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Evaluation of Collagen Building, UV Protective and Antioxidant Properties of CollaBZen™ on Human Collagen Type I,II & Type IV

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Abstract
CollaBZen™ from Zenherb labs, a proprietary blend of polyphenols extracted from *Phyllanthus emblica*, *Camellia sinensis*, and *Coffea arabica*, was assessed for its anti-ageing potential. Human dermal fibroblast and chondrocyte cells were exposed *in vitro* to hydrogen peroxide and UVA to induce ageing manifested as ROS production and collagen breakdown respectively.
CollaBZen™ revealed no cytotoxicity up to 0.16 mg/ml in both NHDF and HCH cells. DCFDA assay revealed that CollaBZen™ demonstrated a dose-dependent reduction in H_2O_2-induced ROS levels in both cell types. Exposure to UVA rays led to collagen depletion but CollaBZen™ pre-treatment showed higher collagen levels, offering protection. At 0.04 mg/ml, it conferred a significant shield against UVA rays, with a 48.13%, 39.47%, and 30.5% increase in COL1A1, COL4A1, and COL2A1, respectively. Beyond promoting collagen as documented in the previous studies, CollaBZen™ emerges as a potent food supplement safeguarding skin and joint cells from ageing consequences, reinforcing its role in age-related preventive care.

Keywords: Ageing, senescence, skin health, bone health, nutrition

Introduction
In humans, the increase in the number of senescent cells due to ageing can lead to a domino effect in the skin [1]. Characterized by the following events; increased levels of reactive oxygen species as a consequence of ageing extrinsic factors, increased secretion of skin ageing-associated secreted protein (SAASP) [2], increase in the reserves of matrix metalloproteinases (MMPases), depletion of growth factors [3] which together contributes to unbalanced antioxidant reserves, collagen breakdown, skin thinning, and pigmentary disorders [4]. Ageing is characterized by reduced cell proliferation and increased senescence which also causes degradation of the extracellular matrix surrounding the articular cartilage causing osteoarthritis [5]. Moving joints are surrounded by articular cartilage that provides lubrication and serves as a load-bearing tissue in joints. The cartilage is composed to low-density chondrocytes embedded in an extracellular matrix made up of collagen type -2 proteins and glycosaminoglycan. The extracellular matrix is often degraded due to genetics, obesity, and chronological ageing which can lead to osteoarthritis [5].
Ageing can occur naturally, termed chronological ageing, or in a non-programmed fashion due to extrinsic factors or stress such as ultraviolet rays (UVR), pollution, smoking, eating habits, or inappropriate lifestyle choices [6]. Ultraviolet radiations can cause photoageing that leads to damage of damaging the enzymatic and non-enzymatic systems of the skin. This can collectively damage the structural and membrane proteins [7]. The primary event of extrinsic involves the generation of reactive oxygen species (ROS) which triggers the activation of transcription factors such as nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) [8]. The baseline levels of MMPases are increased due to AP-1 that in turn causes the degradation of elastin and collagen [9].
Recent pieces of evidence have shown that a proper diet can prevent the deleterious effects of ageing in the human body due to the presence of strong antioxidants, vitamins, proteins, trace elements such as iodine, zinc, copper, and iron amongst other medicinally important macromolecules. These phytochemicals can combat the harmful effects of ROS on the macromolecules of the cell such as lipids, proteins, polyunsaturated fatty acids, and DNA, and can prevent inflammation and revert cellular damage [10].

According to the free radical theory of ageing, lipid peroxidation, DNA damage, and inflammation caused by the presence of ROS, are the major causes of skin diseases [11]. Dietary supplementation with antioxidants can also prevent early ageing in the joints and subsequent osteoarthritis [5].

Tremendous research exists on the supplementation of diet with functional foods and nutraceutical components that can restore the health of the damaged skin. Chicken collagen peptide have shown significant anti-inflammatory changes, type 1 collagen synthesis, and cell proliferation on human dermal fibroblasts by virtue of its ROS scavenging potential [12]. Gelatin hydrolysate and gelatin from fish source has also shown to alleviate the effects of ROS in UV-B exposed mice models by enhancing antioxidant enzyme activity and thymus index [13]. Protein hydrolysate from plant source such as walnut showed collagen promotion by inhibition of MMP-1 activity elevated due to the exposure of UVA and UVB rays in rat models [14]. Similarly, plant metabolites such as polyphenols, fatty acids, and vitamins, among others have shown beneficial effects in repairing the adverse effects of skin ageing. For instance, oral administration of olive oil promoted collagen synthesis, and reduced stress-induced skin ageing in mice by reducing ROS, lipid peroxidation, and MMP-8 expression in the Swiss mice models [15]. Niacinamide has been proven to protect immortalized human keratinocyte cells (HaCaT) from oxidative stress induced by particulate matter and the subsequent apoptotic effects of ROS [16]. The polysaccharides extracted from *Hizikia fusiformis* showed UV protective effects against UVB rays in human dermal fibroblast cell lines by inhibiting the activation of NFkB and AP-1 implicated in the degradation of elastin and collagen [17]. Oral consumption of flavonoid hesperidin has also shown protective benefits against UV radiations in mice models by inhibiting UVB-induced wrinkling and collagen fibre loss in male hairless mice [18].

