Human Mesenchymal Stromal Cells-Derived Conditioned Medium Based Formulation for Advanced Skin Care: in vitro and in vivo Evaluation

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Abstract

Photo aging of the skin is primarily due to alterations in the dermal extracellular matrix. Apart from physiological aging, some environmental factors accelerate the skin aging and are characterized by wrinkles, laxity, roughness and irregular pigmentation. Mesenchymal stromal cell derived growth factors and cytokines are used in cosmetic products because of their ability to repair and regenerate the damaged skin. In the present study, Conditioned Medium (CM) derived from human mesenchymal stromal cells was produced, formulated and the CM based formulation was evaluated for anti-aging potential and skin rejuvenation. The particle size analysis and HPLC analysis revealed that the proteins were dispersed in a nano-size range in the concentrated conditioned medium. The total protein content analysis and bioassay results showed that the proteins were stable in the pH range of 5.0 to 8.0. The in vitro scratch wound assay and cyclobutane pyrimidine dimer formation assay of the CM based formulation promoted the migration of cells and resulted in significant decrease in the formation of CPD respectively when compared to untreated cells. The histological examination of nude mice sections revealed that treatment with CM based formulation reduced the extent of hyperkeratosis, epidermal hyperplasia and inflammatory cells in the dermis, improved skin moisture level and showed lesser collagen degradation when compared to animals exposed to UVB irradiation. These results suggest the photo protective effects of CM based formulation and its potential in reducing the UV induced photoaging of the skin.

Introduction

Ageing is defined as a physiological process of growing older and it represents a series of functional changes in humans over time [1]. Aging of the skin at the cellular level is accelerated by exposure to environmental toxins as well as exposure to UV radiation. As a protective external covering of the body, the skin tolerates various physical and mechanical stresses while protecting the internal organs from microbial invasions [2]. This protective function afforded by skin is affected by the aging process [3]. The aging of the skin leads to a reduction in the firmness of skin with the onset of wrinkles [4,5].

Chronic exposure of human skin to solar UV radiation is known to damage the structure and function of the skin [6,7]. These changes are collectively known as photoaging, which is characterized by wrinkles, laxity, roughness and irregular pigmentation [8]. Ultraviolet radiation, especially UVB (280-320 nm) from sunlight, is one of the major environmental hazards to induce skin damage [7]. UV exposure can cause edema, erythema, hyper pigmentation, photoaging, inflammation, DNA damage and cancer in the skin [8]. Wrinkles are an outward sign of cutaneous aging appearing preferentially on Ultraviolet B (UVB) exposed areas.

Products and therapies that can reduce (or) block (or) reverse this aging process are very important materials for research & product development. Mesenchymal stem cells are multipotent cells, which are used in many fields of regenerative medicine. The stem cells of mesenchymal origin are widely reported to secrete a wide range of growth factors/cytokines is not only used in regenerative medicine, but also its potential role as a cosmetic ingredient is being explored. Numerous studies have investigated the stem cell derived CM enriched in growth factors/cytokines is not only used in regenerative medicine [9], but also its potential role as a cosmetic ingredient is being explored. Numerous studies have investigated the stem cell derived CM as a skin moisturizing and anti-aging agent. Kwon et al., have reported that the conditioned medium from human bone marrow-derived mesenchymal stem cells promotes skin moisturization and effacement of wrinkles [11]. Liu et al., showed that umbilical cord mesenchymal stem cells CM protects against photo aging induced by UVA and UVB radiation and is a promising candidate for skin anti-photo aging...
treatments [12]. Sohn et al., demonstrated the anti-aging properties of CM of epidermal progenitor cells derived from mesenchymal stem cells [13]. Moreover, CM derived from various stem cells have been used in anti-aging formulations and hair growth promoting cosmetic formulations [13-15]. Therefore, this study aimed to develop the BM-MSC derived CM based formulation and elucidate its UV-protective effects. Comprehensive analysis of mesenchymal stromal cells derived CM was carried out and it was formulated as a topical formulation and tested through validated in vitro and in vivo models for its potential use in the area of cosmetics. Growth factors and cytokines present in CM were enriched by concentrating the CM through tangential flow filtration by tenfold (10X CM) to get a better biological effect of the CM.

