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Original Article

The Photoprotective Effect of Cucurbita moschata Seed Extract on Rat Skin Exposed to Ultraviolet Radiation

Dewi Sartika[1,2]*, GUSBAKTI RUSIP[3], Linda Chiuman[4], Ermy Girsang[5]

ABSTRACT

Photoaging induced by ultraviolet B (UVB) radiation is characterized by DNA damage, oxidative stress, extracellular matrix degradation, and collagen loss. This study evaluated the photoprotective effects of topical pumpkin seed (*Cucurbita moschata*) extract cream against UVB-induced skin damage in Wistar rats. CPD (cyclobutane pyrimidine dimer), MDA (malondialdehyde), MMP-1 (matrix metalloproteinase-1) levels, and collagen density were assessed. UVB exposure significantly increased CPD, MDA, and MMP-1 levels while reducing collagen density. Treatment with pumpkin seed extract cream, particularly at 10% concentration, markedly reduced CPD and MDA levels ($P \leq 0.05$), indicating potent DNA repair support and antioxidant activity likely attributable to phenolic and flavonoid compounds. MMP-1 levels were significantly decreased, approaching normal values, suggesting inhibition of collagen degradation through anti-inflammatory and anti-matrix degradation mechanisms. Histological examination revealed higher collagen density and more organized collagen fibers in treated groups compared with negative control and placebo. These findings indicate that *Cucurbita moschata* seed extract cream exerts strong photoprotective, antioxidant, anti-inflammatory, and collagen-preserving effects, with the 10% formulation showing the greatest efficacy.

Keywords: *Cucurbita moschata*, photoaging, UVB radiation, CPD, collagen density

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INTRODUCTION

Skin aging is a complex biological process influenced by intrinsic factors, such as genetic programming and hormonal changes, and extrinsic factors, predominantly chronic exposure to ultraviolet (UV) radiation[1]. Within the UV spectrum, ultraviolet B (UVB; 280–320 nm) is particularly harmful due to its higher energy compared to UVA[2]. UVB penetrates the epidermis, directly damaging DNA and triggering molecular pathways that accelerate photoaging beyond natural chronological aging[3].

One of the earliest and most critical events in UVB-induced photoaging is the formation of cyclobutane pyrimidine dimers (CPDs), which disrupt DNA replication and transcription, leading to mutations and apoptosis if unrepaired[4]. In parallel, UVB exposure generates reactive oxygen species (ROS), which oxidize lipids, proteins, and nucleic acids, reflected by elevated malondialdehyde (MDA) levels[5]. Persistent ROS further activate transcription factors such as activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB), which upregulate inflammatory mediators and matrix metalloproteinases (MMPs)[6].

Among MMPs, MMP-1 (interstitial collagenase) plays a pivotal role in collagen degradation, breaking down type I collagen the major structural component of the dermis responsible for strength and elasticity[7]. UVB-induced overexpression of MMP-1 accelerates collagen loss, resulting in dermal thinning, reduced structural integrity, and visible signs of photoaging such as wrinkles and sagging[8,9].

To counteract these mechanisms, natural bioactive compounds rich in phenolics and flavonoids have been studied for their ability to attenuate DNA damage, neutralize ROS, suppress MMP expression, and preserve collagen structure[10-12]. These compounds exert antioxidant, anti-inflammatory, and anti-collagenolytic effects, making them promising candidates for photoprotection.

Pumpkin seeds (Cucurbita moschata) are an underutilized natural resource with a rich phytochemical profile, including phenolic acids, flavonoids, carotenoids, tocopherols, phytosterols, and polyunsaturated fatty acids[13]. These bioactives exhibit antioxidant, anti-inflammatory, wound-healing, and regenerative properties[14,15]. The phenolic and flavonoid constituents in pumpkin seeds may counteract UVB-induced oxidative stress by scavenging ROS and enhancing endogenous antioxidant defenses such as superoxide dismutase and catalase[16]. The presence of essential fatty acids may also contribute to barrier repair and maintenance of skin hydration, further supporting resilience against photodamage[17].

While systemic benefits of C. moschata seeds including cardioprotective, hepatoprotective, and antidiabetic effects are well documented, their potential as a topical anti-photoaging and photoprotective agent has not been extensively studied in vivo. This gap in knowledge provides the rationale for the present study.