The natural sources of antioxidants such as catechin hydrate, gallic acid, α-tocopherol, and ascorbic acid were compared for their protective effects against osteoarthritic chondrocytes. The study was conducted for two groups of women; group 1 belonging to the age group of 50-69 years and 70-80 years in group 2. The effect of the nutraceuticals was assessed *in vitro* by culturing the chondrocytes and was assessed for collagen levels, inflammation, and formation of extracellular matrix. It was observed that women of the lesser-age group showed better articular cartilage formation with less inflammation and better formed extracellular matrix. This study explains that dietary supplementation with antioxidants can have potential benefits in reversing ageing in joints when taken in the early years of life [5]. In another study, hydrogen peroxide was used to induce inflammation, and lumen density was estimated using oxygen-derived radicals in chondrocytes. The ROS leads to a reduction in the collagen matrix and initiates cartilage damage [19]. Vitamin E and resveratrol showed a better effect on the reduction in osteoarthritis-like chondrocytes [20].

These few studies are amongst many that have proven the anti-ageing effects of bioactives derived from natural sources. One such product of Zenherb Labs is CollaBZen™ is a collagen-building blend of three plant extracts *viz*., *Phyllanthus emblica, Camellia sinensis,* and *Coffea arabica.* Our recently published article has proven the antioxidant activity and *in vitro* skin collagen benefits of CollaBZen™. The study reveals that the blend showed an IC_{50} value of 0.018mg/ml for DPPH radical scavenging potential and increased collagen levels in human dermal fibroblast by 35% at 0.05 mg/ml with no toxic effects till 0.2 mg/ml [21]. CollaBZen™ has also been evaluated for its anti-ageing effect in human volunteers where it showed increased deep skin hydration, improved elasticity, and firmness [22].

As the previous data suggests CollaBZen™ shows tremendous antioxidant and collagen-building potential, the present work focuses on the ability of CollaBZen™ to revert ageing in skin on normal human fibroblast (NHDF) cells where the collagen breakdown was triggered by exposure to UVA in addition to the ROS scavenging potential in the NDHF cells upon exposure to hydrogen peroxide. The senescence-like condition in chondrocytes to mimic osteoarthritic condition was induced by UVA and H_2O_2 which led to depleted levels of collagen and ROS accumulation without affecting the cell viability. The collagen-restoration potential and ROS scavenging activity on senescent chondrocytes of CollaBZen™ was also tested.

Materials and Methods

The sample Plant based collagen builder is a proprietary named CollaBZen™ of Zenherb Labs [21, 22]. The cells were procured from Promocell NHDF (Normal Human Dermal Fibroblast (NHDF), Cat No: C-12302, Promocell and HCH (Human Chondrocytes), Cat No: C-12710, Promocell) along with fibroblast growth medium (Promocell: Cat No C-23020) and chondrocyte growth medium (Promocell: Cat No: C-27101). The ELISA kits were procured from Elabsciences.

Maintenance of cell cultures

The NHDF and HCH (Human Chondrocytes (HCH), Cat No: C-12710, Promocell) cells were maintained in the fibroblast growth medium and chondrocyte growth medium with 10% FBS in a humidified atmosphere of 5% CO_2 and 37 °C until confluency. The viability of the cells was constantly recorded using conventional hemocytometer using trypan blue dye.

Sample preparation

The sample was prepared in growth fibroblast growth medium for HDF and chondrocyte growth medium for HCH without serum to obtain a stock of 32mg/ml. Further dilutions were made in the fibroblast growth medium for NHDF cells and chondrocyte growth medium for the HCH cells without serum to give a range of 0.01 mg/ml - 0.320 mg/ml.

Cytotoxicity on HDF and HCH cells

The cells were seeded in a 96-well plate with 100 µl of cell suspension for HDF in the fibroblast growth medium and HCH with chondrocyte growth medium with 10% FBS adjusted to a density of 3 x 10^5 cells/ml followed by incubation for 24 hours in a humidified atmosphere of 5% CO_2 and 37 °C. After incubation, the media was removed, and the cells were treated with 100 µl of CollaBZen™ at different concentrations (0.010-0.32 mg/ml) and the plates were incubated at 37 °C for 24 hours in 5% CO_2 atmosphere. After incubating the plates for 24 hours, the media was removed from all the wells, and 100 µl of 0.5mg/ml of MTT in PBS was added to each well. The plates were further incubated for 4 hours at 37 °C in a 5% CO_2 atmosphere. The supernatants were removed and the formazan crystals were solubilized in 100 µl of DMSO by intermittent shaking. The absorbance was measured using a microplate reader at a wavelength of 590

nm. The percent growth inhibition and percent viability were calculated using the formula given below:

% Cell toxicity = (OD of control - OD of sample / OD of control) x 100
% cell viability = (OD of sample/OD of control) x 100

The concentration of CollaBZen™ responsible for inhibiting the cell growth by 50% termed as IC_{50} was calculated using a dose-response curve value for CollaBZen™ on HDF and HCH cell.

