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Original Research

Plant-Derived Extracts Plus Vitamin E and/or Aloe Vera Protect Against Intrinsic/Extrinsic Stressor in Human Skin: *In Vitro* and Clinical Evidence

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Abstract

Background: Humans are exposed to physical, biological, chemical, and psychological stressor throughout their life span. In recent years many medicinal plants have been shown to induce stress adapting and protective functions. Plant-derived extracts and vitamin E exhibit stress protection or resistance by normalizing cellular homeostasis and enhancing resistance to toxic stimuli to overcome cellular damage. Here we report the evaluation of a topical preparation (product test materials; PTM) containing an ingredient blend of Rhodiola Rosea, Eleutherococcus Senticosus (Siberian Ginseng), Rhaponticum Carthamoides, Inonotus Obliqus, and Slegainella Lepidophylla as the base formula and tested the addition of Lespedeza Capitata (leaf/stem) extract plus vitamin E and/or Aloe Vera to determine the induced protective functions in human skin when challenged with intrinsic and extrinsic stressors. **Methods**: The base topical preparation plus Lespedeza Capitata extract plus vitamin E or the base topical preparation plus vitamin E and Aloe Vera were assayed *in vitro* on (a) intrinsically stressed excised abdominoplasty skin, (b) full thickness (FT) skin equivalent models post-treated with a combination of ultra-violet (UV) B light (250 mJ/cm^2) and diesel particular matter (DPM) (75 µg/mL) skin, for their effect on antioxidant, inflammation, and stress biomarker geners. Additionally, the bioadaptive activity of the PTMs was confirmed in providing resilience and protection against UV-induced erythema. For example, in a clinical study, daily topical application of the PTMs on the buttocks of 20 woman (18–78 years old), average age of 51.1 years, median body mass index (BMI) of 26.5 for 8 weeks followed by 2 minimal erythema dose (MED) of UVB exposure was accessed 24 hours after irradiation. Statistical analysis was performed by *t*-test and ANOVA, repectively. **Results**: Pretreatment with the topical PTMs on intrsinically stressed skin significantly reduced the expression of the stress gene biomarkers, p53, pro-inflammatory cytokines Interleukin-1β (*IL-1*β) and Tumor Necrosis Factor-α (*TNF*α) and the pro-apoptotic BCL2 associated X, apoptosis regulator (BAX) values compared to controls. Topical application of the PTMs on Full Thickness (FT) human skin treated with UVB light and DPM significantly enhanced the stress response by activating heat shock transcription factor 4 (*HSF4*) and heat shock protein family B (small) member 1 (*HSPB1*) gene levels belonging to the heat shock protein (*HSP*) family by significantly increasing the expression of heme oxygenase 1 (*HMOX1*). At the same time, significantly reducing *IL-1*β levels were observed plus protection of skin cells from toxicity ocurred by significantly increasing the expression of B-cell lymphoma 2 (*BCL2*) (anti-apoptotic gene). In the clinical study, daily topical applications of the PTMs for 8 weeks followed by 2MED of UVB irradiation with clinical assessment 24 hours later revealed a significantly reduced intensity of erythema when compared to the buttock region treated with UVB alone. **Conclusions**: The PTMs containing adaptogen ingredients may confer stress resistance and induce stress protective responses against intrinsic as well as extrinsic stressors as demonstrated by the obtained *in vitro* and clinical evidence.

Keywords: product test materials; adaptogen ingredients; diesel particulate matter; UVB; full thickness skin equivalent models

1. Introduction

Skin is the largest organ of human body and is exposed to a variety of intrinsic stressors such as hormonal changes, fatigue [1], insomnia, and extrinsic stressors such as UV radiation and pollution [2]. Acute, prolonged or chronic exposure to intrinsic or extrinsic stressors perturbs the state of cellular homeostasis in skin. For example, a variety of disorders such as atopic dermatitis, dyspigmentation, hyperpigmentation, increased fragmentation and disorganization of extracellular matrix proteins collagen and elastin, and thinning of the basement membrane results in significant aesthetic deterioration on the face and body leading to an aged appearance [3,4]. Most treatments for improving stress are focused on prevention and protection, and some claim to treat some characteristics of stress induced disorders such as hyperpigmentation and dermal matrix fragmentation by increasing extracellular matrix protein levels [5]. Less attention is given to the induction of stress response, which could be the first line of treatment before application of treatment regimen that involves prevention and treatment. Several plants extracts have been found to be beneficial in maintaining and restoring cellular homeostasis, promoting immunomodulatory activity, improving endurance against fatigue, and

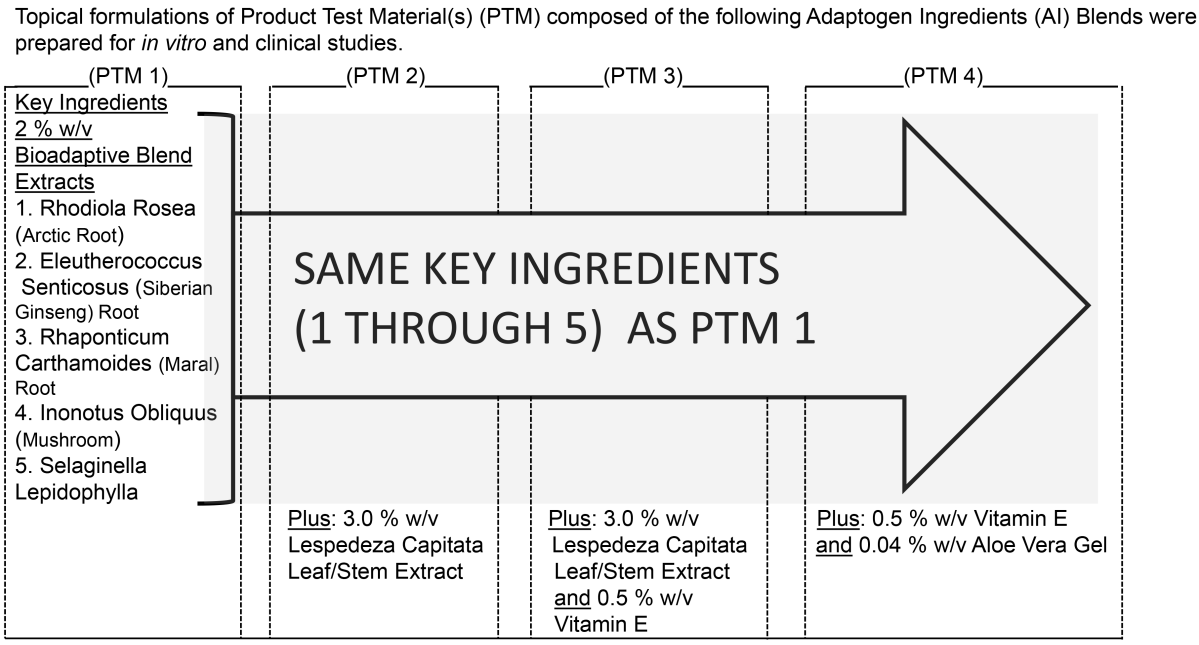

Fig. 1. Schematic of the five Product Test Materials (PTM), PTM1, PTM2, PTM3, PTM4, PTM5 and their ingredient composition.