Therefore, the present study aimed to evaluate the anti-photoaging and photoprotective effects of Cucurbita moschata seed extract cream in rats exposed to UVB radiation. We assessed four key endpoints: (1) CPD levels as indicators of DNA damage, (2) MDA levels as markers of oxidative stress, (3) MMP-1 levels as indicators of collagen degradation, and (4) collagen density as a histological measure of dermal integrity.

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MATERIALS AND METHODS

Study Design

This was a true experimental study employing a post-test only control group design. Thirty male Wistar rats (*Rattus norvegicus*), meeting inclusion criteria, were randomly allocated into five experimental groups using simple randomization.

Material and Tools

This study used *Cucurbita moschata* seed extract prepared at the Faculty of Pharmacy, Universitas Sumatera Utara, formulated into creams at 5% and 10%. UVB exposure was delivered with a 311 nm narrow band UVB lamp. Male Wistar rats (*Rattus norvegicus*), aged 8–12 weeks and weighing 190–250 and bottled water (Aqua®, Danone Indonesia) ad libitum. CPD levels were measured using an ELISA kit (Solarbio®), MDA and MMP-1 with kits from Elabscience® (China), and DNA extracted using the DNeasy® Blood & Tissue Kit (Qiagen®, Germany). Histology used 10% buffered formalin (Merck®, Germany) and Masson's trichrome reagents (Sigma-Aldrich®, USA). Key equipment included a rotary evaporator (Heidolph®, Germany), analytical balance (Mettler Toledo®, Switzerland), micropipettes (Eppendorf®, Germany), microplate reader (Bio-Rad®, USA), and light microscope (Olympus®, Japan).

Ethical Approval

All experimental procedures were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Prima Indonesia (No: 023/KEPK/UNPRI/I/2025), and conducted in accordance with the principles of the 3Rs (Reduction, Refinement, Replacement) and the 5 Freedoms for animal welfare.

Animals

Male Wistar rats, aged 8–12 weeks, weighing 190–250 g, healthy, and active were included. Exclusion criteria were illness during the study, aggressive behavior, or death prior to study completion. Each rat was housed in a cage (30 × 40 × 15 cm) with 2–3 rats per cage, maintained at 22–24 °C under a 12 h light/dark cycle, and fed a standard rodent diet and water ad libitum.

Sample Size

Sample size was calculated using the Federer formula (n−1) (t−1)≥15(n-1)(t-1) \ge 15(n−1)(t−1)≥15, with t = 5 groups and $n \geq 5$ per group. Each group contained 5 animals plus 1 reserve, totaling 30 rats.

Preparation of *Cucurbita moschata* Seed Extract

Five kilograms of pumpkin seeds were separated from pulp and shell, dried at 50 °C until moisture content was <10%, powdered, and sieved (20 mesh). A total of 500 g powder was macerated in 70% ethanol (3.75 L) for 5 days with intermittent shaking. The residue was remacerated twice (1.25 L ethanol, 2 and 3 days, respectively). The combined filtrates were concentrated under reduced pressure at 50 °C using a rotary evaporator to yield the crude extract.

Cream Formulation

Extract was incorporated into a cream base at 5% and 10% concentrations. The base formula consisted of stearic acid (12 g), cetyl alcohol (0.5 g), triethanolamine (1 g), methylparaben (0.1 g), sodium metabisulfite (0.1 g), and distilled water ad 100 g. Oil-phase ingredients (stearic acid, cetyl alcohol) were melted at 70–75 °C, aqueous-phase ingredients dissolved in heated water, and both phases combined with constant stirring until homogenous[18].

Experimental Groups

The study was conducted on male Wistar rats randomly assigned into five groups:

a. Negative control (K–): No UVB exposure, no treatment

b. Positive control (K+): UVB exposure only

c. T1: UVB + cream base only (placebo)

d. T2: UVB + 5% pumpkin seed extract cream

e. T3: UVB + 10% pumpkin seed extract cream

Before the experiment, all rats underwent a 7-day acclimatization period under controlled environmental conditions. Dorsal hair within a 3 × 3 cm area was shaved to ensure uniform UVB penetration. Anesthesia was administered intramuscularly using ketamine (60 mg/kg) and xylazine (20 mg/kg) prior to UVB exposure to minimize discomfort. UVB irradiation was delivered with a narrow-band 311 nm UVB lamp positioned 20 cm above the skin at an intensity of 390 mJ/cm² per session (15 minutes/session), five days per week for four weeks. Topical treatments were applied once daily, including on non-irradiation days, to maintain consistent exposure to the active compounds. At the end of the 4-week protocol, rats were sacrificed 24 hours after the last UVB exposure for tissue collection and further biochemical and histological analyses[19].