Materials and Methods

Mesenchymal stem cells derived from the bone marrow of healthy donors (Stempeutics Research Pvt Ltd, India), DMEM-KO (GIBCO, Carlsbad, USA), FBS (HyClone, Waltham, MA, USA), bFGF (Sigma-Aldrich, St. Louis, Missouri, USA), 1× Glutamax™ (GIBCO, Carlsbad, USA), VEGF ELISA kit (R&D Systems, SVE00), TGF-β1 ELISA kit (R&D Systems, SB100B)

Mesenchymal stem cell culture and conditioned media preparation

Bone-marrow-derived mesenchymal stem cells from three healthy human volunteers were pooled in equal proportions and cultured using 1X DME-KO medium supplemented with 2 ng/ml bFGF, 1X Glutamax and 10% FBS. After allowing sufficient time for cell growth and expansion, the growth medium (conditioned medium) was collected from the tissue culture flask, filtered and concentrated using the tangential flow filtration system (Merck-Millipore, NJ, USA). The concentrated medium was stored in cold storage (-80°C) until further evaluation.

Particle size analysis

The concentrated CM was analyzed for particle size and zeta potential measurement by dynamic light scattering method (Malvern Zetasizer, Malvern, UK) at 25°C.

HPLC qualitative analysis of conditioned medium

The concentrated conditioned medium was qualitatively analyzed for the presence of growth factors using isocratic HPLC method (Shimadzu, LC- 2010 CHT, Kyoto, Japan) at 214 nm. The chromatographic conditions used for the analysis of CM were Luna C18 (250 mm × 4.6 mm, 5 µm) column with pH 3.1 phosphate-buffered solution with acetonitrile and methanol mixture as a mobile phase. The flow rate was kept at 1.0 mL/min and the column temperature was maintained at 25°C.

Analysis of growth factors

The growth factors and cytokines present in the CM were previously reported by our research group [10]. The conventional indirect ELISA and Millipore Luminex Multiplex method were utilized for identification of different growth factors and cytokines in the CM.

Pre-formulation studies of concentrated CM

The pre-formulation studies for the CM concentrate were carried out to find out the suitable pH level for the formulation development. To confirm the pH suitability for the formulation development, the concentrated CM was pH varied from 3.0-9.0. This pH varied sample was analyzed for the total protein content by Bradford method at 595 nm spectrophotometrically. Simultaneously, the pH varied concentrated CM was analyzed for the VEGF and TGF-β content by the conventional ELISA method.

Formulation development

The CM concentrate was used for preparing the semi-solid serum formulation. Xanthan gum was used as a base polymer in the serum formulation. First, the polymer xanthan gum was dissolved in purified water and other excipients were (glycerin, PEG, Euxyl PE and Optasense) added subsequently and mixed to form a serum formulation. Finally, the concentrated conditioned medium was added and mixed for 10 min to get a homogenous final active formulation. The prepared formulation was stored in a cool, dark environment, away from direct light and was subjected to various in vitro and in vivo evaluations.

Migration assay

Human dermal fibroblast (HFF-1) was used for this study. The HFF-1 cells were seeded at a density of 2×10⁵ cells/well in a 6 well plate in media supplemented with DMEM and 15 % FBS. After plating, the cells were maintained in a CO₂ incubator (Binder, Germany) at 37°C, 5% CO₂ and 95% humidity until they were approximately 90% confluent. The cells were then serum starved with 1X DMEM + 0.1% FBS for 24 h. After 24 h post serum starvation, a straight line scratch (wound) was created in the middle of cell layer using the tip of sterile 200 µl pipette tip. The cell monolayer was washed twice using 1X DMEM followed by treatment with 0.25 %, 0.5% and 1% of the formulation prepared using concentrated CM and incubated in a CO₂ incubator at 37°C, 5% CO₂ and 95% humidity for 24 h. Images of the scratch/wound were taken at 0 h, 24 h and 48 h at three points in a single well and distance of wound closure were measured using Image J software.

Cyclobutane Pyrimidine Dimers (CPD) assay

HFF cells were seeded at a density of 2×10³ cells / well in a 96-well culture plate in 1X DMEM containing 15% FBS and incubated overnight in a CO₂ incubator (Binder, Germany) at 37°C, 5% CO₂ and 95% humidity. After overnight incubation, HFF-1 cells were serum starved with 0.1% FBS containing 1X DMEM medium and incubated in a CO₂ incubator and after 24 h post serum starvation, cells were photo-damaged by UVB irradiation (300 mJ/cm²). The UVB exposed cells were treated with 0.25%, 0.5% and 1% of formulation for 48 h. The percentage decrease in CPD formation in formulation treated irradiated HFF-1 cells was calculated and compared with UVB irradiated photodamaged cells. An ELISA kit (Cell Biolabs, Inc., Catalog Number: STA-326) was used for the analysis.

