Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i5.6213

EVALUATION OF COLORLESS CAROTENOIDS FROM WHITE HEIRLOOM TOMATO FOR REGENERATION OF SKIN CELLS VIA REDUCTION OF INFLAMMATION AND STRESS

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Abstract
The article delves into the emerging concept of photoprotection through dietary interventions, emphasizing the pivotal role of plant constituents, specifically carotenoids and flavonoids, in shielding plants from excessive light and preventing UV damage in humans. Tomatoes, globally consumed and known for their flavonoid content, are undergoing efforts to standardize and enhance flavonoid concentrations. Carotenoids, essential for nutrition and health, act as precursors for retinoids, providing health and cosmetic benefits when accumulated in human skin. Colorless carotenoids, particularly phytoene and phytofluene, have gained attention for their bioavailability and potential health benefits. The study introduces heirloom white tomatoes, enriched in phytoene and phytofluene, as a unique source to explore improved MDA and glutathione levels, potentially enhancing antioxidant properties and skincare benefits. The research further investigates the potential of colorless carotenoids derived from white heirloom tomatoes in promoting skin cell regeneration by mitigating inflammation and stress. Methodology involves the preparation of tomato fruit powder, culturing cell lines, and conducting various assays to evaluate cell viability, antioxidant capacity, and skincare benefits. Results demonstrate the cytotoxicity of heirloom tomato powder, its impact on LDH activity, angiogenic potential, reduced MMP-12 and inflammation, decreased MDA, and increased glutathione reductase levels in post-treated cells. The study concludes that heirloom tomato powder, rich in carotenoids, could significantly boost antioxidant production and regeneration. This enhancement may lead to improved skin tone and long-term skincare benefits by promoting skin cell regeneration and reducing inflammation and stress.
Keywords: phytofluene, carotenoids, Heirloom, MMP-12, glutathione and MDA.

1. Introduction
The emerging concept of photoprotection through dietary interventions underscores the pivotal role of plant constituents, especially carotenoids and flavonoids, in shielding plants from excessive light and averting UV damage in humans (Kiefer, Weibel, Smits, Juch, Tiedtke, et al., 2010; Stahl & Sies, 2007b). Tomatoes, globally consumed and renowned for flavonoid content, including quercetin and quercetin glycosides, are undergoing efforts to standardize and enhance flavonoid concentrations

(Crozier, Lean, McDonald, & Black, 1997; Slimestad, Fossen, & Verheul, 2008; Rune Slimestad & Michèl J Verheul, 2005; Stewart et al., 2000).

Carotenoids, vital for nutrition and health, act as precursors for retinoids, providing both health and cosmetic benefits when accumulated in human skin (Biskanaki et al., 2023; Meléndez-Martínez et al., 2022; Udensi, Loughman, Loskutova, & Byrne, 2022). Notably, colorless carotenoids including PT (phytoene) and PTF (phytofluene), have gained attention for their bioavailability and potential health benefits (Khalil et al., 2021; Kiki, 2023; Meléndez-Martínez et al., 2022). However, their oversight in studies using typical colored carotenoid detection wavelengths poses challenges (Rodriguez-Amaya, 2001b). Tomatoes, rich in carotenoids, particularly lycopene, are linked to reduced risks of chronic diseases. Phytoene and phytofluene, colorless carotenoids present in tomatoes, are believed to participate in antioxidant mechanisms, prompting discussions about incorporating PT and PTF into nutricosmetics for both skin appearance and safety (Canene-Adams, Campbell, Zaripheh, Jeffery, & Erdman Jr, 2005; Engelmann, Clinton, & Erdman Jr, 2011; Etminan, Takkouche, & Caamaño-Isorna, 2004; Giovannucci, 2005).

Carotenoids, essential in nutrition and health, are processed into retinoids with vitamin A production and accumulate in human skin, offering health and cosmetic benefits. Among the nearly 800 carotenoids reported, the colourless carotenoids PT and PTF have received considerable attention due to their bioavailability and potential health advantages. More specifically, phytoene and phytofluene may engage in antioxidants (Ben-Dor et al., 2005; Shaish et al., 2008). Their unique absorption wavelengths pose challenges in detection methods, but recent studies have explored their antioxidant capacity and isomerism (Melendez-Martinez, Paulino, Stinco, Mapelli-Brahm, & Wang, 2014; Melendez-Martinez, Stinco, Liu, & Wang, 2013; Stinco, Heredia, Vicario, & Meléndez-Martínez, 2016). Nutricosmetics, bridging food, nutrition, health, and cosmetics, consider PT and PTF as potential components for "beauty from within," aligning with the growing interest in dietary compounds promoting skin appearance and safety (Madhere & Simpson, 2010).

Tomatoes, which contain carotenoids such as lycopene, are linked to a lower risk of chronic disease, while colourless carotenoids such as phytoene and phytofluene are thought to have biological activity. Glutathione, a potent antioxidant with anti-melanogenic properties, has become a common skin-lightening agent, although its efficacy and safety remain a subject of debate (Exner, Wessner, Manhart, & Roth, 2000; Sonthalia, Daulatabad, & Sarkar, 2016b).

The study (patent: WO/2022/238727) emphasizes the importance of glutathione in reduced form (GSH) and introduces heirloom tomatoes, grown without crossbreeding for over 50 years, as a unique source enriched in phytoene and phytofluene, potentially offering antioxidant benefits to cells (Huang & Yin, 2020; Khachik et al., 2002). The same study introduces heirloom white tomatoes, enriched in phytoene and phytofluene, as a unique avenue to explore increased glutathione levels, potentially enhancing antioxidant properties and skincare benefits.