DNA Extraction and CPD Measurement

Skin biopsies (about 25 mg) from the dorsal area were promptly snap-frozen in liquid nitrogen and preserved at −80 °C until analysis. Genomic DNA was extracted utilising the QIAGEN DNeasy Blood & Tissue Kit (Qiagen, Germany) in accordance with the manufacturer's procedure, encompassing tissue lysis, binding to a silica membrane, washing, and elution in nuclease-free water. The concentration and purity of the extracted DNA were assessed spectrophotometrically at 260/280 nm. CPD (cyclobutane pyrimidine dimer) levels, a key marker of UVB-induced DNA damage, were quantified using a commercial ELISA kit according to the kit instructions. Results were expressed as ng/mL based on the standard curve generated from known CPD concentrations[20].

MMP-1 and MDA Measurement

Serum MMP-1 concentrations were measured with a rat-specific ELISA kit, adhering to the manufacturer's guidelines, with absorbance assessed at 450 nm. MDA concentrations were quantified using a commercial TBARS assay kit, derived from a standard curve, and reported in nmol/mL[21,22].

Histopathology and Collagen Density Analysis

Skin samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4–5 µm, and stained with Masson's trichrome. Collagen fibers were visualized as blue/green under light microscopy (Olympus C). Collagen density was quantified using calibrated image analysis[23].

Statistical Analysis

Data were presented as mean ± standard deviation (SD) for normally distributed data, or median (interquartile range) if non-normal. Between-group comparisons were analyzed using one-way ANOVA followed by Tukey's post hoc test for normal data, or Kruskal–Wallis test for non-normal data. A p-value < 0.05 was considered statistically significant.

RESULT AND DISCUSSION

Phytochemical Screening and GC–MS Analysis

Phytochemical screening of *Cucurbita moschata* seed extract in Table 1 revealed the presence of alkaloids (positive with Bouchardat, Mayer, and Dragendorff reagents), flavonoids, triterpenoids/steroids, glycosides, saponins, and tannins. These secondary metabolites are widely reported to contribute to antioxidant, anti-inflammatory, and tissue-protective activities that are critical for mitigating UVB-induced photoaging.

Gas chromatography–mass spectrometry (GC–MS) analysis detected 54 volatile and semi-volatile compounds, with dominant peaks corresponding to 2-hydroxy-(Z)9-pentadecenyl propanoate, 3-methyl-2-(2-methylene-cyclohexyl)-butan-2-ol, methyl linoleate, and methyl oleate (Figure1). These compounds indicate a high proportion of unsaturated fatty acids, esters, long-chain alcohols, sterols, and phenolic derivatives components that contribute to oxidative stability and free radical scavenging activity[24].

Flavonoids, sterols, and long-chain fatty acid esters identified here have been reported to neutralize reactive oxygen species (ROS), suppress CPD formation, and reduce MDA levels (Table 2), thereby protecting against DNA damage and lipid peroxidation[25]. Additionally, their ability to downregulate MMP-1 expression helps prevent collagen degradation, a hallmark of photoaging. Chu et al. (2020) and Gaweł-Bęben et al. (2022) demonstrated that pumpkin seed oil in topical formulations enhances UV protection, reduces inflammation, and preserves collagen structure—findings consistent with the expected biological activity of this extract[26,27].

Total Phenolic, Flavonoid Content, and Antioxidant Activity

The extract showed high total phenolic content (85.13 ± 1.38 mg GAE/g) and flavonoid content (36.01 ± 1.52 mg QE/g), alongside strong antioxidant activity (IC_{50} = 84.31 ppm) as determined by the DPPH assay (Table 3).

Phenolic and flavonoid compounds act as hydrogen or electron donors to neutralize free radicals, inhibit lipid peroxidation, and reduce CPD formation in UVB-exposed skin. Their antioxidant action also contributes to MMP-1 inhibition, supporting collagen preservation[28]. The combination of a rich phytochemical profile (Table 1), dominant GC–MS-identified bioactives, and high phenolic/flavonoid content (Table 3) provides strong biochemical evidence for the extract's anti-photoaging potential. These chemical characteristics support the hypothesized mechanisms of action antioxidant defense, anti-inflammatory modulation, and collagen matrix preservation making *C. moschata* seed extract a promising candidate for topical photoprotection against UVB-induced skin damage.