resisting cellular damage from stressors [6,7]. Rhodiola Rosea [8], Eleutherococcus Senticosus [9] Rhaponticum Carthamoides [10], Inonotus Obliquus [11] and Selaginella Lepidophylla [12] have been found to exhibit adaptgenic functions by boosting antioxidant and anti-inflammatory functions [6,8–13]. Phytochemicals such as stilbenes, anthocyanins, procyanidins, epicatechin, gallocatechingallate, acetogenins, isorhamnetin, sulforaphane, allyl isothiocyanate, lycopene, tomatine, lectin, curcumin, 6-shogaol, and 6-gingerol have been found to activate stress response signaling associated with oxidative stress, inflammation, and heat shock proteins [14]. The mechanism of stress protective response by adaptogenic plants in enhancing stress resistance and adaptation in humans have been shown to involve regulation of cortisol, nitric oxide, stress-activated protein kinase JNK, the forkhead box O transcription factor, and upregulation of heat shock protein pathway [15,16].

Also, the biological activities of the legume crop Lespedeza Capitata have been shown to have skin care and pharmaceutical applications [17]. For example, some evidence from the topical use of Lespedeza Capitata have reported skin moisturizing properties along with protecting against photoaging [17]. Also, the known skin benefits of vitamin E [18] and Aloe Vera [19] are well established. For example, Vitamin E's antioxidant and anti-inflammatory properties have been reviewed [18]. Moreover, Aloe Vera contains multiple vitamins, enzymes, minerals, sugars, and fatty acids that contribute to it healing activities [19]. In our review of the literature, we found that all studies showing stress protective function with adaptogenic plant extracts involved oral administration of botanical extracts. Notably, to date, no studies have shown topical application of botanical actives showing adaptogenic response to intrinsic or extrinsic stressors have been reported.

Plants with adaptogenic function have been described in the literature to provide resilience, resistance, and adaptogenicity against intrinsic and extrinsic stress by enhancing stress protective function including antioxidant, anti-inflammation, anti-fatigue, antidepressive, neuroprotection, and CNS stimulating activity [14]. Oral administration of the tincture extract from Rhodiola rosea roots inhibited pro-inflammatory enzymes cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and phospholipase A2 (PLA2) by preventing the release of arachidonic acid from cell membranes, thereby, rendering membrane stabilization and enhancing anti-fatigue functions [20]. In a clinical study, repeated oral administration of Rhodeola rosea for 4 weeks reduced symptoms of fatigue and improved attention span and cognitive function [20,21]. Further, oral administration of ADAPT-232 capsules 199 containing fixed combination of standardized extracts of adaptogens, Rhodiola Rosea, Eleutherococcus senticosis, and Schisandra Chinesis extract characterized for the content of active markers eleutherosides, schisandrins, salidroside, tyrosol, and rosavin to BALB/c mice prior to stress exposure increased alertness and endurance leading to reduced fatigue. This study found that the adaptogen blends stimulated levels of stress response proteins Heat Shock Protein 70 (HSP70) and Heat Shock Protein 72 (HSP72) in the serum of mice suggesting increased protection and tolerance to stress [22]. In recent years, activation of autophagy mechanisms as an adaptive response to stressors by natural substances, which

includes resveratrol (vitis vinifera) and curcumin (curcuma longa). Resveratrol activated autophagy mechanisms by inhibiting mammalian Target of Rapamycin (mTOR) signaling [23], and curcumin stimulated autophagy by inducing caspase-3 signaling [24]. Both resveratrol and curcumin were shown to exert anti-tumor activity by stimulating autophagic death of tumor cells. In addition, many other polyphenols were also shown to exhibit adaptogenic effects. Quercetin glycosides stimulated glucose update, and thus, overcoming mitochondrial dysfunction to improve response to treatment of type 1 diabetes [25]. Hydroxytyrosol (HT), found in olives, significantly upregulated mitochondrial biogenesis pathway genes PPARG Coactivator 1 Alpha (*PGC1-α*) and Nuclear respiratory factor 1 and 2 (NRF-1 and NRF-2) in retinal pigment epithelial (RPE) cells, and thus, protects from macular degeneration [26].

To the best of our knowlege, there are no studies in the literature that have shown stress adapting and stress protective functions of adaptogen ingredients alone or in combination, when applied topically to intrinsically or extrinsically stressed skin. Since many of these ingredients have been associated with adaptogenic response when administered orally or systemically, we hypothesized that the adaptogen ingrediens may provide stress response activity, when applied topically against intrinsic and extrinsic stressors. Therefore, the purpose of this study was to determine whether or not different adaptogen plant-derived extracts as active ingredients provide protection against against intrinsic and extrinsic stressor via *in vitro* and clinical testing.

2. Materials and Methods

2.1 Product Test Materials

The product test materials (PTMs) with adaptogen ingredients (AI) are shown in Fig. 1.