Inhibition of ROS by CollaBZen™ using DCFDA method

The cells were seeded in a 96-well plate with 100 µl of cell suspension for HDF in the fibroblast growth medium and HCH with chondrocyte growth medium with 10% FBS adjusted to a density of 3 x 10^5 cells/ml followed by incubation for 24 hours in a humidified atmosphere of 5% CO_2 and 37 °C. The monolayer was washed with medium and was incubated for 2 hours at 37 °C and 5% CO_2 before the testing of CollaBZen™ The media was removed and incubated with 100µl of 25µM DCFDA and the plates were further incubated at 37 °C and 5% CO_2. The solution of DCFDA was removed and the cells were washed with 1X PBS without disturbing the monolayer. The cells were then treated with different concentrations of CollaBZen™ and NAC as a positive control for ROS scavenging potential at a 5mM concentration. The plates were incubated at 37 °C and 5% CO_2 for 2 hours. After the incubation period, the plates were treated with 100 µl of 500µM H_2O_2 solution and the plate was further incubated at 37 °C and 5% CO_2 for 2 hours. The fluorescent signal was read using two wavelengths i.e., 484 for excitation and 535 for emission. The percent protection offered by CollaBZen™ against ROS induced by H_2O_2 was calculated using the formula given,

% Inhibition of ROS = (OD of control - OD of sample / OD of Control) x 100

Effect of UVA exposure on collagen levels in NHDF and HCH cells

The effect of UVA exposure for different time periods on the cell's collagen level was estimated. The cells were seeded at a density of 1 x 10^6 cells/ml in a 96-well plate in the fibroblast growth medium and chondrocyte growth medium with 10% FBS for NHDF and HCH cells respectively. The plates were further incubated for 24 hours in 37 °C and 5% CO_2. After incubation, the media was removed and fresh fibroblast growth medium and chondrocyte growth medium with 10% FBS was added for NHDF and HCH cells respectively, and the plates were further incubated for 48 hours at 37 °C and 5% CO_2. After the incubation period, the plates were exposed to UVA light (20mJ/cm^2) for 5, 10, 15, 20, 25, and 30. The plates were further incubated for 24 hours at 37 °C and 5% CO_2. The COL1A1 and COL4A1 levels were estimated for the NHDF cells and COL2A1 levels were measured for the HCH cells using the manuals' instructions given in ELISA KITs (Elabscience). The collagen levels were estimated from the cell suspension after homogenization with ice cold homogenization buffer 1X PBS containing 0.1% triton X-100 and phenylmethylsulfonyl fluoride in ice for 10 mins at 4 °C. The supernatant was carefully transferred to fresh tubes and stored at -20 °C until further use.

Protection by CollaBZen™ in collagen-breakdown induced by UVA in NHDF cells

The same experimental procedure was conducted as mentioned before, only this time the NHDF cells were treated with different concentrations of CollaBZen™ before exposing to UVA light. For COL1A1 and COL4A1 >50% reduction in collagen level due to UV light was observed when the exposure time was 20 respectively. Hence, after treating the cells with 0.005-0.16 mg/ml of CollaBZen™ the plates were exposed to 20mins of UVA light with an intensity of 20mJ/cm^2 to determine the protection offered by CollaBZen™ against reduction in COL1A1 and COL4A1. The estimation of collagen levels was done as per Kit instructions.

Protection by CollaBZen™ in collagen-breakdown induced by UVA in HCH cells

The same experimental procedure was conducted as mentioned before, only this time the HCH cells were treated with different concentrations of CollaBZen™ before exposing to UVA light. For COL2A1 >50% reduction in collagen level due to UV light was found at 25 mins. Hence, after treating the cells with 0.005-0.16 mg/ml of CollaBZen™ the plate was exposed to 25 mins of UV-light with an intensity of 20mJ/cm^2 to determine the protection offered by CollaBZen™ against reduction in COL4A1 levels. The estimation of collagen levels was done as per Kit instructions.

Statistical Analysis

All the experiments were performed in triplicates and the values in the graph are given as average ± standard deviation calculated in the Microsoft Excel Software.

Results

Cell toxicity on NHDF and HCH cells exhibited by CollaBZen™

The sample CollaBZen™ was tested for cytotoxicity potential in normal human dermal fibroblast cells (NHDF) and human chondrocytes (HCH) using MTT assay. It was found that till 0.16 mg/ml the samples did not exhibit a cytotoxic effect as the cell growth for NHDF and HCH was inhibited by 18.52% and 15.83% respectively. However, at a higher concentration, CollaBZen™ evidently affected the cell viability by 27.72% and 23.00% at the concentration of 0.32 mg/ml on NHDF and HCH cells respectively. Hence for the ROS prevention and collagen stimulatory assays, the concentrations of the sample used were below 0.16 mg/ml (Figure 1).