Result of CPD, MMP-1 and MDA Concentration

The effects of *Cucurbita moschata* seed extract cream on biomarkers of photoaging—cyclobutane pyrimidine dimers (CPD), matrix metalloproteinase-1 (MMP-1), and malondialdehyde (MDA) in UVB-exposed Wistar rats are presented in **Figure 2**. UVB irradiation significantly elevated CPD (Figure 2A), MMP-1 (Figure 2B), and MDA (Figure 2C) levels compared with the normal control group ($P \leq 0.05$ to $P \leq 0.0001$). Treatment with the extract cream at both 5% and 10% concentrations reduced these elevations, with the 10% formulation consistently showing the greatest reductions. Statistical comparisons indicate that these decreases were significant for CPD and MDA in both extract-treated groups, while MMP-1 reduction reached significance only with the 10% concentration. The placebo group did not show significant improvement compared with UVB-only rats, confirming that the observed effects were attributable to the active extract.

UVB exposure markedly increased CPD concentration in the untreated group (63.00 ± 4.82 ng/mL) compared with the normal control (24.53 ± 5.89 ng/mL, $P \leq 0.05$), confirming substantial DNA photodamage (Table 4.4). The placebo group (60.74 ± 5.61 ng/mL) showed no significant reduction, indicating that the cream base alone had no protective effect. Application of *C. moschata* seed extract cream at 5% reduced CPD to 54.25 ± 5.29 ng/mL ($P \leq 0.05$ vs. UVB+), while 10% further decreased levels to 50.44 ± 3.99 ng/mL ($P \leq 0.05$ vs. UVB+). Although CPD values in both treatment groups remained higher than the normal control, the reductions were statistically significant, demonstrating partial protection against UVB-induced DNA lesions.

These results align with findings by Kim et al. (2018), who reported that phenolic compounds such as sinapic acid reduce CPD formation in UVB-irradiated HaCaT cells in a dose-dependent manner[29]. The mechanism is attributed to the antioxidant action of phenolics and flavonoids, which donate electrons or hydrogen atoms to neutralize ROS, thereby limiting oxidative stress and CPD formation. Additionally, flavonoids can enhance nucleotide excision repair (NER) pathway activity, facilitating the removal of UVB-induced DNA photoproducts[30]. The Nrf2–HO-1 pathway, previously shown to be activated by pumpkin seed extracts[31], also contributes to cytoprotective gene expression, further reducing DNA damage[28].

UVB irradiation significantly increased serum MMP-1 concentration from 225.99 ± 30.11 pg/mL in the normal control to 310.01 ± 33.11 pg/mL in the UVB+ group ($P \leq 0.05$), reflecting UVB-induced collagenolytic activity (Table 4.5). The placebo group (271.75 ± 35.76 pg/mL) showed no significant difference from UVB+. Treatment with 5% extract cream yielded 276.61 ± 48.91 pg/mL (NS vs. UVB+), whereas 10% extract cream reduced MMP-1 to 231.33 ± 41.26 pg/mL ($P \leq 0.05$ vs. UVB+), approaching normal control values (NS vs. normal).

UVB-induced MMP-1 upregulation is mediated through activation of MAPK signaling pathways (ERK, JNK, p38) and transcription factor AP-1, which drive collagen degradation.[32] ROS generation further amplifies MMP-1 expression via inflammatory cytokines such as IL-1 and TNF-α .[33] The significant reduction in MMP-1 with 10% extract cream suggests that bioactive components in *C. moschata* flavonoids, fatty acid esters, sterols likely inhibit oxidative stress signaling, thereby reducing AP-1 activation and MMP-1 expression, preserving dermal collagen.

MDA levels, a biomarker of lipid peroxidation, rose sharply in the UVB+ group (243.05 ± 32.50 ng/mL) compared with the normal control (32.60 ± 8.21 ng/mL, $P \leq 0.05$) (Table 4.6). The placebo group showed a modest, non-significant reduction (210.22 ± 25.11 ng/mL). Both 5% and 10% extract creams significantly lowered MDA to 171.18 ± 36.59 ng/mL and 151.94 ± 33.62 ng/mL, respectively ($P \leq 0.05$ vs. UVB+), with the 10% dose showing greater efficacy.

