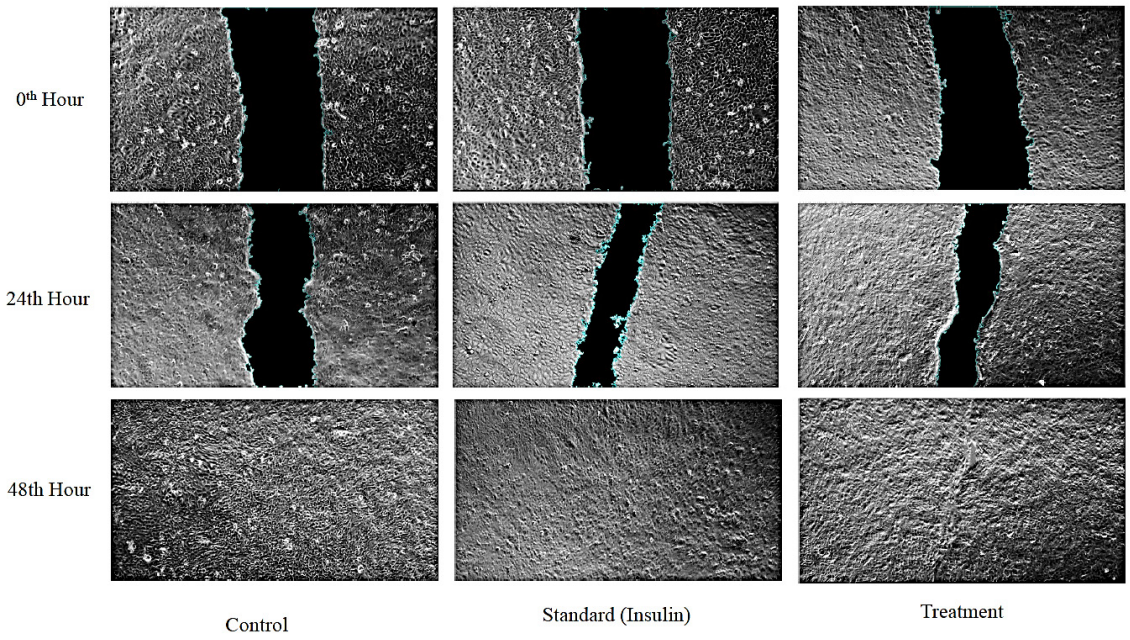

Figure 3: Scratch Wound Assay on HaCaT Cells at different time points.

DISCUSSION

The study found that PH was not harmful to cells and different coloured prana affected wound healing in HaCaT cells compared with the control group. Integration of multiple colour prana was found to be more effective in wound closure than single colour prana. A study showed that administering biofield healing treatments to cells from a close range, both before and after exposure to hydrogen peroxide, resulted in significant variations in cell death rates between the treatment and control groups.[17] External Qi from Yan Xin in Qigong was used to treat fibroblasts, resulting in a short-term activation of ERK and Pkb. This treatment had no negative effects on normal cells or cancer cells.[18] Furthermore, Healing Touch may enhance the immune system by slightly increasing natural killer cell cytotoxicity.[19] Therapeutic touch treatments were also found effective in increasing proliferation in cells.[20]

PH was safe for cells in the trial, with no significant difference in cytotoxicity compared to the control group. Collectively, these findings suggest PH might positively contribute to cellular health. Cytotoxicity studies are essential in pranic healing research. They validate the science, ensure safety, measure effectiveness, optimise techniques, understand health benefits and promote integrative medicine.

In the wound healing area, the influence of different colours of *prana* on the speed of wound closure was observed at the twenty-fourth hr. It was found that certain colours, such as