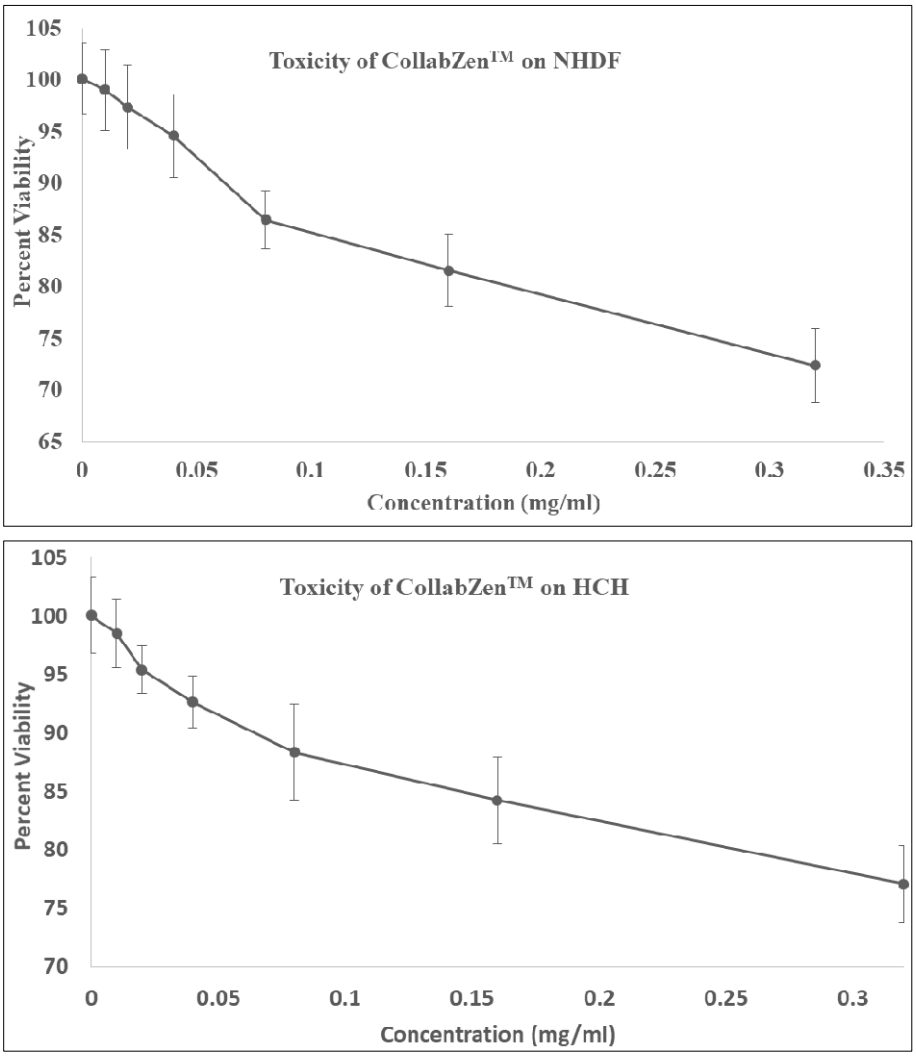
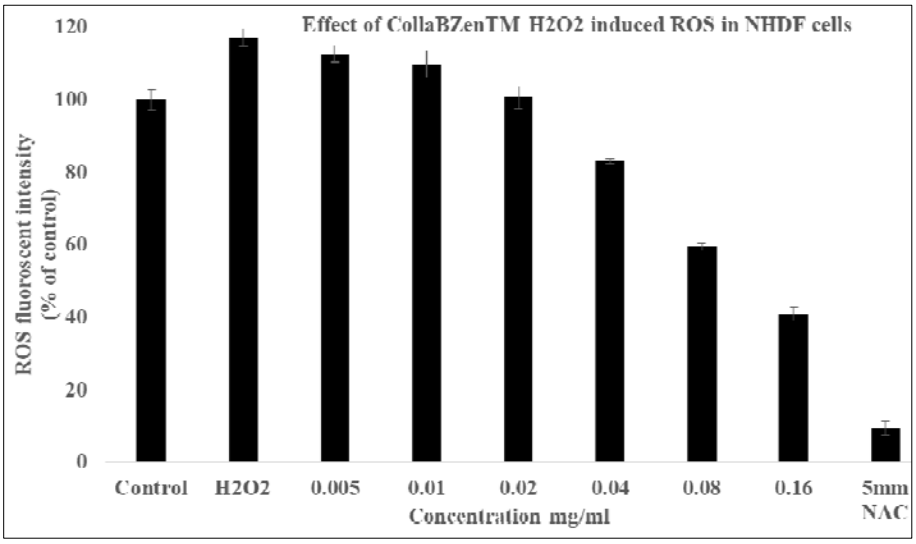


Fig 1: Effect of CollaBZen™ on NHDF and HCH cell viability

Based on initial cytotoxicity experiments conducted on NHDF and HCH it was found that CollaBZen™ showed no cell growth inhibitory capacity till 0.16 mg/ml. Hence, the ROS generation assay by H_2O_2 and protection offered by CollaBZen™ were tested on concentrations below 0.16 mg/ml. The NHDF and HCH cells were treated with a concentration of 0.005-0.16 mg/ml of CollaBZen™ for two hours followed by treatment with 500µM of H_2O_2 for 2 hours. The control without H_2O_2 was used to calculate the percent ROS generated in the wells when treated with H_2O_2. It was found that 17.17% and 20.37% levels of ROS were produced when NHDF and HCH cells were exposed to H_2O_2 for 2 hours. The inhibitory action of CollaBZen™ against ROS induced by H_2O_2 was calculated and it showed a dose dependent decrease in the ROS formation for both NHDF and HCH cells (Figure 2).