2.2 Effect of Product Test Materials (PTMs) on Intrinsically Stressed Skin: Assessment of Stress Gene Markers

Human skin explant used in the assay was a surgical waste from an abdominal procedure performed on a Caucasian female of 50 years of age in a plastic surgery clinic in Beverly Hills, CA, USA. The explant skin was partitioned into ~2 cm^2 × 2 cm^2 sections, left with a thin (~1 mm) layer of hypodermis and incubated in OPTI-MEM (Gibco/Thermo Fisher, Waltham, MA, USA) supplemented with preadipocyte growth medium (Cell Applications, San Diego, CA, USA), and 10% fetal bovine serume (FBS) (Sigma Aldrich, St. Louis, MO, USA) in a 6 well plate format. Test materials (PTM1, PTM3 and PTM4) were topically applied on the tissues at 2 mg/cm^2 of the tissue area and incubated in a tissue culture incubator set at 37 °C for 24 hours, which was followed by recovery of three 4 mm^2 dermal punch biopsies (The PTM2 was not tested because the PTM3 AI blend was similar to PTM2 execpt for the addition of 0.5% vitamin E). Control samples consist of skin tissues treated with water alone. All samples underwent similar incubation period of 24 hours at 37 °C and skin tissue extraction using dermal punch biopsy. Collected skin biopsies were then immediately disrupted with a portable homogenizer (VWR, Radnor, PA, USA) and RNA extraction was completed with RNeasy Plus Mini kit cat #74134 from Qiagen (Qiagen, Germantown, MD, USA), using QiaCube Connect robotic station (Qiagen Germantown, MD, USA). Purified total RNA was assessed at 260 nm and 280 nm via NanoDrop Lite (Thermo Fisher Scientific, Waltham, MA, USA), to quantify RNA content. For PCR reactions, cDNA was prepared using AzuraQuant cDNA kit (Azura Genomics, Raynham, MA, USA) and the expression of the genes of interest (**Supplementary Table 1**) was measured by real-time quantitative PCR with BioRad iCycler iQ Detection System using PCR primers from Realtime primers (Elkins Park, PA, USA) and Fast Green qPCR Master Mix – Fluor (Azura Genomics, Raynham, MA, USA). Efficiency $\Delta\Delta$Ct method was used for quantification of results, after the normalization of gene expression to three housekeeping genes (ACTB, GAPDH and 18S). Genes were considered differentially expressed if the level of expression was reasonably high (less than 30 cycles to detect), p value, as determined by the two-tailed t-test was $\leq$0.05 and the modulation was greater than two fold.

2.3 Evaluation of PTMs Containing AIs on Extrinsically Stressed (UVB (250 mJ/cm^2) and Diesel Particulate Matter (DPM) 75 µg/cm^2 Treated) Skin Model: Assessment Using Thermofisher Open Array (OA) Gene Panel

The study was conducted to understand how stress gene expression in the skin is influenced by specific Product Test Materials (PTMs) under the influences of stressors such as UVB exposure and Diesel Particulate Matter (DPM). The study was performed using a full-thickness *in vitro* skin culture model containing epidermal and dermal cell layers (EFT-400, MatTek Corp, Ashland, MA, USA). Four separate groups of tissues had PTMs applied to the surface four hours prior to DPM treatment. One-hour post-DPM treatment, the tissues were then exposed to 250 mJ/cm^2 of UVB. One group received both stressors (i.e., DPM and UVB), but no PTMs application (“Stress Alone” group). One group of skin tissues were not treated with PTMs, not exposed to any stressors (“Naïve” group) and it was used as an Untreated control. Tissues were collected for gene expression analysis 18 hours post-UVB exposure.

The following groups were included in the study (N = 3/group):

PTM + UVB + DPM group: In this group, skin tissues were pre-treated with each of the test materials, PTM1, PTM2, PTM3, PTM4 followed by UVB + DPM; Stress Alone Group: Treated with UVB + DPM only and Naïve Group: Not treated with UVB, DPM and topicals. Gene expression was assessed on a custom Open Array (OA) panel format 112 (**Supplementary Table 2**). The panel

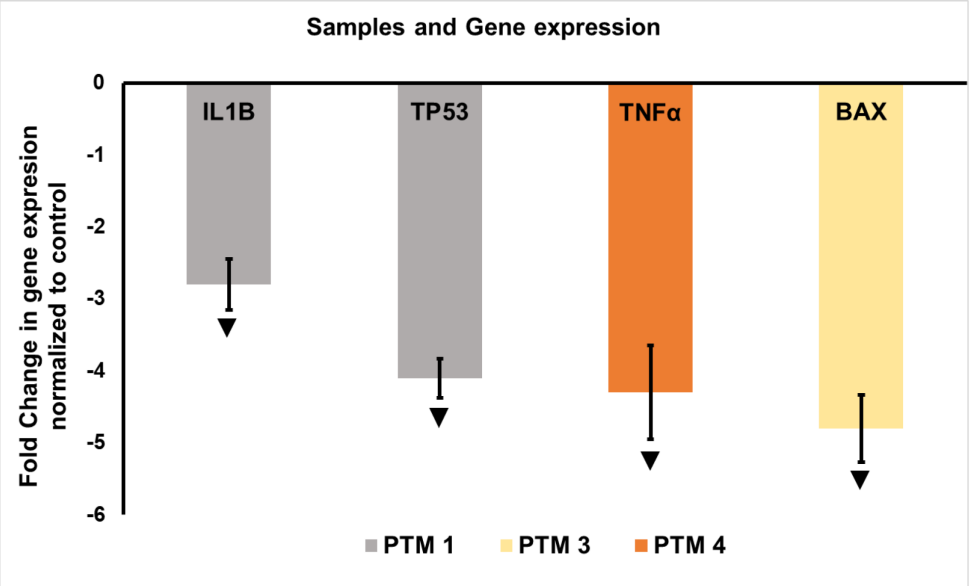

Fig. 2. Effect of adaptogen ingredients (AI) topical materials (PTM1, PTM3, PTM4) on intrinsically stressed skin: Assessment of stress gene markers normalized to control. ▼ = Significant change in gene expression in product test materials (PTM) treated samples compared to control. Topical PTM samples were treated to excised abdominoplasty skin tissues for 24 hours following which RNA was extracted and cDNA synthesized. The RNA samples were assayed for gene expression on a panel of genes that affect stress response. Results showed downregulation of pro-inflammatory cytokine Interleukin -1β (IL-1β) and stress gene Tumor Protein-53 (*TP53*) by PTM1. Further, downregulation of pro-inflammatory cytokine TNFα by PTM4 and downregulation of pro-apoptotic gene *BCL2* Associated X-protein (*BAX*) by PTM3. The figure is a representation of two independent experiments.

consisted of 103 target genes belonging to stress response pathway along with 9 endogenous control genes (Ubiquitin C (*UBC*), Transferrin receptor (*TFRC*), Peptidylprolyl isomerase A (*PPIA*), hypoxanthine phosphoribosyltransferase 1 (*HPRT1*), glucuronidase beta (*GUSB*), Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), Actin beta *(ACTB*), Eukaryotic 18S rRNA (*18S*), and beta-2-microglobulin (*B2M*). The UV light source and Diesel Particulate Matter (DPM) preparation used for extrinsic stress protection assessment is described below.

2.3.1 UV Light Source

SOL500 Sun Simulator (Dr Hönle AG, Munich, Germany) with an H2 lamp filter was used for generating UVB light for extrinsic stress. The full thickness (FT) skin tissues were placed on an irradiation platform 30 cm from the H2 lamp filter and irradiated with 250 mJ/cm^2 UVB with the H2 filter (UVB spectra). The UVB dosage of 250 mJ/cm^2 was confirmed using PMA2106 UVB detector (SolarLight) [27].

