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The Effect of Stevia (*Stevia rebaudiana* Bertoni) Leaf Extract Cream on Transforming Growth Factor-β1 Levels and Collagen Amount in the Skin of Male Wistar Rats (*Rattus norvegicus*) Exposed to Ultraviolet B

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ABSTRACT

Ultraviolet B (UVB) exposure is a major cause of skin aging, leading to collagen degradation and wrinkles due to free radical damage. Stevia (*Stevia rebaudiana* Bertoni) has antioxidant properties that may counteract this by preserving Transforming Growth Factor-β1 (TGF-β1), essential for collagen synthesis. This study evaluated the effects of stevia leaf extract cream on TGF-β1 and collagen levels in male Wistar rats. Thirty rats were divided into five groups: K0 (no UVB, no cream), K1 (base cream), P1 (2.5% stevia cream), P2 (5% stevia cream), and P3 (10% stevia cream). Groups K1, P1, P2, and P3 were exposed to a cumulative UVB exposure of 840 mJ/cm^2 over four weeks. However, TGF-β1 levels measured by ELISA did not differ significantly among groups ($p>0.05$). However, collagen levels, assessed using Picro Sirius Red staining, varied significantly ($p<0.05$). The K1 exhibited significantly lower collagen compared to K0 ($p=0.003$), whereas the P2 group effectively prevented collagen degradation compared to K1 (K1: 65.87% vs. P2: 77.92%; $p = 0.005$). While stevia leaf extract cream did not prevent TGF-β1 reduction, it demonstrated a clear protective effect against UVB-induced collagen loss. Among the tested concentrations, 5% stevia leaf extract cream was the most effective in preserving dermal Collagen, highlighting its potential as a topical agent for preventing photoaging and maintaining skin structural integrity.

1. Introduction

Ultraviolet B radiation promotes the breakdown of the extracellular matrix (ECM). This also includes Collagen and elastin, contributing to skin aging (photoaging). Transforming Growth Factor-β1 and Smad proteins regulate type I collagen synthesis. However, Smad7 negatively regulates this pathway. Ultraviolet exposure increases Smad7 expression, disrupting the TGF-β/Smad signaling pathway. It acts as an inhibitor of sirtuin synthesis, accelerating skin aging (Park *et al.* 2017, 2018).

One way to prevent UV-induced skin aging is to use UV-chemical substances that neutralize free radicals by either donating or accepting electrons, thereby reducing the presence of unpaired electrons (Uwa 2017). Although the electron skin has protective antioxidant networks, continuous UV radiation exposure and the aging process can deplete antioxidants in all layers throughout the

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skin, as observed in UV-exposed layers B (Pandel *et al.* 2013). Antioxidant application on the skin has been shown to provide extra protection against damage caused by sunlight, slow down the skin aging process, lower irritation, and improve (Uwa 2017). Plants contain active compounds that can produce significant amounts of antioxidants to counteract reactive radicals and protect the skin from harmful ultraviolet rays (Hidayah *et al.* 2023).

Stevia (*Stevia rebaudiana* Bertoni) is a traditional herb native to South America, developed and used to produce a sweetener. The sweet steviol glycosides contain, among others, stevioside and rebaudioside A. It has a sweetness level of about 250-300 times higher than sucrose (Gaweł-Bęben *et al.* 2015; Sinta & Sumaryono 2019). Growing to about 65–80 cm in height, *Steviadiana* Bertall is a small perennial plant with sessile leaves arranged in opposite pairs. It is the sweetest among the various Stevia species due to its high concentration of natural sweetening compounds. Adapted to semi-humid, subtropical conditions, it can be cultivated with ease, similar to common vegetable crops, even in a kitchen garden. It requires well-drained red or clay soils that also contain sand with a pH between 6.5 and 7.5 for optimal growth (Pillwan *et al.* 2020).

Studies have investigated the medicinal applications of stevia, including antidiabetic, antihyperlipidemic, antihypertensive, antiobesity, anti-inflammatory, antioxidant, anticancer, antiviral, and antimicrobial properties, as well as the enhancement of liver and kidney functions (Latarissa *et al.* 2020). Besides functionally glycosides, stevia leaf also contains various other phytoconstituents, including phenolic acids and flavonoids, which have high antioxidant capacity (Gaweł-Bęben *et al.* 2015).