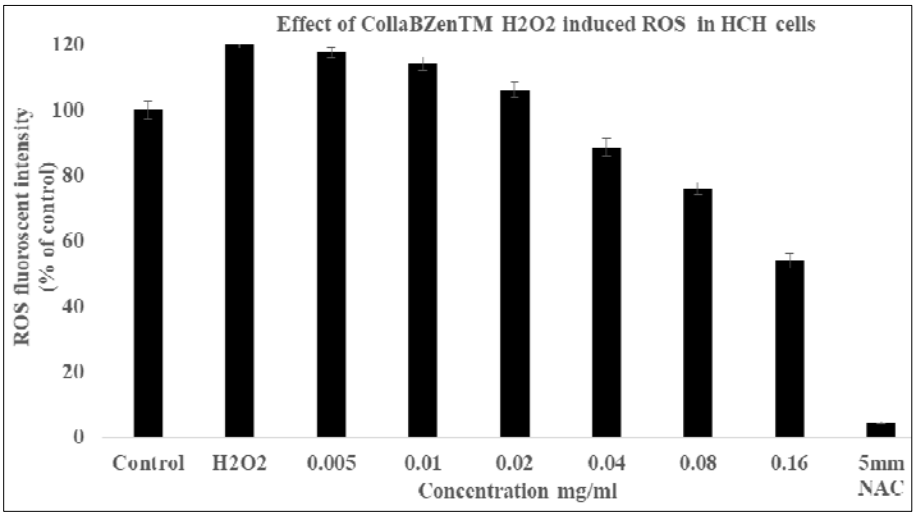


Fig 2: Effect of CollaBZen^{TM} on H_2O_2 induced ROS in NHDF and HCH cells

Effect of UVA treatment on Collagen levels in HDF Cells

The NHDF cells were exposed to UVA light for different time periods ranging from 0-30 min at an interval of 5 mins. The collagen content for the markers COL1A1 and COL4A1 was estimated after incubating the exposed and unexposed cells for 24 hours. There was a dose-dependent decrease in the collagen levels for both markers with an increase in the intensity of UVA rays. At an exposure period of 20 mins, the collagen content for markers COL1A1 (Figure 3) and COL4A1 (Figure 4) estimated with respect to absorbance obtained at 450 nm was reduced by 55.82% and 49.26% respectively. Since 20 min exposure reduced to collagen level to half (T_{50}), 20 mins exposure for COL1A1 and COL4A1 was finally used to estimate the protective effect offered by CollaBZen^{TM} against collagen breakdown induced by UVA light. The standard curve for COL1A1 and COL4A1 was used to determine the collagen levels in ng/ml. The control cells showed 7.72 ng/ml of COL1A1 which was reduced to 2.40 ng/ml when exposed to 20 min to UVA light (Table 1). Whereas, the COL4A1 levels in the control NHDF cells was 1.50ng/ml which further reduced to 0.517 ng/ml upon exposure to UVA light for 20min (Table 2).