Considering the positive effects of a significant presence of phytoene (PT) and phytofluene (PTF) in heirloom white tomatoes, as well as a prior investigation demonstrating increased glutathione levels through the enhancement of glutathione reductase (Patent No: WO/2022/238727), the current study investigates the potential of colourless carotenoids derived from white heirloom tomatoes (TomesOral) in promoting skin cell regeneration by mitigating inflammation and stress.

2. Methodology

2.1. Preparation of Tomato Fruit Powder

Tomesoral White Tomato is an exclusive tomato powder derived from Heirloom tomatoes recognized for their diverse color, shape, and flavor attributes, originating from plant seeds aged at least 50 years. These tomatoes are rich in phytoene and phytofluene, identified as colorless carotenoids, obtained from the fruit of Organic white Heirloom tomatoes. The tomato fruits underwent meticulous sampling and decontamination through rigorous washings. Subsequently, they were subjected to the freeze-drying method as outlined by Gaware in 2010, resulting in the production of the powdered form (Gaware, Sutar, & Thorat, 2010).

2.2. Culturing of Cell line

The immortalized human keratinocyte cell line, HaCaT cells were maintained in Dulbecco's Modified Eagle Medium-High Glucose (DMEM-HG) supplemented with 10% fetal bovine serum (FBS) and antibiotics until reaching the subculturing phase, at which point they were utilized in ensuing experiments.

2.3. Stock and Dilution Preparation

The tomato fruit powder stock was prepared by dissolving it in distill water. After mixing, passed by syringe filter followed by its different dilutions preparations (100ug/ml, 200ug/ml, 500ug/ml, 1000ug/ml and 2000ug/ml) in plain medium (DMEM).

2.4. Treatment of Cell line with Tomato Fruit Powder

The HaCaT cell line at the second passage was seeded onto a 96-well plate to assess cell proliferation. The cells were exposed to the specified concentrations of tomato fruit powder for a duration of 24 hours. Subsequently, a cell viability assay was conducted on the cells, and the collected medium was utilized for subsequent experimental analyses.

2.5. Cell Viability Assay

The cellular viability was assessed utilizing the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, conducted on cells cultured in a 96-well plate. The cells were subjected to various concentrations of fruit powder for a duration of 24 hours. Following the treatment period, 25 µl of MTT solution was introduced, and after an additional 2-3 hours, the resultant purple-hued crystals were dissolved using a 10% sodium dodecyl sulfate (SDS) solution. Subsequently, absorbance measurements were acquired at a wavelength of 570 nm after 3 hours of incubation (Maqbool et al., 2019).

2.6. LDH released assay

The lactate dehydrogenase (LDH) activity in treated HaCaT cells was quantified in the supernatant collected from all experimental cohorts in accordance with the manufacturer's guidelines (AMP Diagnostics, Austria). A volume of 5 µl of medium from each experimental group was employed for the LDH assay. In brief, 5 µl of cell culture medium from both groups was amalgamated with 100 µl of working reagent in a 96-well plate and subsequently incubated for 5 minutes. Absorbance was then assessed using a spectrophotometer at a wavelength of 340 nm.

2.7. Enzyme Linked Immuno-sorbant assay (ELISA)

The Solid Phase Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) was conducted to assay Vascular Endothelial Growth Factor (VEGF), Matrix Metalloproteinase-12 (MMP-12), and Tumor Necrosis Factor-alpha (TNF-α) following a previously established protocol with minor adjustments (Wajid et al., 2015). In summary, a 96-well plate (Corning, USA) was coated with rabbit polyclonal antibodies against VEGF, MMP-12, and TNF-α (Santa Cruz Biotechnology, USA), followed by a 120-minute incubation. Subsequent to three washes with tris-buffered saline (TBS), blocking was carried out using 1% Bovine Serum Albumin (BSA). Following blocking, 100µl of medium from both cell groups was applied and incubated for 60 minutes. Post-incubation, samples were aspirated, and wells were thrice rinsed with TBS-T before overnight incubation with Horseradish Peroxidase (HRP) conjugated donkey anti-rabbit secondary antibody (Santa Cruz Biotechnology, USA) for 90 minutes. After washing, 100µl of the chromogenic substrate tetramethyl benzidine (TMB) (Invitrogen Inc., USA) was introduced, and the reaction was halted with 0.18M sulfuric acid. Absorbance readings were obtained at a wavelength of 450nm.

2.8. Estimation of MDA

To assess the presence of malondialdehyde (MDA) in tomato powder subjected to pre-treatment and post-treatment, the harvested medium was employed, and MDA quantification was conducted using a commercially available kit in accordance with the manufacturer's prescribed protocol (Sigma Aldrich).

2.9. Estimation of GSH and Glutathione Reductase

In the assessment of glutathione (GSH) and glutathione reductase (GR) enzyme levels within the pre-treated and post-treated medium containing heirloom powder, samples from various experimental groups were analyzed to quantify the concentrations of glutathione reductase (GR) and GSH using their respective assay kits, following the manufacturer's protocol (Sigma Aldrich)

2.10. Statistical analysis

The data from experimental groups were presented as the mean ± standard error of the mean (SEM) across triplicate experiments. Statistical comparisons between group means were conducted using one-way analysis of variance (ANOVA), and Bonferroni's test was employed to discern differences between specific groups. Quantitative data derived from distinct experimental groups underwent statistical analysis through GraphPad software, utilizing two-way ANOVA. Statistical significance was determined by a p-value less than 0.05. References were incorporated using EndNote.

3. RESULTS

3.1. Cytotoxicity of Heirloom Tomato Powder and Optimal Dose Indication

The MTT test, which is a reliable and easy method for determining cell cytotoxicity, was used. Figure 1 (HaCaT cell line) depicts the percentage (%) cytotoxicity of heirloom powder at various doses. On the cell lines, almost all tomato powder concentrations had no appreciable cytotoxic effect. However, larger concentrations, such as 1mg or 2mg, showed cytotoxicity. Based on the cytotoxicities, the optimal dose (OC) was determined as 100ug/ml (Figure 1A & B).