The study seeks to determine whether topical application of stevia leaf extract cream can lessen UVB-induced skin damage by reducing TGF-β1 expression and maintaininTGF-β1ancing dermal collagen production. This study is based on the knowledge that UVB radiation contributes to photoaging and the breakdown of the dermal extracellular matrix, primarily by reducing invol levels and downregulating the β1 integrin, which is essential for Collagen synthesis and TGF-β1 (Ke & Wang 2021). Given the well-documented antioxidant and anti-inflammatory properties of stevia, it is hypothesized that the topical application of stevia leaf extract cream will significantly prevent the reduction of TGF- β1 levels and collagen amount in UVB-exposed skin compared to untrTGF-β1ontrols. Thus, stevia may counteract the detrimental effects of UVB radiation and support the regeneration and structural integrity of the skin.

2. Materials and Methods

2.1. Materials

Thirty male Wistar rats (*Rattus norvegicus*), aged 10 to 12 weeks, and weighing between 200-250 grams, were obtained from the Integrated Biomedical Laboratory Unit of Universitas Udayana, Denpasar, Bali, Indonesia, and used as the animal model. Stevia leaves used in this study were fresh leaves picked from a stevia plantation in Wanagiri Village, Sukasada District, Buleleng, Bali, Indonesia, in September 2024. TGF-β1 ELISA Kit was obtained from Reed Biotech Ltd, China, Catalog No.: RE10013. Picro-Sirius Red Stain Kit (For Collagen) was obtained from ScyTek Laboratories Inc., USA.

2.2. Methods of Plant Determination

The plant was identified as *Stevia rebaudiana* (Bertoni) Bertoni, also known by its synonym *Eupatorium rebaudianum* Bertoni, by the Laboratory of the Functional Implementation Unit for Traditional Health Services in Tawangmangu, Central Java, Indonesia. The identification was documented under the reference number TL.02.04/D.XI.6/22566.1103/2024.

2.3. Plant Materials and Extractions

The processing of stevia leaf extract began with the careful selection of fresh and intact leaves. The leaves were thoroughly cleaned and washed, then oven-dried at 50±3°C for 12 hours until the moisture content was reduced to less than 10%. The dried leaves were then crushed into small pieces, blended into a fine powder, and sieved using a 40-mesh sieve to produce high-quality dry stevia leaf powder.

The extraction of stevia leaf was carried out by maceration, with 300 grams of stevia leaf powder soaked in a 96% ethanol solution at a 1:10 ratio. The mixture was intermittently stirred and allowed to sit at room temperature for 2 × 24 hours. After filtration, the filtrate was subjected to solvent removal using a vacuum rotary evaporator at 40°C for 3 hours, yielding 36 g of crude extract, corresponding to a 12% extraction yield. The percentage represents the number of active compounds contained in the sample (Hesturini *et al.* 2023). Phytochemical analysis of stevia leaf extract revealed the existence of active compounds, including flavonoids (3708.43 mg QE/100 g), phenols (923.89 mg GAE/100 g), tannins (509.52 mg TAE/100 g), antioxidant capacity

(5157.32 GAEAC mg/L), and an IC_{50} value of 165.62 ppm.

2.4. Stevia Leaf Extract Cream Preparation

The concentrations of stevia leaf extract used in this study were selected based on previous studies by Das *et al.* (2009, 2012, 2013), which demonstrated that topical formulations containing 2.5% and 5% stevia extract are safe and non-toxic. The 2.5% formulation showed better physical stability, and neither concentration caused skin irritation in animal or human studies. Additionally, a clinical trial using 5% stevia gel applied for 21 days showed improved skin hydration, brightness, and smoothness, and reduced fine wrinkles and mild acne. These findings support the potential of stevia extract for safe and effective skincare applications and justify its use in evaluating a possible dose-dependent protective effect against UVB-induced skin damage while maintaining formulation stability and tolerability (Das *et al.* 2009, 2012, 2013).

The cream formulation utilized an oil-in-water (O/W) emulsion, comprising a base cream and three variations with stevia leaf extract concentrations of 2.5%, 5%, and 10%, as presented in Table 1. In the water phase, methylparaben, propylparaben, glycerin, and Tween 80 were dissolved, followed by the addition of triethanolamine (TEA) with thorough mixing. For the oil phase, liquid paraffin, stearic acid, and cetyl alcohol were melted in a water bath and cooled to 40°C. The water and oil phases were combined in the mortar, stirred vigorously to form the emulsion, and blended with TEA to ensure uniformity. Once the cream base was prepared, 25% of the mixture was reserved as a base cream. The remaining 75% was divided into three equal portions, with each portion mixed with stevia extract at concentrations of 2.5%, 5%, and 10% (2.5 g, 5 g, and 10 g per 100 g of cream, respectively). Each cream was mixed thoroughly, transferred to sterilized containers, cooled, and sealed.