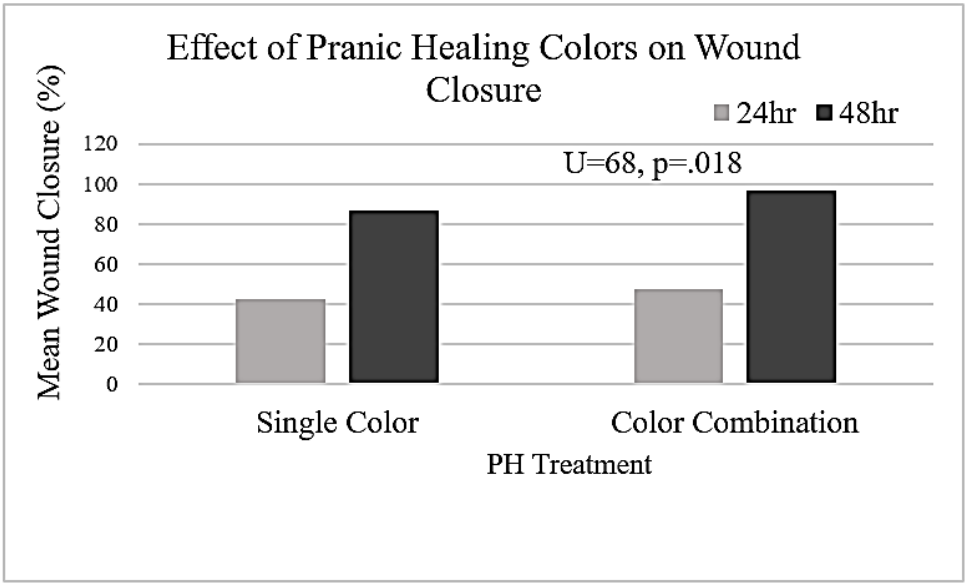

Figure 4: Varied Effects of Pranic Healing Colours on Wound Closure Using the Scratch Wound Assay on HaCaT Cells.

LWO-LWY, exhibited significantly slower healing rates compared to the control group. Interestingly, at the 48 hr, the wound closure percentage for LWO-LWY surged to 90.91%, indicating a remarkable enhancement in healing compared to earlier stages. On the other hand, Electric Violet, often associated with divine energy, showed a lesser enhancement (83.34%) in wound closure at 48 hr. Ordinary energy like LWO-LWY showed substantial healing. The EV colour yields modest improvements, reinforcing Master Choa Kok Sui's preference for ordinary prana in skin treatments. While highly intense, the EV colour prana might be overly potent for skin cells. It is found to potentially suppress cancer cells.[9,21] Moreover, LWO-R and LWG-LWO-LWR found complete healing of the wound at the 48th hr. These results support therapeutic touch on wound area contraction and fibroblast proliferation in rat skin.[22] White blood cells involved in the immune system were increased after Therapeutic Touch treatment.[23]

Repeatedly applying intentional healing to a specific area could potentially boost cell growth. However, external factors may introduce variations in the number of cells observed on different days.[24] The mean rank of the control was less than the LWG-LWO-LWR, pre-treated insulin with PH and positive controls were higher. However, there was no significant difference in the wound area after 24 hr. By 48hr, the healing process was completed in LWR-LWY, LWO-R, LWG-LWO-LWR, STD and CC treatments.. All treated wells had complete closure at 72 hr. Studies investigating *pranic* or *chi* energy perception have assessed the experiential impacts of prana and its therapeutic effects on wound healing in human subjects.[12,25] In this context, the observable influence of PH was apparent, although support from bodily systems was lacking. The interaction of the cell with colour prana is also influenced by the intention of the healer.[26] Pranic Healers focus their intention on projecting *prana*, different colours of prana affected differently. For analysing hand emissions, gelatin filters split light into blue, green, yellow and red bands. Studies on biophoton emissions have shown the emergence of varying intensities and colours of light from hands. External factors, like seasons such as summer and autumn, can impact the strength of these emissions.[27] They are naturally found in the sun, ground and air and can aid in wound healing as a complementary therapy. In the 24th hr, combining EV, along with pre-treated insulin, demonstrated enhanced wound closure compared to the control group. Further investigation is warranted to explore the potential mechanism behind wound closure.

CONCLUSION

The study showed that applying PH colour-based treatment did not have any negative effects on skin-based cell viability, proving that it is safe. The research highlights PH's notable impact on wound healing in HaCaT cell line. Multi-colour prana exhibits greater effectiveness in enhancing wound closure compared to single-colour prana.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

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ABBREVIATIONS

COPD: Chronic Obstructive Pulmonary Disease; **CO_2:** Carbon-di-oxide; **DMEM:** Dulbecco's Modified Eagle's Medium; **DMSO:** Dimethyl Sulfoxide; **ERK:** Extracellular Signal-Regulated Kinase; **EV:** Electric Violet; **FBS:** Fetal Bovine Serum; **HaCaT:** Human Epidermal Keratinocyte Cells; **LWB:** Light Whitish Blue; **LWG:** Light Whitish Green; **LWO:** Light Whitish Orange; **LWR:** Light Whitish Red; **LWV:** Light Whitish Violet; **LWY:** Light Whitish Yellow; **MTT:** Methyl Thiazolyldiphenyl-Tetrazolium bromide; **NCSS:** National Centre for Cell Sciences; **OD:** Optical Density; **PBS:** Phosphate-Buffered Saline; **PH:** Pranic Healing; **PKB:** Protein Kinase B; **SPSS-16:** Statistical Package for the Social Sciences Version 16.

ETHICAL APPROVAL

The study was approved by the Independent Ethics Committee-World Pranic Healing Foundation, India Dated 3/1/2023 with a letter with Ref no 4/2022/29/12/2022.

SUMMARY

Pranic Healing colour-based treatment is safe for skin cells, with no harmful effects. The study demonstrates PH's positive impact on wound healing in HaCaT cell line. Multi-colour prana is more effective in promoting wound closure than single-colour prana.

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