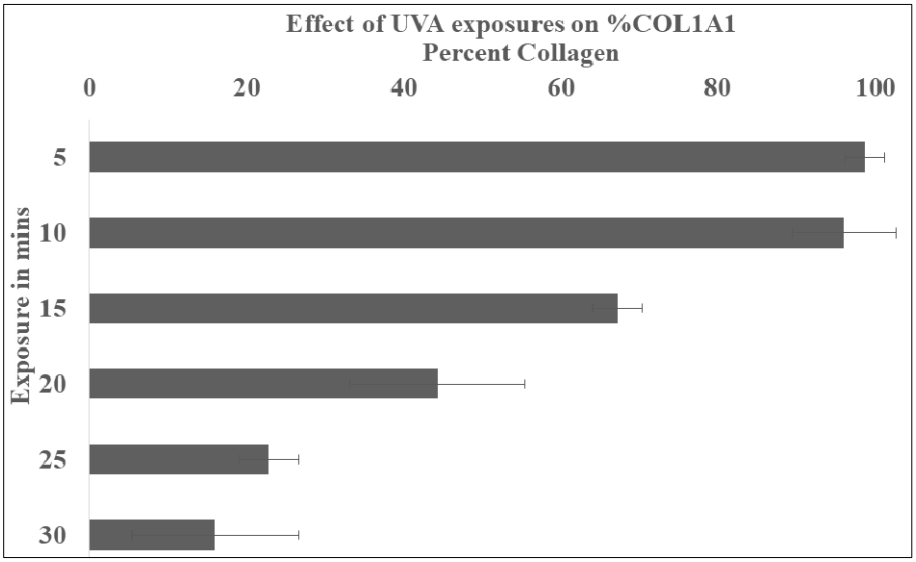


Fig 3: Decrease in COL1A1 content upon exposure to UVA on NHDF cells

Table 1: Effect of UVA on COL1A1 content upon in NHDF Cells

Exposure to UVA in mins	COL1A1 levels (ng/ml)	STDEV
Control	7.267	0.454
5	7.120	0.220
10	6.893	0.580
15	4.390	0.272
20	2.393	0.971
25	0.533	0.327
30	-0.063	0.926

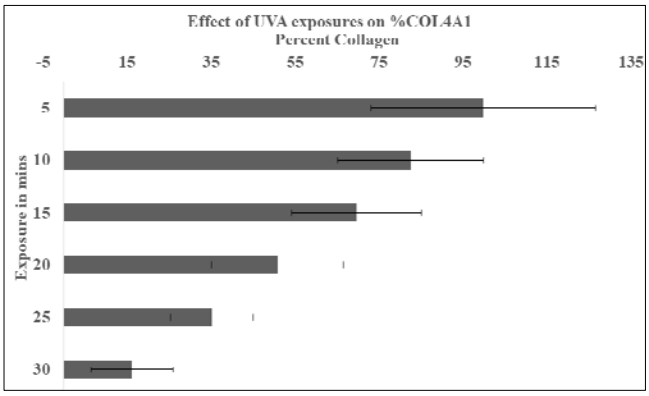


Fig 4: Decrease in COL4A1 content upon exposure to UVA on NHDF cells

Table 2: Effect of UVA on COL4A1 content upon in NHDF Cells

Exposure to UVA in mins	COL4A1 levels (ng/ml)	STDEV
Control	1.500	0.482
5	1.470	0.490
10	1.153	0.346
15	0.890	0.311
20	0.517	0.314
25	0.207	0.197
30	-0.173	0.195

Effect of UVA treatment on Collagen levels in HCH Cells

The collagen marker used for the HCH cells was COL2A1. As mentioned earlier, the cells were exposed for different time points ranging from 0-30 mins to UVA light. The decrease in the collagen content due to UVA insult was recorded using ELISA kit at the end of an incubation period of 24 hours. There was a dose-dependent decrease in collagen content as the time of exposure to UVA light increased. There was a decrease by 56.15% collagen content when the cells were exposed to UVA for 25 mins (Figure 5). As per the standard curve obtained for COL2A1 levels for different concentrations of the protein, cell control revealed 2.93 ng/ml of COL2A1 levels (Table 3). Upon exposure to UVA light for 25 mins, the levels dropped to 0.21 ng/ml. Hence, this exposure period was considered to study the protective effect of CollaBZen^{TM} against collagen inhibitory action of UVA light.

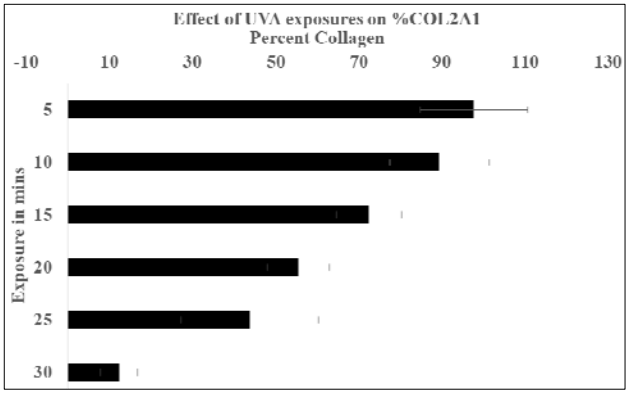


Fig 5: Decrease in COL2A1 content upon exposure to UVA on HCH cells

Table 3: Effect of UVA on COL2A1 content upon in HCH Cells

Exposure to UVA in mins	COL2A1 levels (ng/ml)	STDEV
Control	2.930	0.602
5	2.820	0.622
10	2.420	0.580
15	1.590	0.380
20	0.737	0.430
25	0.213	0.663
30	-1.297	0.235

Effect of pre-treatment of CollaBZen^{TM} collagen levels in UVA-exposed NHDF cells

The cell control with no exposure to UVA light revealed a collagen content for the marker COL1A1 of 4.85 ng/ml and upon exposure to UVA light for 20 mins, the collagen content decreased to 1.8 ng/ml (Table 4). The cells were pre-treated with CollaBZen^{TM} at various concentrations ranging from 0.005-0.160 mg/ml for 48 hours before exposure to UVA. At a lower concentration of 0.005 mg/ml, no protection against collagen reduction was observed, however from 0.01mg/ml to 0.04 mg/ml increase in collagen content from 2.36 to 3.38 ng/ml was noticed. However, at 0.08 mg/ml the collagen dropped to 2.76 ng/ml, but still higher than the UVA control, but at 0.16 mg/ml, the collagen level decreased even further to 2.21 ng/ml. CollaBZen^{TM} showed 48.43% collagen stimulation for COL1A1 marker at 0.04 mg/ml (Figure 6).