Table 1. Formulation of *Stevia rebaudiana* Bertoni leaf extract cream and base cream

Ingredients	Base (%)	I (%)	II (%)	III (%)
Stevia leaf extract	-	2.5	5	10
Stearic acid	18	18	18	18
Liquid paraffin	10	10	10	10
Cetyl alcohol	2	2	2	2
Tween 80	1	1	1	1
Triethanolamine(TEA)	2	2	2	2
Methylparaben	0.18	0.18	0.18	0.18
Propylparaben	0.02	0.02	0.02	0.02
Glycerin	5	5	5	5
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100

2.5. Animal Preparation

This research adhered to the ethical standards outlined by the International Conference on Harmonization–Good Clinical Practice (ICH-GCP) and was approved by the Research Ethics Committee, Faculty of Medicine, Universitas Udayana, under ethical clearance number 2458/UN14.2.2.VII.14/LT/2024. The study utilized 30 male Wistar rats (*Rattus norvegicus*). They are 10 to 12 weeks old and weigh between 200 and 250 grams as the animal model. The rats were acclimatized for a week while being fed a commercial pellet diet (Hi-Pro-Vite 594) and provided with free access to boiled water. They were housed in 10 plastic cages, each measuring 50 × 40 × 20 cm, with three rats per cage to ensure comfort and safety. Environmental conditions were maintained under a 12-hour light-dark cycle. In addition, the temperature is set at 25±2°C, with humidity at 50±10%. At the beginning of the study, the dorsal hair of the rats was shaved over a 2 × 2 cm area.

2.6. Experimental Design of Animal Treatment

This research used a randomized post-test-only control group design. The Federer formula $(t - 1)(n - 1) \geq 15$) was used to calculate the sample size, where t represents the number of groups and n the number of animals per group (Rukmana *et al.* 2022). This computation indicated that the study needed at least five rats per group. The sample size was expanded to six rats per group to account for a possible 10% dropout rate. This adjustment ensured that the final number of analyzable subjects would still meet the minimum requirement, even in the event of animal loss or exclusion during the experimental period.

Here, five groups were created to divide the rats by six in each group (n = 6): a normal control group that received no treatment (K0); a negative control group that received a base cream and was exposed to UVB (K1), and three treatment groups (P1, P2, P3) receiving stevia leaf extract cream at concentrations of 2.5%, 5%, and 10% respectively, which were also exposed to UVB. The base cream and stevia leaf extract creams were applied twice daily at doses up to 0.05 g/cm^2: first, 20 minutes before UV exposure to allow adequate absorption into the skin, and then again 4 hours after irradiation, as reactive oxygen species (ROS) began to form at that time. The creams were also applied on days without irradiation. UVB exposure using a UVB Lamp PL-S

9 Watt (Phillips, Poland) was administered to the rats in groups K1, P1, P2, and P3 on Monday, Wednesday, and Friday at a vertical distance of 5 cm at 10:00 AM. UVB exposure began at 50 mJ/cm^2 for 50 seconds in the first week, increased to 70 mJ/cm^2 for 70 seconds in the second week, and then to 80 mJ/cm^2 for 80 seconds during the following two weeks. This resulted in a total UVB exposure of 840 mJ/cm^2 per group over four weeks (Wiraguna *et al.* 2019).

On the 30th day, skin biopsies were conducted on five groups of rats, performed 2 × 24 hours after the last irradiation. Its purpose is to eliminate the acute effects of radiation exposure. All of the rats were anesthetized using an intramuscular injection of Ketamine 10 mg/kg BW and also Xylazine 2 mg/kg BW, followed by euthanasia through cervical dislocation. Skin samples were then taken from the dorsal area of each rat using a punch biopsy technique, 10 mm in diameter and 0.2 mm in depth. The specimens were divided into two parts: the first was stored in phosphate-buffered saline (PBS) at -20°C for 24 hours for TGF-β1 measurement, while the second was kept in 10% neutral-buffered formalin at room temperature for histological preparation with Picrosirius Red staining to assess collagen content.

2.7. Measurement of TGF-β1 Levels

Skin tissue samples collected on the 30th day were prepared for TGF-β1 measurement by rinsing with cold PBS, weighing, cutting into small pieces, and homogenizing in a 1:9 tissue-to-PBS ratio on ice. Further cell disruption was performed using a sonicator, followed by centrifugation to collect the supernatant containing soluble proteins. ELISA measured TGF-β1 levels. It's followed by the protocol of the TGF-β1 ELISA Kit (Reed Biotech Ltd, China), Catalog No.: RE10013. The procedure involved 100 µL of the sample or standard in each well, followed by sequential incubations with Biotinylated Detecting Antibody, HRP conjugate, and Substrate Reagent at 37°C, with washing steps between each addition. To terminate the reaction, a Stop Solution was applied. Finally, absorption is measured at 450 nm, and TGF-β1 levels are determined using a standard curve.