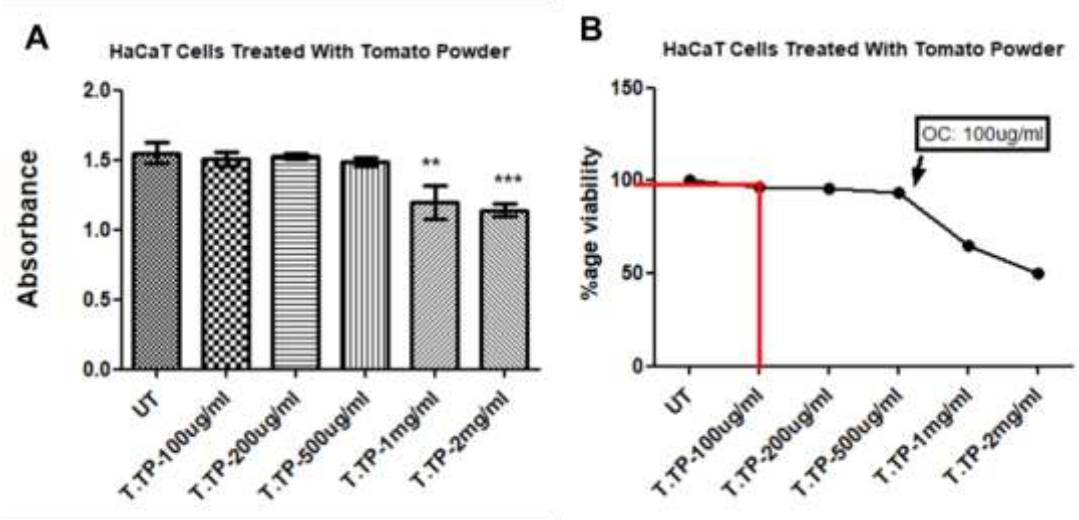

Figure 1: A) Bar graph show the relative values of absorbance after MTT assay. b) Line graph show the relative percentage of viabilities on different concentration of tomato powder while box represent the OC value for tomato powder. Values were taken as mean ± SEM, with * indicating a significant difference between viabilities of treated groups and untreated controls ($p<0.05$).

3.2. Injury reduction in Post Treated Groups

HaCaT cells further treated with 6mM H_2O_2 for 3 hours and then injured cells were treated with the same doses of heirloom tomato as were used in previous experiment. There was a significant decrease in cell viability when cells treated with H_2O_2 and a significant increase in viability after tomato powder treatment was also observed showing significant recovery from injury.

To examine the levels of injury and recovery, the cytoplasmic enzyme lactate dehydrogenase (LDH) produced by cells was measured using the LDH test. LDH is released in the cytosol when cells are injured or stressed. Our findings revealed lower levels of LDH released in HaCaT cell treatment groups when compared to wounded cells, although levels of LDH were still high at considerably greater doses. Based on these LDH release findings and cell viability results, an effective dose range of tomato powder for recovery from H_2O_2 caused stress was determined to be 100ug/ml-500ug/ml with an OC of 100ug/ml (Table 1, Figure 2).

Table 1: Relative absorbance LDH of post treated cells

Groups	UT	H_2O_2 injury	T.TP-100ug/ml	T.TP-200ug/ml	T.TP-500ug/ml	T.TP-1mg/ml	T.TP-2mg/ml
Absorbance (SEM)	0.064±0.00045	0.0984± 0.00682	0.058±0.0011	0.060±0.00023	0.056±0.0016	0.073±0.00065	0.073±0.00053

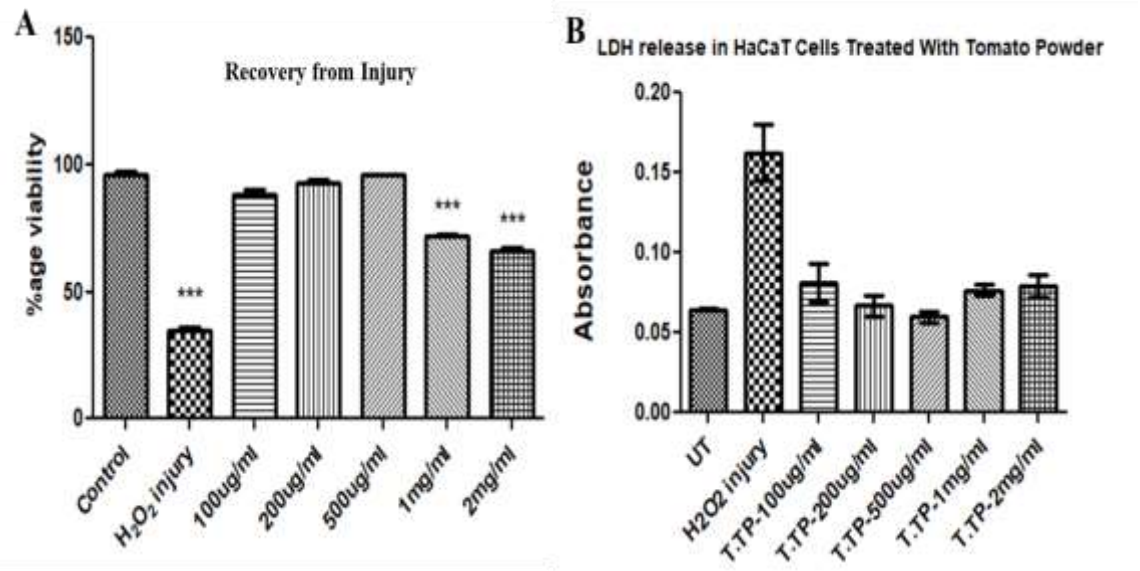

Figure 2: A) Bar graph shows the recovery of HaCaT cells after inflammation injury. B) Graph shows the levels of LDH release in post treated cells. Values were obtained as mean ± SEM, and * indicates a significant difference in cellular viability and LDH levels between treated groups and untreated controls ($p<0.05$).