These findings are consistent with reports that UVB-induced ROS initiates lipid peroxidation, generating MDA as a stable end product[34]. Flavonoids, phenolics, sterols, and vitamin E in *C. moschata* extracts act

Table 1. Phytochemical screening of *Cucurbita moschata* seed extract

Parameter	Reagent	Result
Alkaloid	Bouchardat	+
	Mayer	+
	Dragendorff	+
Flavonoid	Mg-HCl + H_2SO_4	+
Triterpenoid/Steroid	Lieberman–Burchard	+
Glycoside	Molish + H_2SO_4	+
Saponin	Aquades	+
Tannin	$FeCl_3$	+

Table 2. GCMS analysis results

Peak #	R. Time	Area %	Name
1	2.138	0.10	Methyl 2,5-dihydro-5-oxofuran-3-carboxylate
2	2.265	0.12	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
3	2.524	0.01	Germacrene-D
4	2.650	0.08	Dodecanoic acid, silver(1+) salt (CAS)
5	3.039	0.04	2,5-Pyrrolidinedione, 3-ethyl-3-methyl- (CAS)
6	3.246	0.05	5-Eicosene, (E)-
7	3.454	0.03	Tetracosanoic acid, 23-oxo-, methyl ester (CAS)
8	3.557	0.01	Estran-3-one, 17-(acetyloxy)-2-methyl-, (2α,5α,17β)- (CAS)
9	3.682	0.30	Cytidine (CAS)
10	4.045	0.24	2-Chloro-1-isopropyl-4-methylcyclohexane
11	4.204	2.12	2-Octylfuran
12	4.410	1.38	β-Santalene (CAS)
13	4.521	0.14	Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl ester, (R)-
14	5.115	0.97	2,3-Diazabicyclo[2.2.1]hept-2-ene, 4-methyl-1-(pent-4-en-1-yl)-
15	5.371	0.23	Isosativene
16	5.527	0.33	Bicyclo[3.2.2]nona-2,6-dien-5-ol-4-one
17	6.090	0.03	Borinic acid, diethyl-, 1-cyclododecen-1-yl ester
18	6.625	1.16	9-Octadecenoic acid (Z)- (CAS)
19	6.951	1.47	Camphor-10-sulfonic acid, (3-methylenecyclopentyl)methyl ester
20	7.257	0.62	β-Santalene (CAS)
21	7.908	0.07	3-Methylbutylpyrazine
22	8.421	0.23	3-Methylbutylpyrazine
23	8.768	0.61	9-Octadecenoic acid (Z)-, methyl ester (CAS)
24	9.090	1.06	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
25	10.114	1.51	13-Octadecenal, (Z)-
26	10.393	2.63	Dicyclohexyl-4,4'-diol
27	10.552	2.22	9,12-Octadecadienoic acid, methyl ester
28	10.772	1.46	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-
29	11.807	0.81	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-
30	12.268	0.19	(-)-Isoaromadendrene-(V)
31	12.865	0.12	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (CAS)
32	13.208	0.47	3,6-Dimethyl-1-heptyn-3-ol
33	13.361	0.20	Cyclopentene, 1,3-dimethyl-2-(1-methylethyl)-
34	13.567	0.23	13-Methylpentadec-14-ene-1,13-diol
35	13.714	0.20	Cyclopentane, 1-methyl-1-(2-methyl-2-propenyl)-
36	14.124	0.12	E6-Tetradecenylacetate
37	14.965	0.18	2-Isopropenyl-5-methylhex-4-enal
38	15.643	2.79	3-Methyl-2-(2-methylene-cyclohexyl)-butan-2-ol
39	16.183	11.62	2-Hydroxy-(Z)9-pentadecenyl propanoate
40	16.584	7.58	Acetic acid, 6-hydroxymethyl-cyclodecyl ester
41	17.206	6.46	3-Pentanol, 2,3,4-trimethyl- (CAS)
42	17.623	18.62	2-Hydroxy-(Z)9-pentadecenyl propanoate
43	17.954	6.86	6-Nitro-cyclohexadecane-1,3-dione
44	18.803	13.58	3-Methyl-2-(2-methylene-cyclohexyl)-butan-2-ol
45	19.097	2.78	p-Menthane-1,3-diol (CAS)
46	19.375	1.51	9-Octadecenal, (Z)- (CAS)
47	19.799	0.14	Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- (CAS)
48	19.923	0.13	3-Pentanol, 2,3,4-trimethyl- (CAS)
49	20.180	1.49	7-Octen-3-ol, 2,3,6-trimethyl-
50	20.485	1.48	3a,6,6,9a-Tetramethyldodecahydronaphtho[2,1-b]furan-2-ol
51	21.120	0.89	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (CAS)
52	21.496	1.77	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester (CAS)
53	22.588	0.41	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-
54	23.862	0.16	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-