2.3.2 Diesel Particulate Matter (DPM) Preparation

Diesel particulate matter NIST 1650b (Sigma-Aldrich, Inc. St. Louis, MO, USA) was used to prepare stock solution of 25 mg/mL in dimethyl sulfoxide (DMSO) and sonicated for 30 min to avoid agglomeration of the suspended Particulate Matter 2.5 (PM2.5) particles. DPM was diluted to 100 µg/mL in DMSO within 1 h of stock preparation to avoid variability in the NIST1650 composition for testing on skin models.

2.4 Clinical Methods

To demonstrate the bioadaptive capacity of test materials, a clinical study design to investigate the functional activity of test formulations to provide resilience and protection against UV induced erythema was developed. The study endeavored to demonstrate that pre-treatment of test materials suppresses development of erythema following UV exposure in healthy volunteers. IRB approval was obtained for the study protocol prior to study initiation at the clinical site. The study was performed on 20 female healthy volunteers at 18 to 78 years of age (average age 51.1 years) median BMI of 26.5 at Dermatology Consulting Services, PPLC, NC under the supervision of Zoe Draelos, MD (see Table 1). Subjects who signed informed consent and met all of inclusion criteria and none of the exclusion criteria were enrolled in the study. Subjects were dispensed a bar of Dove soap for cleansing of the entire buttock area and body. The subjects were instructed to apply one skin care product/regimen to the randomized right buttock and a second skin care product/regimen to the randomized left buttock and nothing to the central buttock which was defined as the untreated control site. The total number of sites for product application was 40 with each topical applied to 10

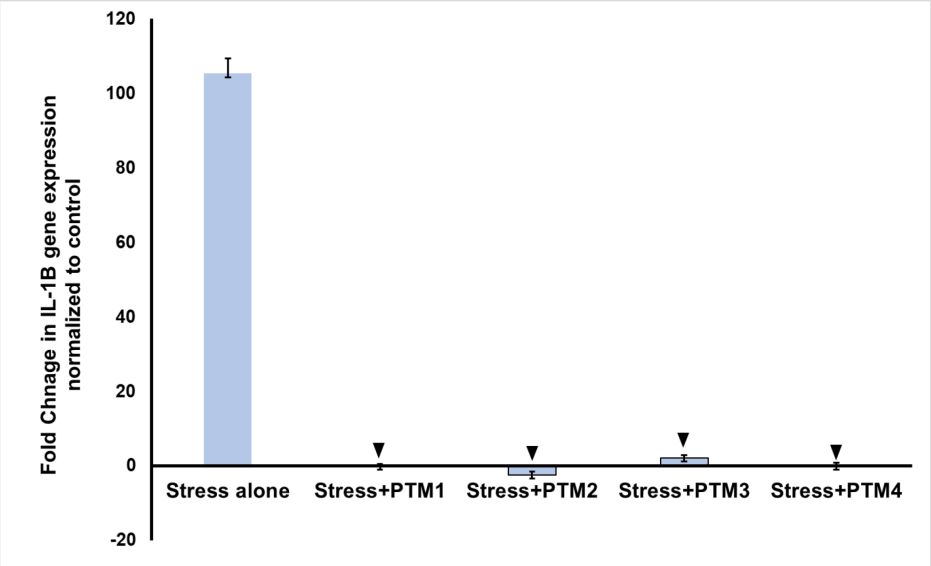

Fig. 3. Effect of AI topical materials PTM1, PTM2, PTM3, PTM4 on extrinsically stressed skin: Assessment of stress gene markers normalized to control. ▼ = Significant reduction in IL-1β gene expression in PTM treated samples compared to control.The study was performed using a full-thickness *in vitro* skin culture model containing epidermal and dermal cell layers (EFT-400, MatTek). Four separate groups of tissues had TMs applied to the skin surface four hours prior to diesel particular matter (DPM) (100 µg/mL) treatment. One-hour post-DPM treatment, the tissues were then exposed to 250 mJ/cm^2 of ultra-violet B (UVB). One group received both stressors (i.e., DPM and UVB), but no PTM application ("Stress Alone" group). The Untreated control group was not treated with PTM, and not exposed to any stressors ("Naïve" group). Tissues were collected for gene expression analysis 18 hours post-UVB exposure on a panel of stress genes. There was significant downregulation of Interleukin -1β (*IL-1β*) gene expression in FT skin model pre-treated with PTM1, PTM2, PTM3 and PTM4 and exposed to combination of UVB and DPM. This figure is a representation of two independent experiments.

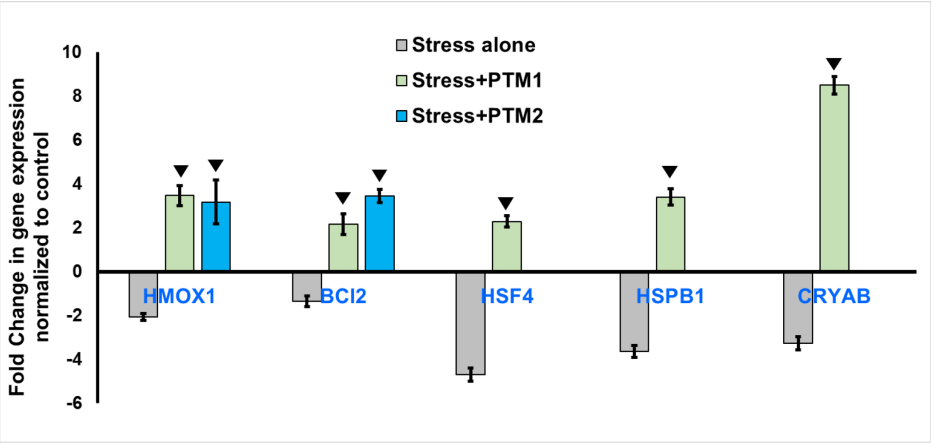

Fig. 4. Effect of AI topical materials PTM1, PTM2, PTM3, PTM4 on extrinsically stressed skin: Assessment of stress gene markers normalized to control. ▼ = Significant change in gene expression in PTM treated samples compared to control. Methods detailed in Fig. 3. PTM1 and PTM2 significantly stimulated antioxidant gene Heme Oxygenase 1 (*HMOX1*), anti-apoptotic B-cell lymphoma 2 gene (*BCL2*), and HSP family genes heat shock transcription factor 4 (*HSF4*), heat shock protein family B (small) member 1 (*HSPB1*) and Crystallin Alpha- B (*CRYAB*). This figure is a representation of two independent experiments.

sites on either left or right side of buttock in a randomized design. A compliance check was conducted at 4 weeks.