3.3. Potential of Angiogenesis in HaCaT cells after treatment with Heirloom tomato

After treating HaCaT cells with heirloom tomato, increased angiogenesis was observed via estimating the level of angiogenetic protein VEGF in post treated HaCaT cells. According to our findings, treating the cell line with heirloom tomato improves angiogenesis in HaCaT cells. VEGF levels were determined using ELISA. ELISA analysis of angiogenic secreted protein levels revealed higher amounts of VEGF in post-treated HaCaT cell groups than in H2O2 stressed HaCaT cells (Figure 3A).

3.4. Decreased MMP-12 (elastase) and inflammation (TNF- α) after treatment with heirloom tomato

After treating HaCaT cells with heirloom tomato, reduced MMP-12 and inflammation was observed in stressed cells via estimating the level of enzyme elastase and inflammatory markers TNF- α in post treated HaCaT cells. According to our results, treatment of cell line with heirloom tomato lowers the elastase activity and inflammation levels in HaCaT cells. Levels of elastase and inflammation were estimated by ELISA showed reduced elastase and inflammation levels in heirloom tomato post treated groups as compared to H_2O_2 stressed cells. Whereas in post treated group the levels of elastase and inflammation showed no significant difference from untreated group (Figure 3B & C).

3.5. Decreased MDA in Post Treated HaCaT cells

HaCaT cells' intracellular MDA activity was measured (n = 5 replicates) before and after treatment with heirloom tomato powder. MDA activity was significantly decreased in post treated cells. The presence of heirloom tomato powder in the media significantly lowered the MDA activity (Figure 3D).

Table 2: Absorbance values of VEF, MMP-12, TNF-α and MDA

Groups	Control	H_2O_2 stress	100ug/ml
VEGF	0.598± 0.0199	0.245±0.0159	0.578±0.0196
MMP-1	0.245 ± 0.0217	0.820 ± 0.0327	0.321 ± 0.0543
TNF-α	0.377 ± 0.0375	0.733 ± 0.0184	0.446 ± 0.0233S
MDA	0.443 ± 0.0166	0.884 ± 0.0380	0.532 ± 0.0220

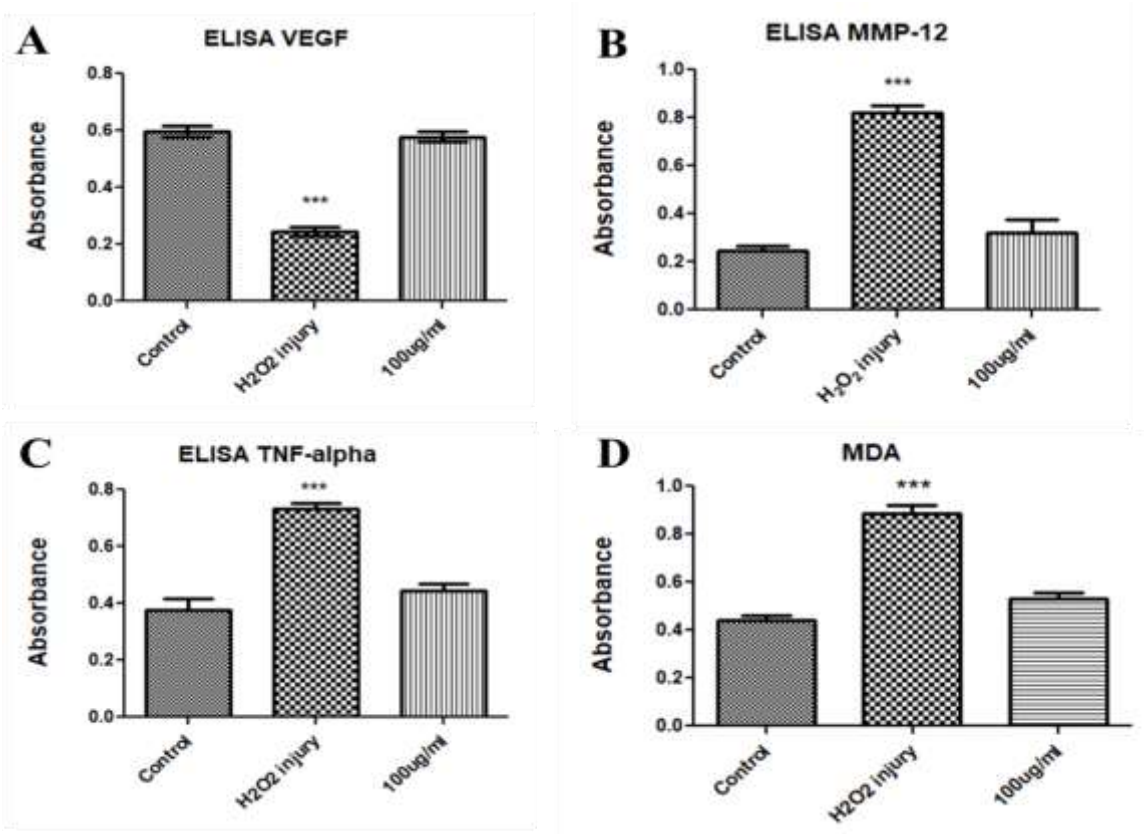

Figure 3: A) The graph depicts the angiogenesis levels in post-treated HaCaT cells. B) The graph depicts the MMP-12 levels in post-treated cells. C) The graph displays the TNF-α level in post-treated cells. D) A bar graph depicts the MDA levels in post-treated HaCaT cells. The values were taken as mean ± SEM, and * indicates a significant difference between treated groups and untreated controls ($p < 0.05$).