2.8. Histological Examination for Collagen

Skin tissues were blocked using a paraffin-embedded block cut into five µm slices and stained with Picro-Sirius Red Stain for collagen (ScyTek Laboratories Inc., USA). The amount of dermal Collagen was calculated by measuring the pixel area of the dermis containing bright red Collagen. The evaluation was performed on JPEG photographs of the specimens, captured with an Optilab Pro (Miconos, Indonesia) camera and an Olympus CX41 (Japan) microscope using a 10 × 40 objective. Each specimen was photographed at three sites per subject. Then, the collagen content in the dermis was quantified using Adobe Photoshop CS6 and ImageJ. The results were determined using the following formula and presented as a percentage (%).

$$\text{Collagen area percentage} = \frac{\text{Collagen - Stained area}}{\text{Total tissue area}} \times 100$$

2.9. Data Analysis

For the analysis, SPSS v27.0 for Windows was used. The data analysis began with descriptive statistics to summarize characteristics, such as mean, median, standard deviation, and range, and with normality testing using the Shapiro-Wilk test. In addition, the homogeneity of variance was evaluated through Levene's test. The differences between groups were analyzed using One-Way Analysis of Variance (ANOVA). When significant differences were identified, pairwise comparisons were conducted using a post hoc Least Significant Difference (LSD) test. In statistical assessment, a p-value below 0.05 is considered significant.

3. Results

3.1. TGF-β1 Levels

Table 2 shows the One-Way ANOVA test for TGF-β1 levels, yielding a p-value of 0.813 ($p \geq 0.05$). This suggests that there are no statistically significant differences in TGF-β1 levels among the groups. Consequently, a post hoc multiple-comparison test was not conducted, as the primary test did not reveal any significant differences between the samples. However, when the TGF-β1 levels were ranked, the order was as follows: P2 (5% stevia) > P1 (2.5% stevia) > K0 (standard control) > K1 (negative control) > P3 (10% stevia). This result indicates that the P2 group had the highest TGF-β1 levels, followed by P1, K0, K1, and P3, which had the lowest levels.

3.2. Collagen Amount

The significance analysis result of collagen amount was conducted using a One-Way ANOVA test, shown in Table 3. The analysis yielded a p-value of 0.024, indicating a significant difference in collagen amount among the five groups ($p<0.05$). Post-hoc analysis was carried out through the LSD test. The pairwise

Table 2. One-Way ANOVA Analysis Result of TGF-β1 Levels

Groups	N	Minimum (pg/mL)	Maximum (pg/mL)	Mean±SD (pg/mL)	P Value
K0	6	79.58	709.58	364.44±296.63	0.813
K1	6	67.08	797.08	301.81±291.75	
P1	6	55.42	728.75	368.06±305.97	
P2	6	101.25	722.92	368.89±247.40	
P3	5	137.92	287.08	204.58±56.88	

K0: normal control, K1: negative control, P1: 2.5% stevia cream + UVB, P2:5% stevia cream + UVB, P3:10% stevia cream + UVB

Table 3. One-way ANOVA analysis result of collagen amount

Groups	N	Minimum (%)	Maximum (%)	Mean±SD (%)	P Value
K0	6	69.78	83.79	78.50±5.36	0.024
K1	6	57.59	72.05	65.87±6.56	
P1	6	66.45	82.32	73.77±7.13	
P2	6	66.05	87.42	77.92±6.86	
P3	6	64.45	82.82	72.79±7.67	

K0: normal control, K1: negative control, P1: 2.5% stevia cream + UVB, P2:5% stevia cream + UVB, P3:10% stevia cream + UVB

comparison showed statistically significant differences between K0 and K1 ($p = 0.003$) and between K1 and P2 ($p = 0.005$), as shown in Table 4 and Figure 1. Specifically, the mean collagen amount in group K1 was significantly lower than in group K0 ($p = 0.003$), suggesting that UVB exposure significantly reduced Collagen in the skin. Meanwhile, group P2 exhibited a significantly higher mean collagen amount than group K1 ($p = 0.005$), suggesting that 5% stevia leaf extract cream effectively inhibited collagen degradation in UVB-exposed rat skin. Meanwhile, no significant differences were observed among groups K1, P1, and P3 ($p>0.05$).