In vivo evaluation of the formulation in nude mice model

The present study was conducted to evaluate the efficacy of the test formulation against pathophysiological changes induced by UVB irradiation in nude mice (Study no. ES 098/13). The in vivo evaluation of prepared formulations was tested in 6-8 week old nude mice (NU/NU). The animals were divided into different groups (n=4/group). All mice, except the ones from the control group, were irradiated with UVB (UVB emitting system - Sankyo Denki G1578 E, Japan) at a...
dose of 150 ml/cm², once daily, for seven days. The animals were kept at a fixed distance of 35 cm from the light source and the irradiation intensity at the skin surface was measured using a UV light meter (Lutron-340A). Irradiation procedure was carried out in aseptic conditions every day. Immediately post UVB irradiation, the test formulation was topically administered to the entire dorsal back of each animal in the respective groups. Since UVB exposure leads to pathophysiological changes in animal skin, all the animals were closely observed for the appearance of wrinkles; the roughness of the skin; loss of water (moisture) and erythema (redness). Hydration of the skin and Erythema Index (E.I.) was measured using Corneometer MPA-5 and Mexameter MX-18 (Courage + Khazaka, Cologne, Germany) respectively each day before UVB exposure. UVB exposure leads to microscopic changes such as an increase in epidermal and dermal thickness; infiltration of inflammatory cells; hyperkeratosis along with a decrease in the amount of dermal collagen. Hence, after completion of irradiation schedule, the skin of all animals was excised and subjected to histological analysis. Hematoxylin and Eosin (H&E) stained sections were analyzed for microscopic changes such as hyperkeratosis, infiltration of inflammatory cells in the dermis and epidermal and dermal thickness. In addition, the collagen content in the dermis was evaluated by Masson Trichrome staining procedure. The sections were visualized by light microscopy and photomicrographs were captured.

Statistical analysis

All the data analysis was performed using Graph Pad Instat (Trial Version). Student’s t-test was used for statistical analysis and significance. The P-value less than 0.05 were considered statistically significant.

Results and Discussion

Particle size analysis of concentrated conditioned medium

The particle size analysis of CM concentrate showed that the dispersed particles were in the nano-size range (18.94 nm). The PDI (0.574) showed that the CM contains uniform-sized particles in the dispersion (Figure 1). The zeta potential measurement (-10.4 mV) using the Malvern Zetasizer revealed that the proteins and peptides in the CM were stable at the storage conditions.

Qualitative analysis of growth factors present in the concentrated conditioned medium

The HPLC method was developed to detect the proteins present in the stem cell-derived CM. The CM contains several cells derived growth factors and cytokines dispersed in the growth medium. To confirm several proteins in dispersed form in the CM, the isocratic HPLC method was developed, and the protein peaks were detected at 214 nm. The HPLC analysis showed many sharp peaks in the chromatogram (Figure 2). This analysis qualitatively confirms that there were many proteins dispersed in the CM in a stable form. The confirmation of many dispersed protein in the medium by this qualitative HPLC analysis was further supported by highly accurate ELISA bio-assays.

Analysis of growth factors

Our research group [10] used the conventional ELISA method and multiplex method for the evaluation of growth factors and cytokines in the concentrated CM. Overall 28 analytes, that include growth factors and cytokines, were determined from the concentrated samples of CM of mesenchymal cells compared to control media. The angiogenic factors like Angiotenin (ANG) Angiopoietin (AGPT-1) were detected in significant quantity in the CM when compared to the control medium. The Bone Morphogenic Protein (BMP-6) was comparatively high in the CM and there was no increase in the BMP-2 quantity in the stem cell-derived CM. The cytokines and chemokines like macrophage CSF (M-CSF), Interleukin (IL6), Transforming growth factor beta (TGF-β), MIF and IL11 were found to be in significant quantities in the CM. Metalloproteases MMP1, MMP2 and MMP9 were tested along with their natural occurring inhibitors TIMP1 and 2. It was found that MMP1, MMP2, TIMP1 and TIMP2 were significantly expressed in the CM. MMP9 an inflammatory marker was found to be suppressed in the CM. There were a significant increase in the quantity of growth factors like Fibroblast Growth Factor 7 (FGF7), Hepatocyte Growth Factor (HGF), SDF1, Fibroblast Growth Factor 2 (FGF2), Vascular Endothelial derived Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF-AA), RANTES and Leukemia Inhibitory Factor (LIF) in the CM compared to the control medium. The extracellular protein such as collagen and laminin were measured in the CM and found that the laminin was significantly increased than the collagen.
The analysis provides evidence that the bone marrow mesenchymal stem cell-derived CM contains many secreted cytokines and growth factors dispersed in the solutions. The cell signaling factors like FGF7, IL6, IL11, TGF-β, and PDGF are important in wound healing process. The PDGF, VEGF, HGF, M-CSF etc. are important in hair growth. The Angiopoietin, PDGF, IL-6, TGF-β, M-CSF, VEGF, Laminin etc. are important for skin health. Due to the presence of various cytokines and growth factors in the CM, the CM might be an important bioactive material in the area of regenerative medicine [10].