3.6. Increased Glutathione Reductase and GSH in Post Treated Groups

H_2O_2 stressed HaCaT cells' intracellular glutathione reductase (GR) activity and GSH levels were measured (n = 5 replicates) before and after treatment with heirloom tomato powder. In tomato powder-treated cells, GR activity increased considerably. White tomato powder increased GR activity from 0.784±0.139 mU/mL before treatment to 5.07±0.323 mU/mL with a dose of 500 ug/mL TomesOral. The addition of more tomato powder to a concentration of 1mg/ml and 2mg/ml will not boost the levels further. The optimum value is observed in 100ug/ml-500ug/ml concentration.

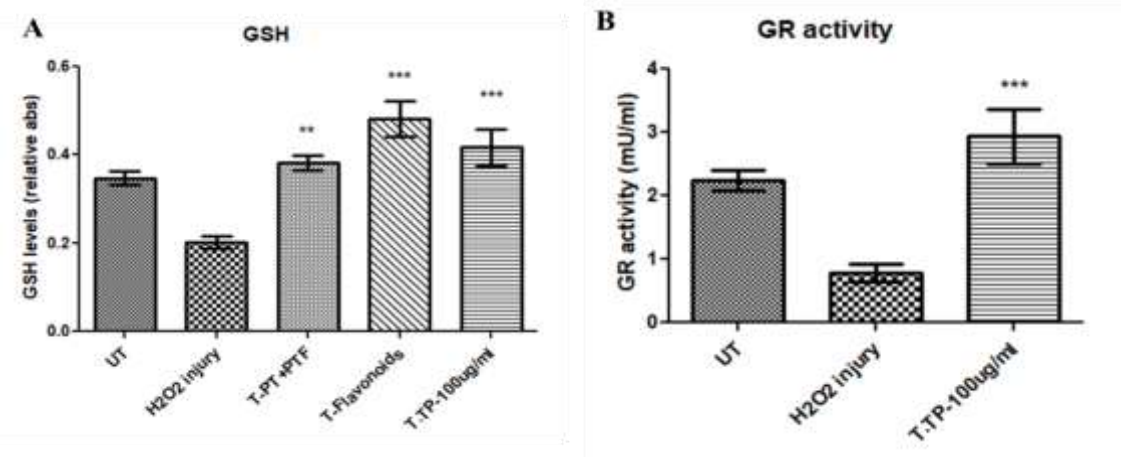

Figure 6: Expression study of GR. The graph displays the GR levels in post-treated cells. Values were taken as mean ± SEM, and * indicates a significant difference in LDH levels between treated groups and untreated controls ($p<0.05$).

4. Discussion

Heirlooms tomatoes, which originated from 50 years old seeds without any cross-breeding are known for their diversity of flovor, shape, color and shape. These are white colored tomato which has been investigated for various biological properties. In this study the role of heirloom tomatos powder was investigated for the increase in glutathione reductase levels. It is basically phytoene and phytofluene rich powder that can cause the increase in glutathione reductase levels. In order to prove this fact, first strategy is to define the non-cytotoxic dose of the tomato powder determined by MTT assay..

The emerging field of photoprotection through dietary interventions highlights the significance of plant constituents, particularly carotenoids and flavonoids, in shielding against UV damage (Kiefer, Weibel, Smits, Juch, Tiedke, et al., 2010; Stahl & Sies, 2007a). Tomatoes, globally renowned for their flavonoid content, are undergoing efforts to enhance their concentrations (Crozier et al., 1997; Slimestad et al., 2008; Rune Slimestad & Michel J Verheul, 2005; Stewart et al., 2000). Carotenoids, essential for health and nutrition, act as precursors for retinoids and offer both health and cosmetic benefits when accumulated in human skin (Biskanaki et al., 2023; Meléndez-Martínez et al., 2022; Udensi et al., 2022). Colorless carotenoids like phytoene (PT) and phytofluene (PTF) have recently gained attention for their bioavailability and potential health benefits (Khalil et al., 2021; Kiki, 2023; Meléndez-Martínez et al., 2022).

MTT dye is used in studies for cell proliferation and cytotoxicity to assess cell viabilities (Shoemaker, Cohen, & Campbell, 2004; Sreelatha, Jeyachitra, & Padma, 2011). Several herbs and phytochemicals were evaluated using the MTT reagent for their cytoprotective or cytotoxic effects (Horakova et al., 2003; Sreelatha et al., 2011). The cytotoxic effect of heirloom tomato powder on HaCaT fibroblast cells was investigated using the MTT reduction test. Heirloom tomato powder significantly inhibits HaCaT cell growth in a dose-dependent manner (Figures 1a and 1b). The summarised results revealed that tomato powder applied to fibroblast HaCaT cells is non-toxic at lower concentrations but has a cytotoxic effect on HaCaT cell growth at concentrations more than 1 mg/ml. So the study advises a dose that has no harmful effects on cells. The dose is further validated by an increase in angiogenesis in post-treated damaged cells.