3.3. Histological Features of Collagen with Picro Sirius Red Staining

The histological examination revealed several differences in collagen fiber structure among the groups presented in Figure 2. In the K0 group (standard control), collagen fibers appeared wide, thick, and intact, with a vibrant red hue, indicating a healthy collagen structure. In contrast, the K1 group (negative control) showed smaller, thinner, shorter, and more fragmented collagen fibers, with fewer intact fibers than in all other groups. Additionally, the Collagen in K1 did not well absorb the Picrosirius Red staining, suggesting reduced collagen integrity. Treatment groups demonstrated varying degrees of improvement in collagen structure. The P1 group (2.5% stevia cream + UVB) exhibited thicker,

Table 4. Post hoc LSD analysis result of collagen amount

Groups	Mean difference	CI 95%		P Value
		Lower bound	Upper bound	
K0 vs K1	12.62	4.58	20.66	0.003*
K0 vs P1	4.73	-3.31	12.77	0.237
K0 vs P2	0.58	-7.46	8.62	0.883
K0 vs P3	5.70	-2.34	13.74	0.157
K1 vs P1	-7.90	-15.94	0.14	0.054
K1 vs P2	-12.04	-20.08	-4.00	0.005*
K1 vs P3	-6.92	-14.96	1.12	0.089
P1 vs P2	-4.15	-12.19	3.89	0.298
P1 vs P3	0.98	-7.06	9.02	0.804
P2 vs P3	5.12	-2.92	13.16	0.201

K0: normal control, K1: negative control, P1: 2.5% stevia cream + UVB, P2:5% stevia cream + UVB, P3:10% stevia cream + UVB. *p<0.05

wider collagen fibers with a more intense red hue than K1. The P2 group (5% stevia cream + UVB) showed even greater improvement, with thicker, wider collagen fibers and a deeper red hue than those observed in K1. The P3 group (10% stevia cream + UVB) displayed thicker, wider, and redder collagen fibers than K1, but some fibers appeared slightly shorter and thinner than in K0 and P2.

4. Discussion

Exposure to ultraviolet radiation, particularly UVB, generates ROS, leading to damage to DNA, proteins, and lipids, as well as activating pathways associated with oxidative stress. Activation of these pathways, including the Mitogen-Activated Protein Kinase (MAPK) and Activator Protein (AP-1) pathways, increases the expression of Matrix Metalloproteinases (MMPs), which accelerate the degradation of Collagen. Ultraviolet also suppresses TGF-β, a key factor in collagen synthesis. This imbalance accelerated collagen degradation beyond its rate of production, disrupting the dermal structure, reducing elasticity, and contributing to the appearance of photoaging (Seol *et al.* 2021). Transforming Growth Factor-β1 regulates procollagen production and is involved in collagen synthesis through the TGF-β/Smad signaling pathway (Chen & Hou 2016). The application of stevia leaf extract cream is expected to prevent or minimize this decline in TGF-β1. While a descriptive analysis revealed higher mean TGF-β1 levels in the standard control and 2.5% and 5% stevia leaf extract compared to the negative control group and the 10% stevia leaf extract group, these differences were not statistically significant. The groups treated with 2.5% and 5% stevia leaf extract