Pre-formulation studies of concentrated CM

In order to validate secretome profiling of MSC derived CM, we initially performed proteomic profiling of CM using human specific ELISA kits and bead based multiplex method and the data of the same is already reported [10]. It is in corroboration with the cytokines/growth factors identified in the CM derived from MSC cultures published by other researchers [13,16-19]. Since the aim of the present study was to evaluate anti-aging potential of human MSC derived CM formulation, we specifically selected two of the widely studied growth factors VEGF and TGF-β which are important for maintaining healthy skin. The activity of these factors ranges from endothelial cell proliferation and migration, angiogenesis and microvasculature, epithelialization, anti-inflammation, ECM remodeling and stimulate collagen synthesis, inhibition of melanin synthesis, all of which are important for skin regeneration, wrinkle effacement, moisturization and cytoprotection. Moreover, we have quantified the level of these growth factors in many of the manufacturing batches (skin care formulation batches) to show the consistency in its expression. The pre-formulation studies of the concentrated CM were carried out under different pH conditions. The total protein content was found to be stable from pH 3.0 to 8.0 (25.36 to 32.80 mg/ml), whereas a sharp decrease was observed at pH 9.0 (7.2 mg/ml) (Figure 3a). The VEGF level was high in pH 5.0 to 7.0 (27.47 and 31.37 ng/ml) and a sharp decrease was observed in pH 9.0 (1.52 ng/ml) (Figure 3b). The TGF-β showed moderate stability in the pH range from 4.0 to 8.0 (10.33 to 11.97 ng/ml) and it was reduced in acidic pH (6.75 ng/ml) and not detected in pH 9.0 (Figure 3c). This experiment revealed the protein stability at different pH conditions and found that the proteins are bound to be stable in the pH range of 5.0 to 8.0.

In vitro analysis of conditioned media based formulation

The effect of CM based formulation on the migration of Human dermal Fibroblast cells (HFF-1) was studied using scratch wound assay. The assay showed that the formulation-treated wound was closed faster and completed in the 48 h time interval. The migration of the HFF cells was not affected by the CM based formulation. This scratch wound assay experiment revealed that the stem cell CM based formulation was non-toxic and promotes the migration of cells towards the wound area to cover up the wounds (Figure 4). The percentage of cell migration after 24 hrs was found to be 90±4.24, 86±3.07 and 81±3.24 at concentration of 0.25%, 0.5% and 1% respectively when compared to control (62±2.38). Due to various important growth factors and cytokines present in the CM, the HFF-1 cell migration was triggered towards the wound area and the wound closure was comparable to the control. Thus, the CM and its growth factors are compatible with the human HFF-1 cells. The in vivo wound healing is a complex process involving many coordinated events [20,21]. The exogenously provided enriched CM may accelerate the wound healing process due to the presence of various wound healing growth factors like KGF/FGF7, IGF-1, IL6, IL11, TGF-β and PDGF [9, 10, 15].