LDH release is a simple and positive cytotoxicity assay. LDH is an enzyme released when there is disruption to the cell membrane, and it could be estimated in cell culture supernatants. (George, Tynga, & Abrahamse, 2015). In many studies control cells showed lesser LDH release and cells treated with heirloom powder showed higher LDH release (Saad et al., 2006). The same thing happened when we treated HaCaT cell line with heirloom powder LDH level was significantly high but in untreated cells it was not affected. Further, as many metalloproteinases (MMPs), elastase (MMP-12) is able to degrade extracellular matrix components such as elastin and is involved in tissue remodeling processes. After treatment with tomato powder, it is clearly seen that levels of MMP-12

and imflammatory marker TNF-α were found to be significantly lowered as compared to injured groups. Thus, this property of tomato powder will impart certain anti-aging properties to fibroblastic cells even in skin.

However, studies have often overlooked colorless carotenoids due to challenges in detection methods using typical colored carotenoid detection wavelengths (Rodriguez-Amaya, 2001a). Nonetheless, recent research has explored their antioxidant capacity and isomerism (Melendez-Martinez et al., 2014; Melendez-Martinez et al., 2013; Stinco, Escudero-Gilete, Heredia, Vicario, & Melendez-Martinez, 2016), aligning with the growing interest in dietary compounds promoting skin appearance and safety (Madhere & Simpson, 2010). Tomatoes, rich in carotenoids like lycopene, are associated with reduced risks of chronic diseases, with colorless carotenoids like phytoene and phytofluene believed to possess biological activity (Canene-Adams et al., 2005; Engelmann et al., 2011; Etminan, Takkouche, & Caamano-Isorna, 2004). Glutathione, a potent antioxidant, has become a common skin-lightening agent, albeit with debate regarding its efficacy and safety (Exner et al., 2000; Sonthalia, Daulatabad, & Sarkar, 2016a). This study emphasizes the importance of glutathione in its reduced form (GSH) and introduces heirloom white tomatoes, enriched in phytoene and phytofluene, as a unique source potentially offering antioxidant benefits to cells (Khachik et al., 2002). By investigating the effects of colorless carotenoids derived from these tomatoes, particularly phytoene and phytofluene, the study aims to promote skin cell regeneration by mitigating inflammation and stress.

Glutathione contributes in various ways to protecting against oxidative stresses. Firstly, it acts by non-enzymatic or enzymatic electrophilic conjugation in a process that utilizes glutathione irreversibly. Secondly, in the presence of enzyme glutathione peroxidase (GPX), it protects by reduction of peroxides against the oxidative stress. Glutathione (GSH, reduced shape) is oxidized in this latter phase into dimers (GSSG, disulfide shape), which are reduced back to glutathione (GSH) by glutathione reductase (Kondo et al., 2016; Lu, 2009). The reduced glutathione (GSH) through its tyrosine inhibition has a skin-whitening effect. GSH is a compound which inhibits the production of melanin. Glutathione reductase enzyme in the body readily reduces GSSG to GSH. Thus, by increasing the amount of glutathione reductase the level of GSH will enhance and it whitens the skin by acting on melanocytes in the epidermis (Watanabe, Hashizume, Chan, & Kamimura, 2014). The whitening effect of GSH on skin is due to its antioxidant potential. It has capacity to scavenge reactive oxygen species (ROS) produced by UV exposure in epidermal cell and to prevent melanogenesis caused by ROS (Briganti, Camera, & Picardo, 2003; Maeda & Hatao, 2004). In our results, the level of glutathione reductase significantly enhanced after treatment with heirloom tomato powder as compare to untreated ones. This shows a strong evidence for the enriched amount of PT and PTF, which is found in heirloom tomato powder, to play a pivotal role in the increase of glutathione reductase which in turn increase reduced glutathione thus enhancing the skin tone to relative long term basis. Many cosmetic products use glutathione as an active component. Glutathione for skin whitening can be found in cream, soap, lotion, nasal spray, and injectable forms (Dickinson & Forman, 2002; Meister, 1988). Topical glutathione lotion is poorly absorbed by skin cells because the thiol group forms disulfide quickly. Enzymes in the gastrointestinal system hydrolyze glutathione after oral absorption, resulting in reduced bioavailability(Exner et al., 2000). When substantial oral doses were given, glutathione levels temporarily increase(Meister, 1988; Villarama & Maibach, 2005). In contrast, intravenous glutathione delivers very high dosages straight into the systemic circulation and is the recommended form of delivery. However, its safety as an intravenous medication has also been questioned (Zubair, Hafeez, & Mujtaba, 2017). Thus the safest approach is to increase the enzyme (glutathione reductase) levels rather than direct administration of its product (reduced glutathione).

Our findings suggest that heirloom tomato powder, enriched in phytoene and phytofluene, could be a potent agent for enhancing glutathione reductase production, contributing to improved skin tone and long-term skincare benefits. The optimal dose range for effective treatment without cytotoxic effects was identified, and post-treatment with tomato powder showed significant recovery from

injury, reduced LDH release, increased angiogenesis, decreased MMP-12 and inflammation, reduced MDA levels, and increased glutathione reductase and GSH levels.

5. Conclusion:

In conclusion, the investigation into the potential of colorless carotenoids from heirloom white tomatoes for skin cell regeneration highlights promising prospects for skincare interventions. The study confirms the non-cytotoxic effects of heirloom tomato powder and demonstrates its efficacy in mitigating inflammation, reducing oxidative stress, and promoting skin cell regeneration.

6. Future Perspectives:

Future studies could dive deeper into the molecular mechanisms that underpin the observed effects of colourless carotenoids on skin cells. Furthermore, clinical investigations on human subjects could validate the skincare benefits of heirloom tomato powder and investigate its use in skincare formulations. Furthermore, studies investigating the synergistic effects of phytoene and phytofluene with other skincare components may yield new insights into skincare product creation. Overall, the study lays the path for novel dietary interventions in skincare, highlighting the potential of natural chemicals to promote skin health and vitality.