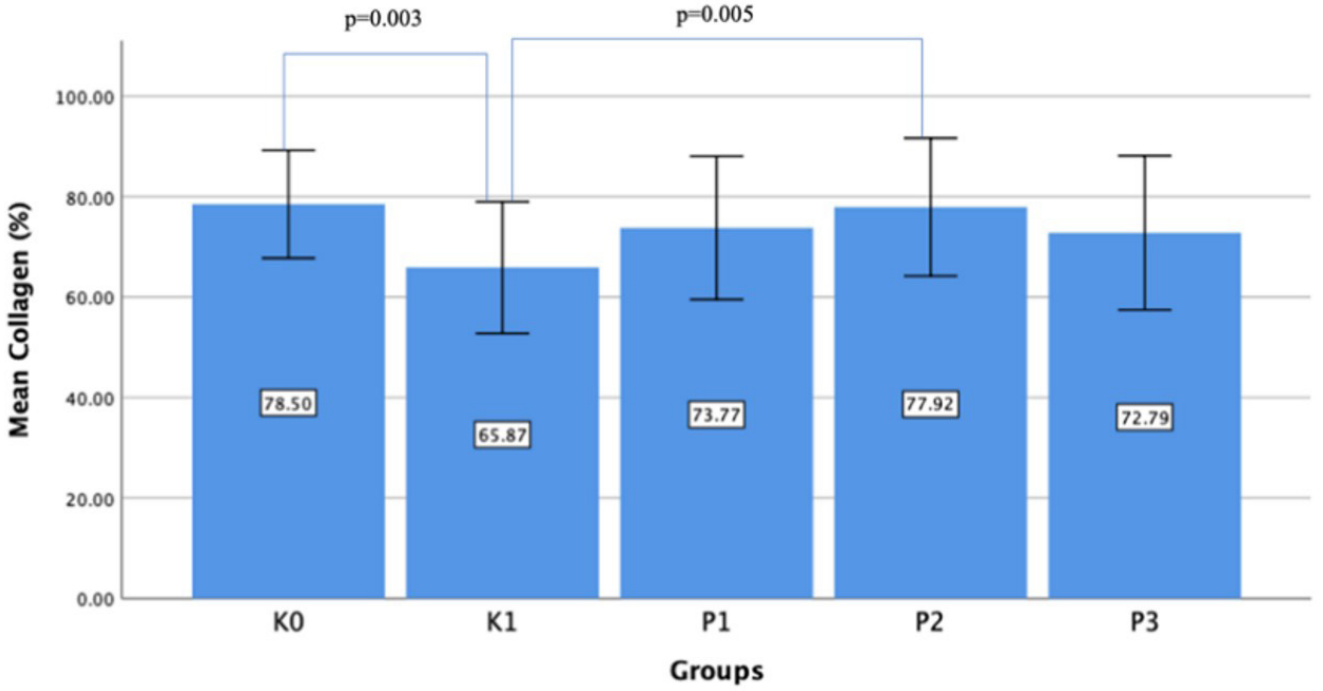

Figure 1. Mean collagen (%) comparison between groups. (K0: normal control, K1: negative control, P1: 2.5% stevia cream + UVB, P2: 5% stevia cream + UVB, P3: 10% stevia cream + UVB)

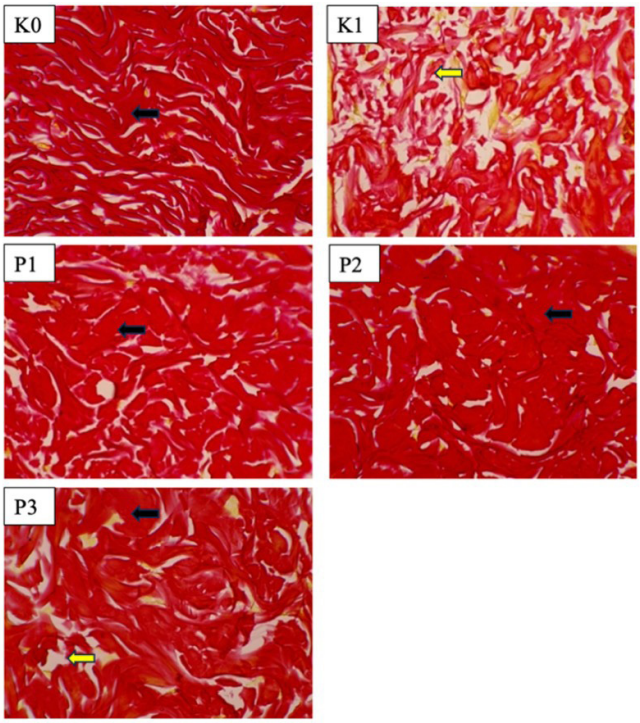

Figure 2. Histological features of collagen assessed using picro sirius red stain, 10 × 40 magnification. (K0) normal control group, (K1) negative control group, (P1) 2.5% stevia cream + UVB, (P2) 5% stevia cream + UVB, (P3) 10% stevia cream + UVB. Black arrows indicate intact collagen, while yellow arrows highlight fragmented collagen

showed, descriptively, higher TGF-β1 levels than the negative control group; however, the concentrations used may not have been optimal to produce a significant effect on TGF-β1 levels. For example, in this study, the 10% stevia leaf extract group exhibited lower TGF-β1 levels due to the body's response to excessively high concentrations, potentially inhibiting its biological activity. Stevia extract, which contains high amounts of flavonoids and phenols and possesses antioxidant activity, shows significant potential as a source of bioactive compounds for the development of dietary supplements and skincare products. However, prolonged use or high concentrations may increase the risk of toxicity due to its abundance of biologically active components, including antioxidants (Gaweł-Bęben *et al.* 2015).

Ultraviolet B radiation induces complex, time-dependent alterations in TGF-β signaling pathways. The timing of measurement is critical, as Smad7 mRNA, an antagonist of TGF-β signaling, is rapidly upregulated shortly after UVB exposure. Additionally, the DNA-binding activity of the Smad3/4 complex is diminished within hours (Quan *et al.* 2002). The expression of TGF-β type II receptor mRNA is also suppressed for several hours but tends to recover by 24 hours. Phosphorylation of Smad2 protein, a key step in TGF-β signaling, is also affected within hours

of exposure (Quan *et al.* 2001, 2002). The timing of measurement further influences observed TGF-β1 protein levels in keratinocytes, which decrease at 24 hours following high UVB doses but increase by 48 hours. These later changes correlate with increased inflammatory cytokines and collagen degradation, contributing to photoaging (Jung *et al.* 2008). Thus, the magnitude and timing of TGF-β pathway alterations depend on factors such as UV dose, exposure duration, and cell type, emphasizing the need for temporal monitoring over several days to fully characterize UVB effects on TGF-β signaling (Quan *et al.* 2002; Ke & Wang 2021). In this context, the lack of significant changes in TGF-β1 observed in the current study may be attributed to the suboptimal timing of measurement relative to the dynamic response window.