The ability of the CM formulation to decrease UVB induced CPD formation was evaluated in photodamaged (UVB dose; 300 ml/cm²) fibroblast cells after 48 h as compared to irradiated untreated control cells. Pyrimidine dimers are DNA lesions formed from thymine/ cytosine bases in DNA via photochemical reactions on UVB exposure. The effect of the formulation at different concentrations (0.25%, 0.5% and 1%) was evaluated on the UVB irradiated photodamaged cells and the percentage decrease in CPD formation was compared with the UVB-irradiated untreated cells. The results showed that the CM based formulation led to a decrease in the CPD formation in the UVB irradiated cells compared to the untreated UVB irradiated cells. The formulation protected the cellular DNA from UV irradiation and reduced the CPD formation (Figure 5). The CM formulation showed higher protective effect at the concentration of 0.5% followed by 0.25% (42±3.59 and 33±3.68 decrease in CPD respectively) when compared to 1% (23±4.21). But all the formulation concentrations showed a comparatively higher protective effect than the control UVB irradiated cells (18%±2.42). The unprotected UV rays are known to cause mutation in the DNA that may lead many pathological
conditions such as skin cancers [22]. The CPD is a photoproduct of the damaged DNA and it is one of the most causative agents for primary lesions in UV-irradiated DNA [23]. The CPD assay confirms that the CM growth factors and the formulation were useful in preventing the CPD formation in the cells by UV irradiation.

This decrease in skin hydration manifested in the form of substantial extent of skin roughness as observed on day 8th of animals in UVB treated animals. The mean corneometer readings of the formulation-treated group ranged from 47.8±3.2 to 37.4±2.5corneometer units. A comparison of corneometer units on day 1 and day 8 revealed that there was a 72.32% decrease in skin moisture content in UVB treated group. But, in the CM formulation-treated group, the skin moisture reduction was only 21.75% (Figure 7). The study showed evidence that the UVB exposure led to decreased moisture capacity in the skin of the animals. However, the formulation gave protection (69.92%) against the UVB rays and significantly decreased the skin moisture reduction from the UVB exposure.

In vivo evaluation of conditioned media based formulation

In this study, the role of test formulations in reducing the degradative changes in the skin upon exposure to UVB radiation was investigated in nude mice. Nude mice do not have any fur on the skin (hairless). They are highly susceptible to photodamage by UVB radiation. The damage instilled in the form of erythema and appearance of wrinkles is easily visible on the animal skin. The degree of protection of the CM formulation was assessed using macroscopic (wrinkles, the roughness of the skin, loss of water and erythema) and microscopic (increase in epidermal thickness, infiltration of inflammatory cells and hyperkeratosis) parameters. The macroscopic visual aspect of the skin of non-irradiated and irradiated animals was compared on days 1, 4 and 8. On day 1, the skin surface of all the animals in all the groups was smooth and even. On day 4, no visible macroscopic changes (redness, swelling, wrinkles) were observed in the control group (non-irradiated). On the other hand, noticeable induction of wrinkles and desquamation were seen in animals of UVB exposed group (Figure 6). Exposure of animals to UVB led to the visible appearance of wrinkles and desquamation beginning from day 4th and this effect got intensified till day 8th as compared to animals in the non-irradiated control group. But the application of CM formulation in formulation treated group appeared to restore the UVB induced damage as the skin showed comparatively better than the UVB irradiated group.

The skin moisture level of the animals was measured with a corneometer on all the days (day 1 to 8) before the schedule of UVB exposure. The animals of non-irradiated control group displayed high mean corneometer readings ranging from 49.6±3.7 to 55.80±1.2 corneometer units. These values indicate good moisture capacities of the skin and reflect high hydration index. However, exposure of animals to UVB resulted in a decrease in corneometer readings beginning from very first exposure, which sequentially dropped further to a considerable extent till day 8th of study (54.60±4.7 to 14.5±1.3).

The Erythema Index (E.I.) in the skin was evaluated using Mexameter every day (for 8 days) before UVB exposure. Mean E.I. was obtained on the dorsal skin of all the animals. An increase in mean E.I. from 286.8±12.9 to 366.2±14.4 was observed from day 1 to day 8 in UVB exposed group; whereas, the mean E.I. was lower in the formulation-treated group (265±14.3 to 271.4±15.9). A comparison of mean E.I. values between days 1 and 8 reveals that UVB exposure led to more than 27.68% increase in E.I. whereas for the CM formulation treated group, the E.I was less than 3%. There was more than 25.68%
reduction of erythema in the CM formulation-treated group than the UVB-irradiated group (Figure 8). This experiment using Mexameter reveals that the CM formulation significantly prevented the increase in E.I. and protected (91.29%) the animal skin from the harmful UVB rays.