Subjects returned to the research center at week 8 for irradiation. The left, right, and central buttock (3 sites) was irradiated with 2MED of UVB from a solar simulator (150W xenon arc bulb, Solar Light, PA, Philadelphia, USA). Subjects returned to the clinic 24 hours after irradiation. At the clinic, dermaspectrophotometer read-

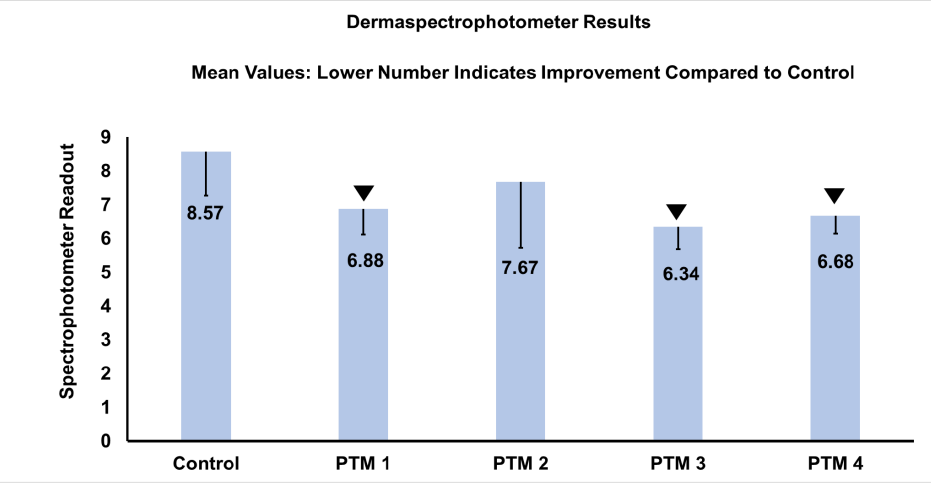

Fig. 5. Assessment of erythema intensity by dermaspectrophotometer measurement (n = 10 site per sample). ▼ = Significant erythema suppression in PTM treated subjects compared to control (subjects treated with UV alone). Twenty Subjects who signed informed consent and met all of inclusion criteria and none of the exclusion criteria were enrolled in the study. Subjects were dispensed a bar of Dove soap for cleansing of the entire buttock area and body. The subjects were instructed to apply one skin care product/regimen to the randomized right buttock and a second skin care product/regimen to the randomized left buttock and nothing to the central buttock which was defined as the untreated control site. The total number of sites for product application was 40 with each topical applied to 10 sites on either left or right side of buttock in a randomized design. Subjects returned to the research center at week 8 for irradiation. The left, right, and central buttock (3 sites) was irradiated with 2MED of UVB from a solar simulator (150W xenon arc bulb, Solar Light, Philadelphia). Subjects returned to the clinic 24 hours after irradiation. At the clinic, dermaspectrophotometer readings and photographs of erythema were obtained from the two-product application treated sites on the right and left buttocks and the one untreated control site on the central buttocks. The photographs of erythema intensity in UVB treated alone group, combination of UVB plus product treatment group were assessed using Image J open-source software.

Table 1. Clinical subject demographics and characteristics.

N = 20
AGE range 18 to 78 (mean 51.5 $\pm$ 6.3) years
Body Mass Index (BMI) 26.5 $\pm$ 2.4
All subjects completed the study without any adverse events.
N = number of subjects

ings and photographs of erythema were obtained from the two-product application treated sites on the right and left buttocks and the one untreated control site on the central buttocks. The photographs of erythema intensity in UVB treated alone group, combination of UVB plus product treatment group were assessed using Image J open-source software (version 1.48; National Institute of Health, Bethesda, MD, USA). Safety parameters which included potential signs of irritation, blistering, and edema were assessed throughout the duration of the study. Subjects who were found to be noncompliant were dropped from the study and excluded from the data analysis. Statistical assessment of the variance of the mean of dermaspectrophotmeteric assessment from control group and topical PTM treated group were analysed using analysis of variance (ANOVA) test. A p value of 0.05 or less between the control and PTMs treated group were considered statistically significant.

2.5 Statistical Analysis

For the gene expression data, genes were considered statistically significant if the level of expression was 2-fold or greater compared to control levels as determined by two-tailed t-test ($p < 0.05$). For the analysis of the clinical data, the means of the dermaspectrohphotmeteic values comparing the controls to the PTM levels were considered significant ($p < 0.05$) by ANOVA (as outlined above).

3. Results and Discussion

We developed a set of 4 topical formulations containing adaptogenic ingredients (AI) blends (Fig. 1) for testing on intrinsic as well as extrinsically stressed human skin models. We used surgically excised ex vivo abdominoplasty skin as a representative model for simulating intrinsic stress since ex vivo skin secrete stress inducers including Adrenocorticotropic Hormone (ACTH), cortisol and pro-inflammatory cytokines TNF-α, IL-1β, Interleukin-2 (IL-2), Interleukin-5 (IL-5), Interleukin-7 (IL-7), Interleukin-10 (IL-10), and Interleukin-17 (IL-17), thereby demonstrating characteristics of injured skin [3,28–30]. Evaluation of stress genes panel (**Supplementary Table 1**) on intrinsically stressed excised abdominoplasty skin following topical application of PTM1 induced downregulation of stress gene marker Tumor Protein-53 (*TP53*) gene (a

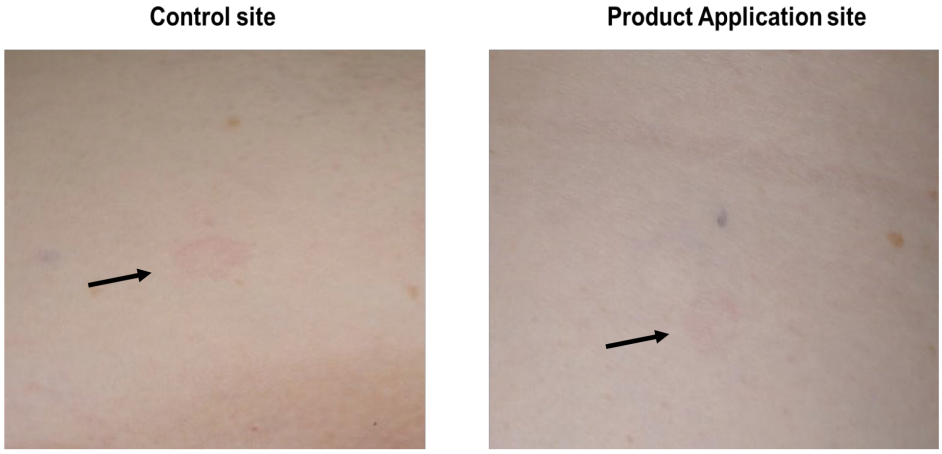

Fig. 6. Photographic assessment of intensity of erythema. Methods detailed in Fig. 4. Pre-treatment with PTM1 for 8 weeks prior to 2 minimal erythema dose (MED) UVB exposure significantly suppressed UV induced erythema. UV induced erythema in control site and product application site are indicated by black arrows.