Transforming Growth Factor-β plays a crucial role in multiple regulatory pathways in the body, including oxidative stress, inflammation, and other factors (Tzavlaki & Moustakas 2020), which may not be entirely influenced by stevia extract. Although UVB exposure is known to reduce TGF-β1 levels through oxidative stress and inflammatory pathways, the absence of this effect in the current study may be attributed to the antioxidant and anti-inflammatory properties of stevia, which could be acting through alternative pathways not directly involving TGF-β1. Stevia could affect other signaling pathways involved in the skin's response to oxidative stress and UVB, such as the Nuclear Factor Kappa B (NF-κB) pathway, which plays an important role in inflammation and stress responses. Reactive oxygen species act as initiators of oxidative stress and are crucial in activating the NF-κB transcription factor. Once activated, NF-κB stimulates the expression of various pro-inflammatory cytokines, including Tumor Necrosis Factor-α (TNF-α), Interleukin-1 (IL-1), and IL-6. These molecules contribute significantly to the processes underlying photoaging-induced skin aging (Ansary *et al.* 2021). Stevioside, a bioactive compound in stevia, has been shown to inhibit the NF-κB pathway. Stevioside may also affect the MAPK pathway, which includes JNK, ERK, and p38 MAPK, involved in regulating cellular responses to stress (Wang *et al.* 2014; Zou *et al.* 2020). UVB exposure activates MAPK signaling, leading to AP-1 activation, which regulates MMP production and collagen degradation. Antioxidant compounds in stevia may interact with these kinases, influencing cellular responses to UVB exposure and potentially reducing MMP activity and collagen degradation (Poon *et al.* 2015).

Quercetin, a flavonoid with antioxidant properties found in stevia, can inhibit the activity of AP-1 and NF-κB, thereby preventing collagen degradation and inflammatory processes (Shin *et al.* 2019). Quercetin also inhibits TNF-α-induced inflammation by targeting key pro-inflammatory signaling pathways. It suppresses ERK and JNK activation, thereby reducing c-Jun and AP-1 activity. Quercetin also stabilizes IκBα, preventing NF-κB nuclear translocation and the transcription of inflammatory genes. Additionally, quercetin enhances PPARγ activity, which antagonizes both NF-κB and AP-1-mediated gene expression. Through these mechanisms, quercetin effectively blocks TNF-α-driven inflammatory cascades (Li *et al.* 2016). Quercetin and rutin were also evaluated as potential topical sunscreens in humans, exhibiting significant protective effects against UVA and UVB radiation (Korać & Khambholja 2011). Ferulic acid, a phenolic compound found in stevia, exhibits potent antioxidant properties and serves as a versatile ingredient. Its excellent UV-absorbing ability further highlights its role in protecting the skin from sun damage (Gaweł-Bęben *et al.* 2015). Ferulic acid also exhibits strong anti-inflammatory activity by targeting both the NF-κB and MAPK signaling pathways. It inhibits NF-κB activation by preventing phosphorylation of the p65 subunit, stabilizing IκBα to block its degradation, and suppressing IKK activity, thereby reducing nuclear translocation and transcriptional activity of NF-κB.

Additionally, ferulic acid interferes with the MAPK pathway by inhibiting the phosphorylation of p38, JNK, and ERK1/2, thereby decreasing the production of pro-inflammatory cytokines (Liu *et al.* 2025). The suppression of inflammatory signaling cascades, including NF-κB and MAPK/AP-1 pathways, reduces MMP expression, enzymes that contribute to collagen breakdown (Feng *et al.* 2024). Together, these bioactive compounds in stevia synergistically inhibit collagen degradation by modulating key inflammatory and oxidative stress pathways.

The application of stevia leaf extract cream has been shown to inhibit the reduction in collagen levels in the skin of male Wistar rats exposed to UVB radiation, though this effect was observed only at a specific dose. The amount of Collagen in the group treated with the base cream and exposed to UVB was significantly lower than in the normal control group. This result indicates that UVB exposure triggers ROS formation, which inhibits collagen synthesis while simultaneously degrading existing Collagen, thereby contributing

to photoaging. These findings are consistent with previous research showing that UV radiation can alter the physical properties of the skin, including the loss of type I Collagen, which contributes to structural damage and decreased skin elasticity (Park *et al.* 2018).