7. References

1. Ben-Dor, A., Steiner, M., Gheber, L., Danilenko, M., Dubi, N., Linnewiel, K., . . . Levy, J. 2005. Carotenoids activate the antioxidant response element transcription system. Molecular Cancer Therapeutics, 4(1), 177-186.
2. Biskanaki, F., Kalofiri, P., Tertipi, N., Sfyri, E., Andreou, E., Kefala, V., & Rallis, E. 2023. Carotenoids and dermoaesthetic benefits: Public health implications. Cosmetics, 10(5), 120.
3. Briganti, S., Camera, E., & Picardo, M. 2003. Chemical and instrumental approaches to treat hyperpigmentation. Pigment Cell Research, 16(2), 101-110.
4. Canene-Adams, K., Campbell, J. K., Zaripheh, S., Jeffery, E. H., & Erdman Jr, J. W. 2005. The tomato as a functional food. The Journal of nutrition, 135(5), 1226-1230.
5. Crozier, A., Lean, M. E., McDonald, M. S., & Black, C. 1997. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. Journal of agricultural and food chemistry, 45(3), 590-595.
6. Dickinson, D. A., & Forman, H. J. 2002. Glutathione in defense and signaling: lessons from a small thiol. Annals of the New York Academy of Sciences, 973(1), 488-504.
7. Engelmann, N. J., Clinton, S. K., & Erdman Jr, J. W. 2011. Nutritional aspects of phytoene and phytofluene, carotenoid precursors to lycopene. Advances in Nutrition, 2(1), 51-61.
8. Etminan, M., Takkouche, B., & Caamano-Isorna, F. 2004. The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. Cancer Epidemiology Biomarkers & Prevention, 13(3), 340-345.
9. Etminan, M., Takkouche, B., & Caamaño-Isorna, F. 2004. The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. Cancer Epidemiology and Prevention Biomarkers, 13(3), 340-345.
10. Exner, R., Wessner, B., Manhart, N., & Roth, E. 2000. Therapeutic potential of glutathione. Wiener Klinische Wochenschrift, 112(14), 610-616.
11. Gaware, T., Sutar, N., & Thorat, B. 2010. Drying of tomato using different methods: comparison of dehydration and rehydration kinetics. Drying Technology, 28(5), 651-658.
12. George, B. P. A., Tynga, I. M., & Abrahamse, H. 2015. In vitro antiproliferative effect of the acetone extract of Rubus fairholmianus gard. Root on human colorectal cancer cells. BioMed research international, 2015.
13. Giovannucci, E. 2005. Tomato products, lycopene, and prostate cancer: a review of the epidemiological literature. The Journal of nutrition, 135(8), 2030S-2031S.

14. Horakova, L., Licht, A., Sandig, G., Jakstadt, M., Duracková, Z., & Grune, T. 2003. Standardized extracts of flavonoids increase the viability of PC12 cells treated with hydrogen peroxide: effects on oxidative injury. Archives of toxicology, 77(1), 22-29.
15. Huang, C., & Yin, Z. 2020. Highly Efficient Synthesis of Glutathione via a Genetic Engineering Enzymatic Method Coupled with Yeast ATP Generation. Catalysts, 10(1), 33.
16. Khachik, F., Carvalho, L., Bernstein, P. S., Muir, G. J., Zhao, D.-Y., & Katz, N. B. 2002. Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. Experimental biology and medicine, 227(10), 845-851.
17. Khalil, A., Tazeddinova, D., Aljoumaa, K., Kazhmukhanbetkyzy, Z. A., Orazov, A., & Toshev, A. D. 2021. Carotenoids: Therapeutic strategy in the battle against viral emerging diseases, COVID-19: An overview. Preventive Nutrition and Food Science, 26(3), 241.
18. Kiefer, S., Weibel, M., Smits, J., Juch, M., Tiedke, J., & Herbst, N. 2010. Citrus flavonoids with skin lightening effects–Safety and efficacy studies. Int J Appl Sci, 132, 46-54.
19. Kiefer, S., Weibel, M., Smits, J., Juch, M., Tiedtke, J., & Herbst, N. 2010. Citrus flavonoids with skin lightening effects–safety and efficacy studies. Int J Appl Sci, 132, 46-54.
20. Kiki, M. J. 2023. Biopigments of microbial origin and their application in the cosmetic industry. Cosmetics, 10(2), 47.
21. Kondo, M., Kawabata, K., Sato, K., Yamaguchi, S., Hachiya, A., Takahashi, Y., & Inoue, S. 2016. Glutathione maintenance is crucial for survival of melanocytes after exposure to rhododendrol. Pigment cell & melanoma research, 29(5), 541-549.
22. Lu, S. C. 2009. Regulation of glutathione synthesis. Molecular aspects of medicine, 30(1-2), 42-59.
23. Madhere, S., & Simpson, P. 2010. COS DERM.
24. Maeda, K., & Hatao, M. 2004. Involvement of photooxidation of melanogenic precursors in prolonged pigmentation induced by ultraviolet A. Journal of investigative dermatology, 122(2), 503-509.
25. Maqbool, T., Awan, S. J., Malik, S., Hadi, F., Shehzadi, S., & Tariq, K. 2019. In-Vitro Anti-Proliferative, Apoptotic and Antioxidative Activities of Medicinal Herb Kalonji (Nigella sativa). Current Pharmaceutical Biotechnology, 20(15), 1288-1308.
26. Meister, A. 1988. Glutathione metabolism and its selective modification. Journal of Biological Chemistry, 263(33), 17205-17208.
27. Meléndez-Martínez, A. J., Mandić, A. I., Bantis, F., Böhm, V., Borge, G. I. A., Brnčić, M., . . . Elgersma, A. 2022. A comprehensive review on carotenoids in foods and feeds: Status quo, applications, patents, and research needs. Critical reviews in food science and nutrition, 62(8), 1999-2049.
28. Melendez-Martinez, A. J., Paulino, M., Stinco, C. M., Mapelli-Brahm, P., & Wang, X.-D. 2014. Study of the time-course of cis/trans (Z/E) isomerization of lycopene, phytoene, and phytofluene from tomato. Journal of agricultural and food chemistry, 62(51), 12399-12406.
29. Melendez-Martinez, A. J., Stinco, C. M., Liu, C., & Wang, X.-D. 2013. A simple HPLC method for the comprehensive analysis of cis/trans (Z/E) geometrical isomers of carotenoids for nutritional studies. Food Chemistry, 138(2-3), 1341-1350.
30. Rodriguez-Amaya, D. B. (2001a). *A guide to carotenoid analysis in foods* (Vol. 71): ILSI press Washington.
31. Rodriguez-Amaya, D. B. (2001b). *A guide to carotenoid analysis in foods*: ILSI press Washington.
32. Saad, B., Dakwar, S., Said, O., Abu-Hijleh, G., Battah, F. A., Kmeel, A., & Aziazeh, H. 2006. Evaluation of medicinal plant hepatotoxicity in co-cultures of hepatocytes and monocytes. Evidence-Based Complementary and Alternative Medicine, 3.
33. Shaish, A., Harari, A., Kamari, Y., Soudant, E., Harats, D., & Ben-Amotz, A. 2008. A carotenoid algal preparation containing phytoene and phytofluene inhibited LDL oxidation in vitro. Plant foods for human nutrition, 63(2), 83-86.