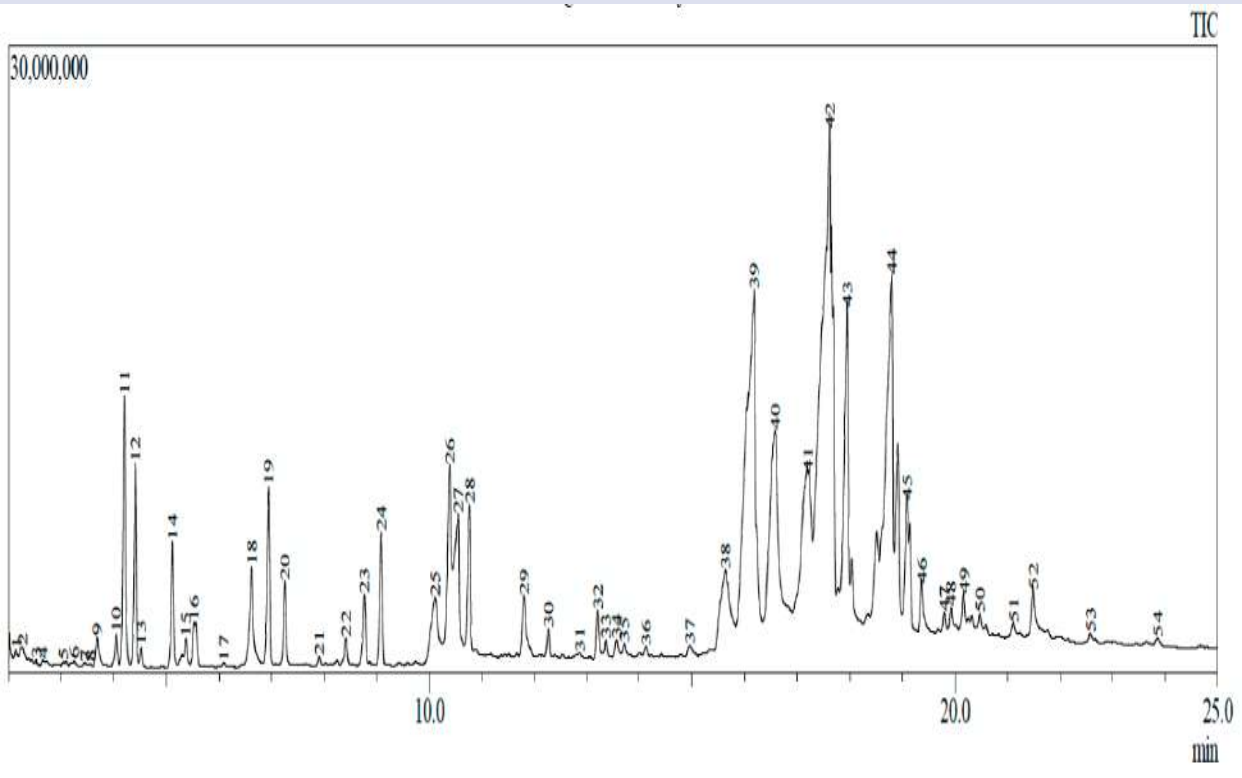

Figure 1. GCMS chromatogram of *Cucurbita moschata* seed extract

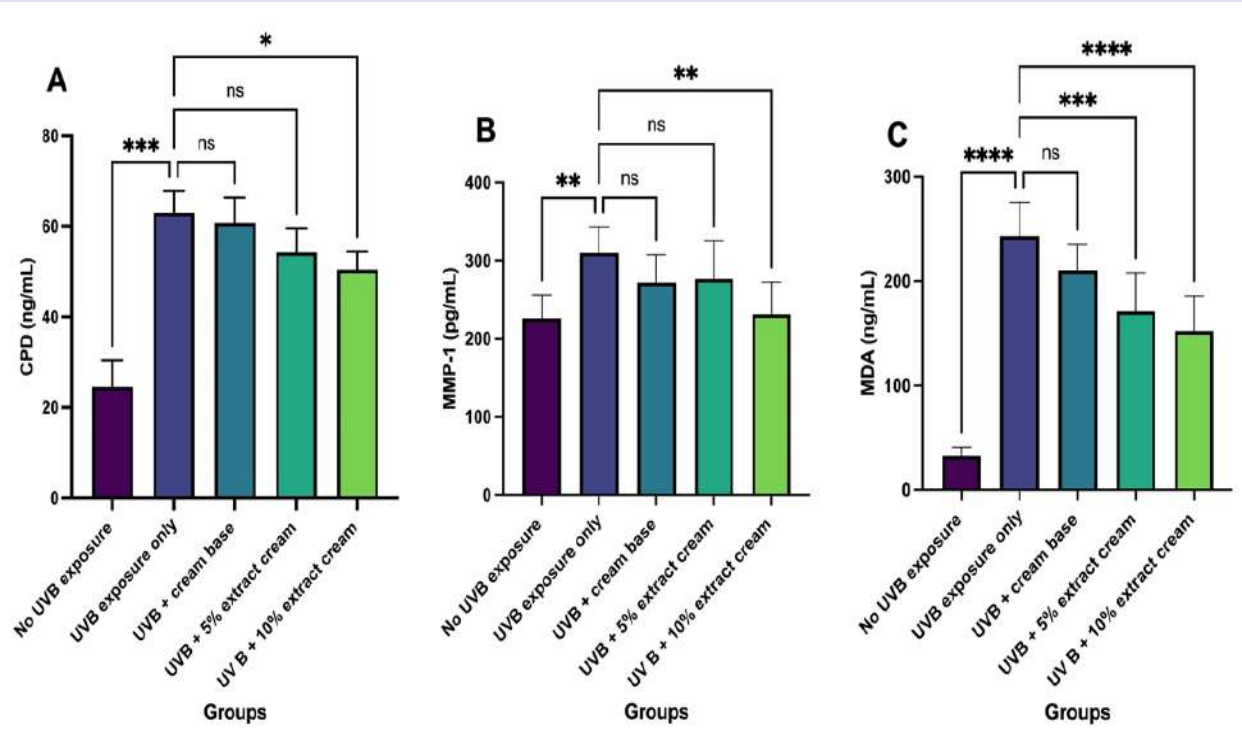

Figure 2. (A) CPD levels, (B) MMP-1 levels, and (C) MDA levels. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 indicate significant differences; ns = not significant.

synergistically to scavenge ROS, reduce membrane lipid peroxidation, and protect cellular integrity. The dose-dependent decrease in MDA underscores the extract's antioxidant potency, corroborating its high phenolic and flavonoid content and strong DPPH-scavenging activity shown earlier. The proposed mechanistic pathway of *C. moschata* seed extract in protecting against UVB-induced photoaging is summarized in Figure 3. UVB irradiation promotes CPD formation, ROS accumulation, and MMP-1 overexpression, which collectively drive collagen degradation and visible signs of skin aging. Pumpkin seed extract mitigates these processes by scavenging ROS, suppressing MMP-1 activity, and preserving collagen integrity, thereby exerting its anti-photoaging effect.

Visual and Histological Analysis

The macroscopic appearance and histological evaluation of dorsal skin from each treatment group are presented in Figure 4 and Table 4. Visual inspection revealed that normal control rats (K1) had smooth skin with uniform pigmentation and no signs of erythema or scaling. In contrast, UVB-exposed untreated rats (K2) exhibited marked erythema, dryness, and scaling, indicative of severe photoaging changes. The placebo group (K3) showed similar damage, suggesting that the cream base alone did not confer protection. Treatment with *C. moschata* seed extract cream at 5% (K4) and 10% (K5) improved skin surface appearance, with the 10% group showing the closest resemblance to the normal control.

Histological scoring based on Nussbaum et al. (2009) criteria demonstrated significant differences between groups (Table 4)[35]. The normal control scored 1, indicating the presence of fibroblasts and minor collagen fibers without degeneration. The UVB+ group scored 0, reflecting an absence of collagen fibers and severe dermal atrophy. The placebo group scored 1, indicating minimal collagen regeneration. Treatment with 5% extract cream improved the score to 2, corresponding to moderate collagen content with both mature and immature fibers. The 10% extract cream achieved a score of 3, denoting dominant mature collagen fibers and a near-complete restoration of dermal structure.