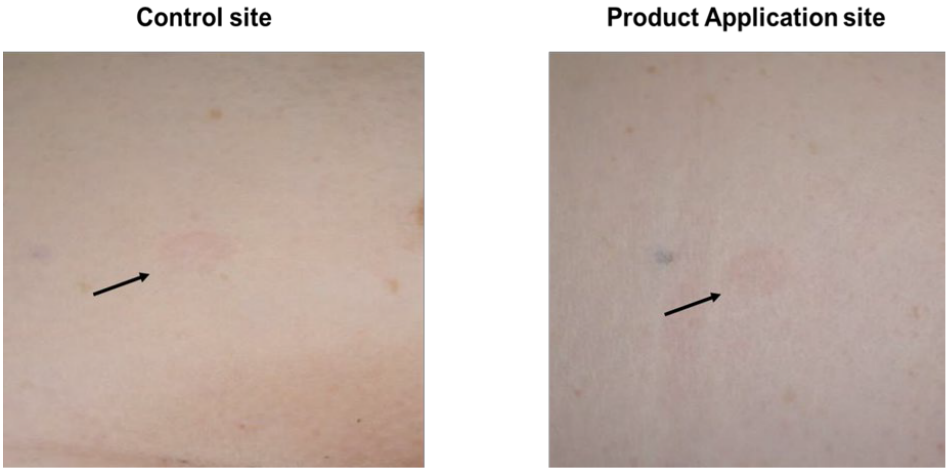

Fig. 7. Photographic assessment of intensity of erythema. Methods detailed in Fig. 4. Pre-treatment with PTM2 for 8 weeks prior to 2 MED UVB exposure significantly suppressed UVB induced erythema. UV induced erythema in control site and product application site are indicated by black arrows.

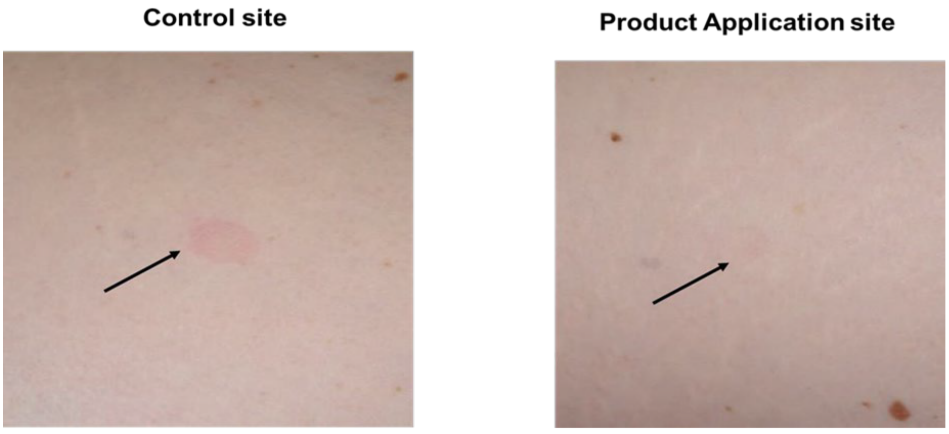

Fig. 8. Photographic assessment of intensity of erythema. Methods detailed in Fig. 4. Pre-treatment with PTM3 product regimen for 8 weeks prior to 2 MED of UVB exposure significantly suppressed UVB induced erythema. UV induced erythema in control site and product application site are indicated by black arrows.

sensor for DNA damage and tumor suppressor) [20] and pro-inflammatory cytokine (*IL-1*β) [22], PM3 significantly reduced expression of pro-inflammatory cytokine TNFα and downregulated expression of pro-apoptotic gene *BCL-2* Associated X-protein (*BAX*) (Fig. 2). PTM3 and PTM4 had no effect on TP53 and IL-1β. Our results indicated differential expression of gene markers of stress induction by the AI topical materials under conditions of intrinsic stress. Since the PTM3 AI blend composition was similar to PTM2 formulation except for the addition of 0.3% vitamin E, PTM2 was not tested on intrinsically stressed skin since it could produce similar target gene expression outcome as PTM3 formulation upon topical application on intrinsically stressed skin.

Based on the capacity of the AI topical formulations to reduce expression of key markers of stress induction on intrinsically stressed skin, we hypothesized that topical application of AI may also provide stress protective response in skin exposed to extrinsic stressors UVB and DPM. UVB is a major environmental stressor associated with DNA damage through induction of Cyclibutane Pyrimidine Dimers (CPD) and perturbation of cellular apoptosis in human skin specifically, when exposed to 250 mJ/cm^2 UVB dose or higher [27,31]. DPM at 100 ug/mL dose was selected based on published reports in which it was shown that human skin reconstruct models exposed to 50–100 ug/mL DPM alone induces significant activation of oxidative stress and pro-inflammatory cytokine response [32–34]. In our experiment, we combined UV and DPM to evaluate the capacity of the PTMs containing adaptogenic ingredients in stimulating stress response using a Thermo Fisher custom Open Array (OA) stress response gene panel (**Supplementary Table 2**). The panel contained genes belonging to Heat Shock Protein (HSP) family, oxidative stress, DNA damage, and pro- inflammatory cytokines.

Pre-treatment with 4 topicals PTM1, PTM2, PTM3 and PTM4 significantly suppressed pro-inflammatory cytokine IL-1β expression to basal levels in the presence of stressors (Fig. 3).

Pre-treatment with PTM1 and PTM2 upregulated 2-fold and higher antioxidant gene Heme Oxygenase 1 (*HMOX1*) expression, anti-apoptotic B-cell lymphoma 2 gene (*BCL2*) and 3 Heat Shock Protein (HSP) family genes heat shock transcription factor 4 (*HSF4*), heat shock protein family B (small) member 1 (*HSPB1*) and Crystallin Alpha B (*CRYAB*) (Part of HSP20 gene family) (Fig. 4). PTM3 and, however, did not induce significant differential (2-fold change) in expression of *HMOX1*, *BCL2* and *HSP* family genes *HSF4*, *HSPB1* and *CRYAB* other antioxidant, apoptotic and HSP family genes in the open array panel.