For the COL4A1 marker, the cell control showed the presence of 1.44 ng/ml which was reduced to 0.550 ng/ml when exposed to UVA for 20 mins (Table 5). A similar pattern was observed for the protection offered by CollaBZen^{TM} for the marker COL4A1 as for COL1A1, where protection was offered from 0.02-0.16 mg/ml. At 0.04 mg/ml there was a 39.44% protection observed (Figure 7).

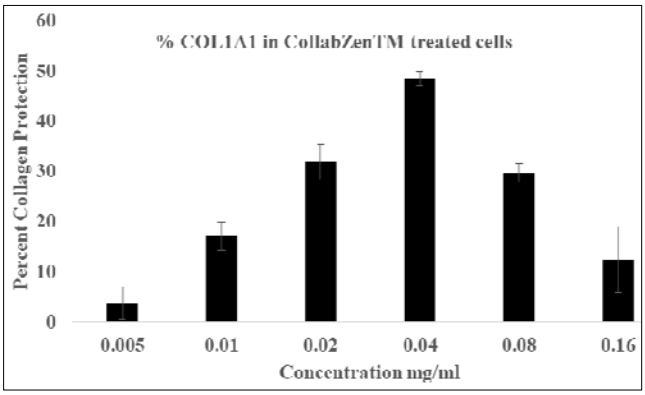


Fig 6: Protection of against UVA-induced decrease of COL1A1 levels in NHDF cells by CollaBZen™

Table 4: Pretreatment of CollaBZen™ reduced the breakdown of COL1A1 in NHDF Cells

Conc (mg/ml)	COL1A1 levels (ng/ml)	STDEV
Control	4.847	0.552
UVA	1.800	0.266
0.005	1.923	0.204
0.010	2.353	0.179
0.02	2.837	0.224
0.04	3.377	0.086
0.08	2.763	0.122
0.16	2.206	0.799

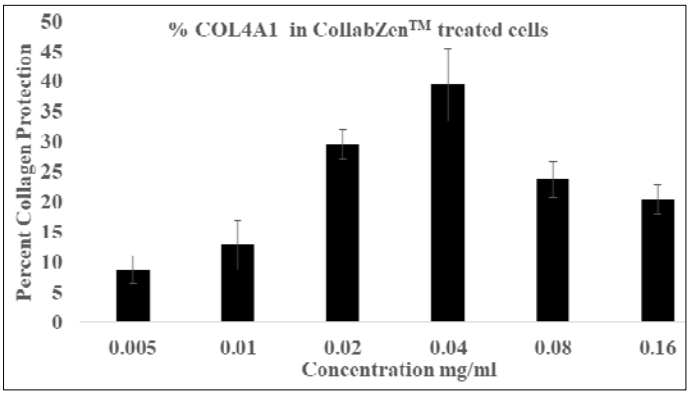


Fig 7: Protection of against UVA-induced decrease of COL4A1 levels in NHDF cells by CollaBZen™

Table 5: Pretreatment of CollaBZen™ reduced the breakdown of COL4A1 in NHDF Cells

Conc (mg/ml)	COL4A1 levels (ng/ml)	STDEV
Control	1.457	0.070
UVA	0.550	0.070
0.005	0.623	0.050
0.010	0.687	0.050
0.02	0.863	0.067
0.04	0.963	0.055
0.08	0.800	0.056
0.16	0.767	0.025

Effect of pre-treatment of CollaBZen™ collagen levels in UVA- exposed HCH cells

The cell control with no exposure to UVA light revealed a collagen content for the marker COL2A1 of 2.37 ng/ml and upon exposure to UVA light for 25 mins, the collagen content decreased to 0.177 ng/ml (Table 6). When the cells were pre-treated with CollaBZen™ at various concentrations ranging from 0.005-0.160 mg/ml for 48 hours before exposure to UVA, the protective effect of CollaBZen™ was recorded on HCH cells. As observed for NHDF cells, at a lower concentration of 0.005 mg/ml, no protection against collagen reduction was observed, however from 0.01mg/ml to 0.04 mg/ml increase in collagen content from 0.497 to 0.807 ng/ml was noticed. However, at higher concentrations such as 0.08 and 0.16 mg/ml the collagen dropped to 0.523 and 0.333 ng/ml respectively, but still higher than the UVA control. CollaBZen™ showed the highest protection of 30.05% at 0.04 mg/ml for the COL2A1 marker on the HCH cells (Figure 8).