The histological features and alterations in the skin of all the animals were determined by staining with H&E. Histological examination of each slide was done for epidermal thickness, hyperkeratosis and infiltration of inflammatory cells in the dermis. It was found that UVB exposure led to induction of pathological features in the skin associated with photo aging. Animals in UVB-irradiated group displayed a substantial increase in epidermal thickness (14.25±1.19 pixels as compared to 3.06±0.41 pixels in untreated control); increase in dermal thickness (99.21±8.73 pixels as compared to 55.18±3.64 pixels in untreated control); hyperkeratosis and infiltration of inflammatory cells in the dermis as compared to untreated control. A lower extent of epidermal hyperplasia as indicated by a reduced value of epidermal thickness (10.98±2.05 pixels as compared to 14.25±1.19 pixels in the UVB-irradiated group) and dermal thickness (76.38±4.60 pixels as compared to 99.21±8.73 pixels in the UVB-irradiated group) were recorded in CM formulation treated animals. Hyperkeratosis was low in CM formulation treated animal. The extent of infiltration of inflammatory cells in the dermis was found to be moderate in all CM formulation treated animals (Figure 9a and Table 1). In addition, collagen degradation in UVB induced mice as compared to non-irradiated control group. The animals treated with CM formulation exhibited lower extent of the epidermal thickness and significant decrease in dermal thickness (10.98 pixels and 76.38 pixels as compared to 14.25 pixels and 99.21 pixels respectively in UVB treated group respectively(mean±SEM, P<0.05) (n=5). Taken together, the detailed histological evidence of the skin proved that the formulation with CM as its active ingredient was able to protect the skin from the UVB induced damage in the in vivo system.

The UVB exposure to the normal skin leads to photo aging and causes various changes in the skin architecture. Photo aging depends primarily on the degree of sun exposure and skin pigment. The photodamaged skin is associated with increased epidermal thickness and alterations in dermis such as erythema, scabs, roughness, and wrinkling of the skin. Wrinkle formation occurs because of accumulated skin damages such as matrix destruction and skin inflammation.

In the present study, the experiments showed evidence that the UVB irradiation for 7 consecutive days led to change in the macroscopic and microscopic levels. Macroscopically UVB exposure led to an increase in the number and depth of wrinkles, loss of moisture content, induction of erythema. Whereas microscopically UVB led to epidermal hyperplasia, hyperkeratosis and an increase in infiltration of inflammatory cells in the dermis as compared to the untreated group animals.

| Group           | Mean Epidermal Thickness (Pixels± SEM) | Mean Dermal Thickness (Pixels± SEM) | Epidermal hyperplasia | Hyperkeratosis | Infiltration of inflammatory cells in dermis |
|-----------------|----------------------------------------|-------------------------------------|-----------------------|----------------|-------------------------------------------|
| Control         | 3.06±0.41                              | 55.18±3.64                          | A                     | A              | A                                         |
| UVB-irradiated  | 14.25±1.19                             | 99.21±8.73                          | +++                   | +++            | +++                                       |
| Formation treated | 10.98±2.05                             | 76.38±4.60                          | +                     | +              | ++                                        |

Table 1: Histological evaluation of nude mice skin after treatment with conditioned medium based formulation A= Absent, +++ = high, + = low

* =As calculated from mean of three different locations using UTHSCSA image tool
These UVB induced toxic effects were reversed by the stem cell CM based formulation. It was found that topical application of CM formulation led to the restoration of UVB induced effects at macroscopic and microscopic levels. Macroscopically the CM formulation led to smoother skin, a decrease in erythema as well as considerable restoration of moisture content in animals. While macroscopically photoprotective effects of the CM formulation were supported by the low extent of hyperkeratosis, epidermal hyperplasia, infiltration of inflammatory cells and lesser collagen degradation as compared to animals exposed to UVB irradiation.

**Conclusion**

The above study showed evidence that the stem cell-derived conditioned medium contains many important growth factors and cytokines which may be useful in skin health care. These stem cells derived proteins were feasible to formulate a stable skin care product. The formulation made from the CM was non-toxic to the human primary cells and it protected the primary cell DNA from CPD formation. This protective effect of the CM formulation was further proved in the in vivo nude mice model. The CM based formulation extensively protected the rat skin from toxic UVB-irradiation. This study provides evidence that the mesenchymal stem cell-derived conditioned medium as a useful skin care bioactive material with many potential pharmacological actions.

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**Conflict of Interest**

The authors confirm that there are no known conflicts of interest associated with this publication.

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