Our data is consistent with the induction of stress response genes such as HSP70 and HSP72 in studies involving oral administration of adaptogenic ingredients [23]. In summary, our data suggests that IL-1β is downregulated by PTM1 both under intrinsic and extrinsic stress, whereas PTM4 downregulated pro-inflammatory cytokines TNFα under intrinsic stress and IL-1β under extrinsic stress. PTM1 and PTM2 were effective not only in suppressing pro-inflammatory cytokines, but also were effective in activating stress protective response through induction of antioxidant *HMOX1* gene and HSP family genes. We did not quantify proteins levels by enzyme-linked immunosorbent assay (ELISA) or Western blot analysis and were are not able to perform these studies due to the limitation of scientific funding resources. However, when skin gene expression was quantified and compared to protein expression levels for such parameters like collagen, elastin, and the matrix metalloprotineases (MMP 1 and MMP 3) and others in a Journal of Cosmetic Dermatology report, there was good correlation between gene and protein expression [35]. So, in part, there is also evidence for correspondence from gene to protein expression [35].

Based on positive input from mechanistic studies that demonstrated capacity of the adaptogen topical compositions to resist development of intrinsic and extrinsic stressors by downregulation of pro-inflammatory cytokines primarily IL-1β, and upregulation of antioxidant genes and heat shock proteins, we decided to evaluate the stress response function of the topical compositions in a clinical study with UVB induced erythema as the primary efficacy endpoint. Erythema is an inflammatory reaction induced by activation of pro-inflammatory cytokines, mainly IL-1β and their activation of downstream prostaglandin synthesis by COX-2 by UVB [33,34].

Assessment of erythema intensity by dermaspectrophotometric analysis at 24 hours following UVB exposure showed that all 4 formulations reduced the intensity of erythema. However, the reduction in spectrophotometric measurement was slightly more pronounced in the areas treated with PTM 3 and PTM 4 (Fig. 5). The improvement of erythema intensity by PTM1, PTM2, PTM3 and PTM4 AI topical materials shown with dermaspectrophotometric analysis was further examined by photographic images taken at 24 hours following UVB exposure. Since PTM3 and PTM4 compositions are almost identical with the exception of Lespedeza Capitata Leaf/Stem Extract, only PTM3 treated location was photographed. The images showed that compared to control site treated with 2 MED of UVB, the sites treated with PTM1 regimen (Fig. 6), PTM2 (Fig. 7) and PTM3 (Fig. 8) showed suppression of erythema intensity. The area of the control UVB treated area assessed by Image J software was 3.1 cm^2 and area treated with product and UVB reduced to 1.7 cm^2, indicative of reduced UVB induced erythema. These results support the mechanistic observation of the capacity of adaptogen compositions to activate stress response mechanisms by suppression of pro-inflammatory cytokines, a hallmark of erythema and activating expression of antioxidant genes and stress mitigating heat shock protein pathway genes.

4. Conclusions

The present results demonstrate that topical application of adaptogen ingredients (AI) activates stress protective response in human skin against intrinsic stressors as well as major extrinsic stressors UVB and DPM by suppressing proinflammatory cytokines, activating antioxidant gene expression and inducing stress response by upregulating HSP gene family. Our data is consistent with the induction of stress response genes such as HSP70 and HSP72 in studies involving oral administration of adaptogenic ingredients. The gene expression results corroborated with our clinical data, which showed the capacity of AI topical compositions to suppress intensity of erythema induced by UVB. In summary, the results provide an indication of potential benefits of including adaptogenic ingredients in topical skin products. More research including mechanistic and clinical studies with long term administration of AI compositions are needed to further characterize the usefulness of topical application of adaptogens on human skin and its clinical benefits in protection against intrinsic and extrinsic stressors.

Abbreviations

PTM, Product Test Materials; AI, Adaptogen Ingredients; DPM, Diesel Particulate Matter; UVB, Ultra-violet B; FT, Full Thickness skin equivalent models.

Availability of Data and Materials

All datasets including table and figures, developed and analyzed during this study are included in this published article. The corresponding author will make available all data published in this manuscript upon reasonable request.

Author Contributions

GD, LB and HK designed and executed the *in vitro* studies with Sunnybio discovery Inc. MR and ZDD designed the clinical study in consultation with LB and executed the study. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work

Ethics Approval and Consent to Participate

The study was carried out in compliance with 21CFR Part 50 Protection of Human Subjects. The primary investigator verbally consented all subjects. Subjects were allowed to take as much time as necessary to read the consent form. The consent form was approved by the Allendale Institutional Review Board (AIRB), Old Lyme, CT. The investigator answered all subject questions. Following answering of all questions, the subjects and the investigator signed the consent form. The subject were provided with a copy of the consent form. The ethics approval number is: DCS-09-20, ethics committee approval date: March 01, 2020.

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Conflict of Interest

Ganesh Diwakar, Lisa Barnes, Melanie Riggs, Helen Knaggs are employees of Nu Skin enterprises, Provo UT USA. Zoe Diana Draelos is an owner and Principal Investigator at Dermatology Consulting Services, PLLC, NC, USA. The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2812366.

References

[1] Cogan E. Hormone therapy of ageing: myths and realities. Revue Medicale De Bruxelles. 2004; 25: A371–A375.

[2] Bonté F, Girard D, Archambault JC, Desmoulière A. Skin Changes During Ageing. Sub-Cellular Biochemistry. 2019; 91: 249–280.

[3] De Tollenaere M, Meunier M, Scandolera A, Sandre J, Lambert C, Chapuis E, *et al*. Well-aging: A new strategy for skin homeostasis under multi-stressed conditions. Journal of Cosmetic Dermatology. 2020; 19: 444–455.

[4] Lee CM, Watson REB, Kleyn CE. The impact of perceived stress on skin ageing. Journal of the European Academy of Dermatology and Venereology. 2020; 34: 54–58.

[5] Zouboulis CC, Ganceviciene R, Liakou AI, Theodoridis A, Elewa R, Makrantonaki E. Aesthetic aspects of skin aging, prevention, and local treatment. Clinics in Dermatology. 2019; 37: 365–372.

[6] Kaur P, Robin, Makanjuola VO, Arora R, Singh B, Arora S. Immunopotentiating significance of conventionally used plant adaptogens as modulators in biochemical and molecular signalling pathways in cell mediated processes. Biomedicine & Pharmacotherapy. 2017; 95: 1815–1829.

[7] Panossian A, Wagner H. Stimulating effect of adaptogens: an overview with particular reference to their efficacy following single dose administration. Phytotherapy Research. 2005; 19: 819–838.

[8] Li Y, Pham V, Bui M, Song L, Wu C, Walia A, *et al*. *Rhodiola rosea L.:* an herb with anti-stress, anti-aging, and immunostimulating properties for cancer chemoprevention. Current Pharmacology Reports. 2017; 3: 384–395.