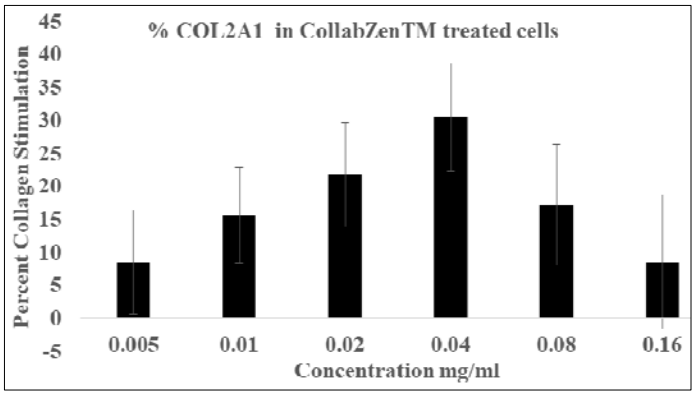


Fig 8: Protection of against UVA-induced decrease of COL2A1 levels in HCH cells by CollaBZen^{TM}

Table 6: Pretreatment of CollaBZen^{TM} reduced the breakdown of COL2A1 in HCH Cells

Conc (mg/ml)	COL2A1 levels (ng/ml)	STDEV
Control	2.373	0.538
UVA	0.177	0.110
0.005	0.343	0.076
0.010	0.497	0.081
0.02	0.623	0.075
0.04	0.807	0.108
0.08	0.523	0.025
0.16	0.333	0.067

Discussions

Reports suggest that the regulation of type 1 collagen in the skin fibroblasts is the underlying mechanism of wrinkle formation in humans. The major factors associated with a decrease in collagen type-1 proteins are UV radiation, chronological ageing, and senescence [23]. Since the COL1A1 and COL1A2 genes are responsible for the production of type-1 collagen, reports have suggested that their transcription is tightly regulated. Hence, in the current study effect of UVA on the breakdown of COL1A1 in NHDF and the protective effects of CollaBZen^{TM} were monitored. The natural blend showed 48.43% protection for COL1A1 at 0.04 ng/ml as compared to the UVA control. The baseline level of COL1A1 was 4.847 ng/ml which decreased to 1.800 ng/ml and it was restored to 3.377 ng/ml with 0.04 mg/ml of CollaBZen^{TM}.

COL4A1 is a major component of the skin basement membrane and dermo-epithelial junction that determines the polarity of keratinocytes and acts as a selective barrier to control molecular and cellular exchange [24, 25]. Reports suggest that COL4 levels decreases because of ageing and it may affect the TGF-β1 signaling which can have implications in skin ageing [24]. In the current study, oxidative stress was triggered in NHDF cells using UVA light and the depletion in the COL4A1 levels were measured. It was found that there was a decrease in COL4A1 levels from 1.457 ng/ml in the baseline untreated control versus 0.550 ng/ml in UVA-treated cells. Pretreatment of NHDF with CollaBZen^{TM} blend restored levels of COL41 to 0.963 ng/ml at 0.04 mg/ml. CollaBZen^{TM} showed COL4A1 protection by 39.47% as compared to the UVA-treated cells.

As mentioned earlier, the chondrocytes are embedded in the extracellular matrix made up of type-2 collagen and glycosaminoglycans. ROS accumulation due to ageing can cause inflammation and collagen degradation [20]. In the current study, the senescence in the human chondrocyte cells was induced by hydrogen peroxide to accumulate ROS. Also, the collagen-degradation was achieved by exposing the HCH cells to UVA. It was found that upon exposure to UVA the baseline levels of COL2A1 reduced from 2.373 ng/ml in untreated cell control to 0.177 ng/ml in UVA-exposed cells. The collagen degradation was prevented in the cells pretreated with CollaBZen^{TM} and it was found that 0.04 mg/ml showed the highest collagen promotion up to 30.5% as compared to UVA-treated cells. The levels of COL2A1 in the CollaBZen^{TM}- treated cells was found to be 0.807 ng/ml.

As mentioned earlier, stress or ageing is a consequence of imbalance between the ROS species and antioxidant defence mechanism in the cells. ROS can trigger activation NF-κB leading to activation of AP-1 culminating into degradation of collagen because of increased MMPs. It thus important to protect the cells from oxidative insult by preventive excessive generation of ROS [8, 9]. When the intrinsic antioxidant system falls insufficient to maintain the balance, it is important to exogenously provide the cells with extrinsic ROS scavengers. CollaBZen^{TM} showed a decreased production of ROS in both dermal and chondrocyte cells.

Thus CollaBZen not only protects further degradation of Collagen via extrinsic factors but also boosts the collagen production.

Conclusion

In the current study, CollaBZen^{TM} was shown to exhibit anti-ageing potential by reversing the oxidative stress in NHDF and HCH cells at concentrations 0.02-0.16 mg/ml. Also, the nutraceutical blend CollaBZen^{TM} inhibited collagen breakdown in the UVA-induced skin fibroblast cells and human chondrocyte cells. It has been determined that agents promoting the synthesis of collagen, scavenging reactive oxygen species, and preventing UV-induced damage will be the future of anti-ageing. Hence, CollaBZen^{TM} serves as a potential nutraceutical agent with anti-ageing benefits for the skin to prevent photoageing, wrinkling, sagging, and thinning as well as for the joints by slowing down the process of collagen-degradation and hence reverting osteoarthritis.

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