The average collagen amount in the group treated with 2.5% stevia leaf extract was descriptively higher than that in the negative control group, but the difference was not statistically significant, suggesting that a concentration of 2.5% might not be sufficient to inhibit collagen degradation after UVB exposure effectively. Similarly, the 10% stevia extract group also had a descriptively higher collagen amount than the negative control group, but the lack of statistical significance suggests that, at this higher concentration, the stevia extract's efficacy might have diminished. This study demonstrated that the application of 5% stevia leaf extract cream effectively prevented a reduction in collagen content, thereby providing significant protection against UVB-induced collagen degradation. The results further indicate that the 5% concentration of stevia leaf extract is more effective than other tested concentrations in preserving Collagen and maintaining skin integrity. These ameliorative effects are primarily attributed to the extract's potent antioxidant activity. The bioactive compounds present in stevia leaf, such as phenolic acids and flavonoids, exhibit strong free radical-scavenging activity, providing sufficient antioxidant defense to neutralize ROS (Shukla *et al.* 2012). In addition to its antioxidant activity, stevia leaf extract may also inhibit UVB-induced upregulation of MMPs, which are responsible for collagen degradation. Its anti-inflammatory properties further contribute to skin protection by reducing UVB-mediated inflammatory responses. Thus, it can be concluded that a 5% concentration of stevia extract is the optimal dose for significantly inhibiting collagen reduction after UVB exposure. The results align with a previous study conducted by Das *et al.* (2012), which showed that applying a 5% stevia leaf extract gel for 21 days effectively reduced wrinkles and mild acne, brightened the skin, and improved skin hydration (Das *et al.* 2012). The preservation of Collagen is biologically relevant, as it can help maintain skin elasticity, prevent wrinkle formation, and slow the structural breakdown associated with photoaging (Liu *et al.* 2024). The findings support the potential application of stevia extract in anti-aging skincare products to maintain dermal integrity and skin youthfulness.

Stevia contains several flavonoids, such as apigenin, which has been shown to enhance the synthesis of type I and III Collagen (Zhang *et al.* 2015). Apigenin demonstrated protective effects against UVA- and UVB-induced skin cancer in SKH-1 mice. Although it did not prevent NF-κB nuclear translocation, apigenin reduced the activity of NF-κB-regulated genes (Korać & Khambholja 2011). Stevia also contains protocatechuic acid, which can increase type I collagen synthesis and inhibit MMP-1 production (Shin *et al.* 2020). The amount and density of Collagen in bodily tissues, particularly the skin, depend greatly on the balance between its synthesis and degradation. Collagen synthesis primarily occurs in fibroblasts, the cells that produce structural proteins, such as type I and III Collagen. TGF-β enhances collagen synthesis, while collagen degradation is mainly regulated by AP-1, which controls the enzyme MMP-1, which breaks down type I and III collagen (Jadoon *et al.* 2015). Therefore, investigating factors that influence collagen synthesis and degradation can help understand how their balance contributes to overall collagen levels and density.

This study demonstrated that the application of stevia (*Stevia rebaudiana* Bertoni) leaf extract cream did not prevent the UVB-induced decrease in TGF-β1 levels in the skin of male Wistar rats (*Rattus norvegicus*). However, it significantly mitigates the reduction in collagen amount, with the 5% concentration being the most effective. These findings indicate that stevia leaf extract, particularly at 5%, shows promise as a natural ingredient for skincare formulations. It can be effectively incorporated into anti-aging creams, moisturizers, or sun protection products to support skin elasticity and reduce the formation of wrinkles, thereby helping protect against photoaging.

Several limitations to this study may affect the interpretation of the findings. The use of rat models poses challenges in translating findings to humans, as rat skin differs from human skin in thickness and permeability, factors that can affect the absorption and efficacy of topical agents (van Ravenzwaay & Leibold 2004). Furthermore, the 28-day treatment duration may be insufficient to fully capture the gradual, cumulative effects of photoaging, which typically develop over extended periods of UV exposure. Another limitation is that collagen and TGF-β1 levels were assessed only at the end of the intervention period (post-test-only design). As a result, the temporal dynamics and onset of

the effects of stevia leaf extract could not be determined. Nonetheless, this approach was chosen to minimize subject morbidity and address ethical concerns related to repeated invasive skin sampling. Therefore, further research using human skin models or clinical trials is required to understand the mechanisms better, evaluate safety, and confirm the efficacy of stevia leaf extract cream in preserving Collagen, ideally through longer-term studies and a broader range of assessment parameters.

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