[9] Choi HR, Nam KM, Lee HS, Yang SH, Kim YS, Lee J, *et al*. Phlorizin, an Active Ingredient of Eleutherococcus senticosus,

Increases Proliferative Potential of Keratinocytes with Inhibition of MiR135b and Increased Expression of Type IV Collagen. Oxidative Medicine and Cellular Longevity. 2016; 2016: 3859721.
[10] Todorova V, Savova MS, Ivanova S, Ivanov K, Georgiev MI. Anti-Adipogenic Activity of *Rhaponticum carthamoides* and Its Secondary Metabolites. Nutrients. 2023; 15: 3061.
[11] Lu Y, Jia Y, Xue Z, Li N, Liu J, Chen H. Recent Developments in *Inonotus obliquus* (Chaga mushroom) Polysaccharides: Isolation, Structural Characteristics, Biological Activities and Application. Polymers. 2021; 13: 1441.
[12] Gechev TS, Hille J, Woerdenbag HJ, Benina M, Mehterov N, Toneva V, *et al*. Natural products from resurrection plants: potential for medical applications. Biotechnology Advances. 2014; 32: 1091–1101.
[13] Diwakar G, Barnes L, Kern D, Olmos C. 25617 Topical formulation with an ingredient blend composed of Rodiola rosea and Eleutherococcus senticosus (Siberian ginseng) induces stress protective response on human skin against intrinsic and extrinsic stressors. Abstract. Journal of the American Academy of Dermatology. 2021; 85: AB63.
[14] Panossian A. Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals. Annals of the New York Academy of Sciences. 2017; 1401: 49–64.
[15] Panossian AG, Oganessian AS, Ambartsumian M, Gabrielian ES, Wagner H, Wikman G. Effects of heavy physical exercise and adaptogens on nitric oxide content in human saliva. Phytomedicine. 1999; 6: 17–26.
[16] Panossian A, Wikman G, Kaur P, Asea A. Adaptogens exert a stress-protective effect by modulation of expression of molecular chaperones. Phytomedicine. 2009; 16: 617–622.
[17] Pastorino G, Marchetti C, Borghesi B, Cornara L, Ribulla S, Burlando B. Bioligical activities of the legume crops Melilotus officinalis and Lespedeza capitata for skin care and pharmaceutical applications. Industrial Crops and Products. 2017; 96: 158–164.
[18] Keen MA, Hassan I. Vitamin E in dermatology. Indian Dermatology Online Journal. 2016; 7: 311–315.
[19] Sánchez M, González-Burgos E, Iglesias I, Gómez-Serranillos MP. Pharmacological Update Properties of *Aloe Vera* and its Major Active Constituents. Molecules. 2020; 25: 1324.
[20] Pooja, Bawa AS, Khanum F. Anti-inflammatory activity of Rhodiola rosea–"a second-generation adaptogen". Phytotherapy Research: PTR. 2009; 23: 1099–1102.
[21] Olsson EM, von Schéele B, Panossian AG. A randomised, double-blind, placebo-controlled, parallel-group study of the standardised extract shr-5 of the roots of Rhodiola rosea in the treatment of subjects with stress-related fatigue. Planta Medica. 2009; 75: 105–112.
[22] Panossian A, Wikman G. Evidence-based efficacy of adaptogens in fatigue, and molecular mechanisms related to their stress-protective activity. Current Clinical Pharmacology. 2009; 4: 198–219.
[23] Park D, Jeong H, Lee MN, Koh A, Kwon O, Yang YR, *et al*. Resveratrol induces autophagy by directly inhibiting mTOR through ATP competition. Scientific Reports. 2016; 6: 21772.
[24] Ravindran J, Prasad S, Aggarwal BB. Curcumin and cancer cells: how many ways can curry kill tumor cells selectively? The AAPS Journal. 2009; 11: 495–510.
[25] Eid HM, Martineau LC, Saleem A, Muhammad A, Vallerand D, Benhaddou-Andaloussi A, *et al*. Stimulation of AMP-activated protein kinase and enhancement of basal glucose uptake in muscle cells by quercetin and quercetin glycosides, active principles of the antidiabetic medicinal plant Vaccinium vitis-idaea. Molecular Nutrition & Food Research. 2010; 54: 991–1003.
[26] Hao J, Shen W, Yu G, Jia H, Li X, Feng Z, *et al*. Hydroxytyrosol promotes mitochondrial biogenesis and mitochondrial function in 3T3-L1 adipocytes. The Journal of Nutritional Biochemistry. 2010; 21: 634–644.
[27] Narda M, Ramos-Lopez D, Bustos J, Trullàs C, Granger C. A novel water-based anti-aging suncare formulation provides multifaceted protection and repair against environmental aggressors: evidence from *in vitro*, *ex vivo*, and clinical studies. Clinical, Cosmetic and Investigational Dermatology. 2019; 12: 533–544.
[28] Ventura SA, Heikenfeld J, Brooks T, Esfandiari L, Boyce S, Park Y, *et al*. Cortisol extraction through human skin by reverse iontophoresis. Bioelectrochemistry. 2017; 114: 54–60.
[29] Wang PW, Hung YC, Lin TY, Fang JY, Yang PM, Chen MH, *et al*. Comparison of the Biological Impact of UVA and UVB upon the Skin with Functional Proteomics and Immunohistochemistry. Antioxidants. 2019; 8: 569.
[30] Özyurt K, Ertaş R, Atasoy M. Biologics for psoriasis: What is new? Dermatologic Therapy. 2019; 32: e12916.
[31] Biniek K, Levi K, Dauskardt RH. Solar UV radiation reduces the barrier function of human skin. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109: 17111–17116.
[32] Valacchi G, Sticozzi C, Belmonte G, Cervellati F, Demaude J, Chen N, *et al*. Vitamin C Compound Mixtures Prevent Ozone-Induced Oxidative Damage in Human Keratinocytes as Initial Assessment of Pollution Protection. PLoS ONE. 2015; 10: e0131097.
[33] Fuller B. Role of PGE-2 and Other Inflammatory Mediators in Skin Aging and Their Inhibition by Topical Natural Anti-Inflammatories. Cosmetics. 2019; 6: 6.
[34] Wang P, Sun M, Voorhees JJ, Fisher GJ, Li Y. Cyclooxygenases mediate early induction of interleukin-6 expression by solar ultraviolet irradiation in human skin. Journal of Dermatological Science. 2017; 87: 201–203.
[35] Lephart ED. Human skin gene expression doesn't correlate with protein expression? Unless both parameters are quantified. Journal of Cosmetic Dermatology. 2018; 17: 244–245.