34. Shoemaker, M., Cohen, I., & Campbell, M. 2004. Reduction of MTT by aqueous herbal extracts in the absence of cells. Journal of Ethnopharmacology, 93(2-3), 381-384.
35. Slimestad, R., Fossen, T., & Verheul, M. J. 2008. The flavonoids of tomatoes. Journal of agricultural and food chemistry, 56(7), 2436-2441.
36. Slimestad, R., & Verheul, M. J. 2005. Content of chalconaringenin and chlorogenic acid in cherry tomatoes is strongly reduced during postharvest ripening. Journal of agricultural and food chemistry, 53(18), 7251-7256.
37. Slimestad, R., & Verheul, M. J. 2005. Seasonal variations in the level of plant constituents in greenhouse production of cherry tomatoes. Journal of agricultural and food chemistry, 53(8), 3114-3119.
38. Sonthalia, S., Daulatabad, D., & Sarkar, R. 2016a. Glutathione as a skin whitening agent: facts, myths, evidence and controversies. Indian journal of dermatology, venereology and leprology, 82, 262.
39. Sonthalia, S., Daulatabad, D., & Sarkar, R. 2016b. Glutathione as a skin whitening agent: Facts, myths, evidence and controversies. Indian Journal of Dermatology, Venereology, and Leprology, 82(3), 262.
40. Sreelatha, S., Jeyachitra, A., & Padma, P. 2011. Antiproliferation and induction of apoptosis by Moringa oleifera leaf extract on human cancer cells. Food and Chemical Toxicology, 49(6), 1270-1275.
41. Stahl, W., & Sies, H. 2007a. Carotenoids and flavonoids contribute to nutritional protection against skin damage from sunlight. Molecular biotechnology, 37, 26-30.
42. Stahl, W., & Sies, H. 2007b. Carotenoids and Flavonoids Contribute to Nutritional Protection against Skin Damage from Sunlight. Molecular Biotechnology, 37(1), 26-30. doi: 10.1007/s12033-007-0051-z
43. Stewart, A. J., Bozonnet, S., Mullen, W., Jenkins, G. I., Lean, M. E., & Crozier, A. 2000. Occurrence of flavonols in tomatoes and tomato-based products. Journal of agricultural and food chemistry, 48(7), 2663-2669.
44. Stinco, C. M., Escudero-Gilete, M. L., Heredia, F. J., Vicario, I. M., & Melendez-Martinez, A. J. 2016. Multivariate analyses of a wide selection of orange varieties based on carotenoid contents, color and in vitro antioxidant capacity. Food Research International, 90, 194-204.
45. Stinco, C. M., Heredia, F. J., Vicario, I. M., & Meléndez-Martínez, A. J. 2016. In vitro antioxidant capacity of tomato products: Relationships with their lycopene, phytoene, phytofluene and alpha-tocopherol contents, evaluation of interactions and correlation with reflectance measurements. LWT-Food Science and Technology, 65, 718-724.
46. Udensi, J., Loughman, J., Loskutova, E., & Byrne, H. J. 2022. Raman Spectroscopy of Carotenoid Compounds for Clinical Applications—A Review. Molecules, 27(24), 9017.
47. Villarama, C., & Maibach, H. 2005. Glutathione as a depigmenting agent: an overview. International journal of cosmetic science, 27(3), 147-153.
48. Wajid, N., Naseem, R., Anwar, S. S., Awan, S. J., Ali, M., Javed, S., & Ali, F. 2015. The effect of gestational diabetes on proliferation capacity and viability of human umbilical cord-derived stromal cells. Cell and tissue banking, 16(3), 389-397.
49. Watanabe, F., Hashizume, E., Chan, G. P., & Kamimura, A. 2014. Skin-whitening and skin-condition-improving effects of topical oxidized glutathione: a double-blind and placebo-controlled clinical trial in healthy women. Clinical, cosmetic and investigational dermatology, 7, 267.
50. Zubair, S., Hafeez, S., & Mujtaba, G. 2017. Efficacy of intravenous glutathione vs. placebo for skin tone lightening. Journal of Pakistan Association of Dermatology, 26(3), 177-181.