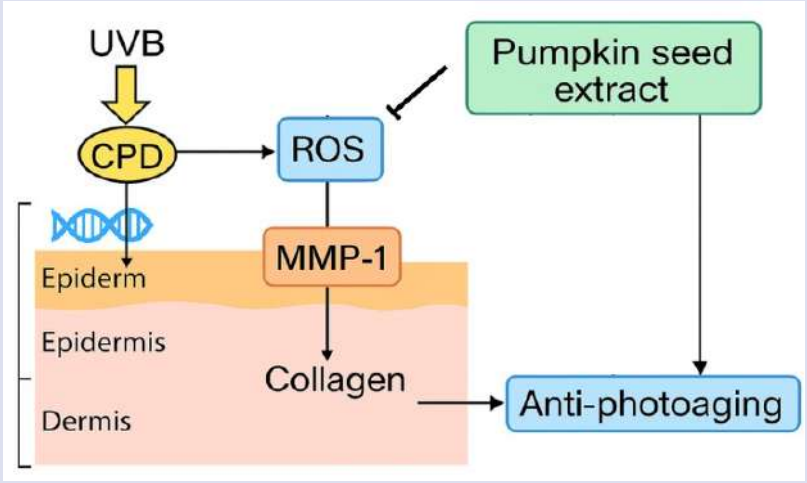

Figure 3. Protective mechanism of *Cucurbita moschata* seed extract against UVB-induced photoaging.

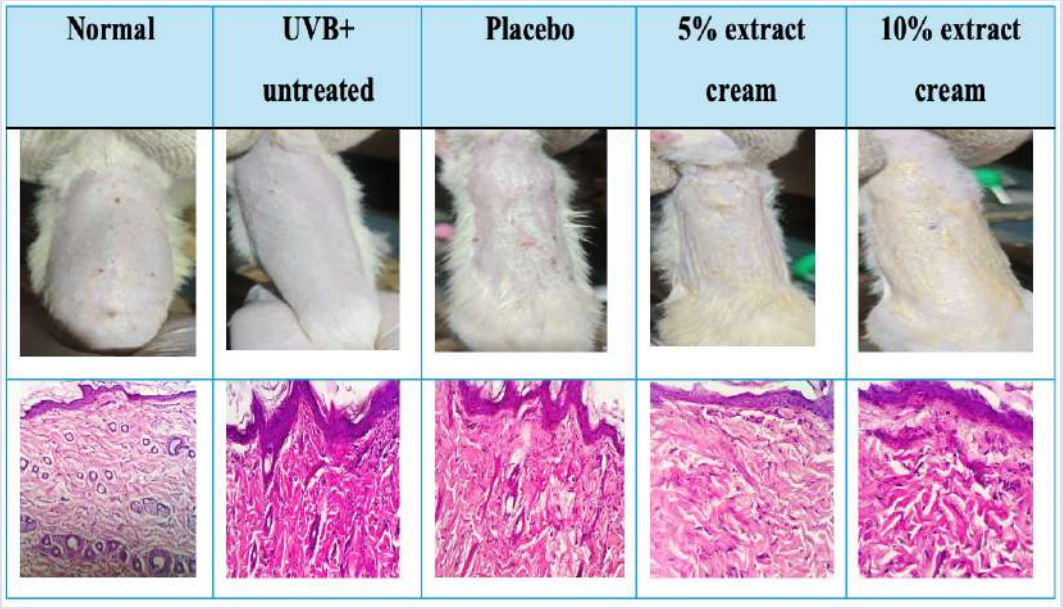

Figure 4. Visual and Histological Analysis

Table 3. Total phenolic, flavonoid content, and antioxidant activity of *C. moschata* seed extract

Parameter	Result (Mean ± SD)
Total phenolic	85.13 ± 1.38 mg GAE/g extract
Total flavonoid	36.01 ± 1.52 mg QE/g extract
Antioxidant activity (IC_{50})	84.31 ppm (Strong)

Table 4. Histological scoring of dermal collagen

Group	Collagen fiber maturity	Score
Normal	Minor collagen, fibroblasts present	1
UVB+ untreated	No collagen fibers	0
Placebo	Sparse immature collagen	1
5% extract cream	Moderate mature & immature collagen	2
10% extract cream	Predominantly mature collagen	3

UVB-induced skin damage is mediated by ROS generation, which activates MMP-1, leading to degradation of type I and III collagen in the dermis (Pittayapruek et al., 2016). This process results in dermal atrophy, thinning of collagen fibrils, and loss of dense connective tissue structure[35]. The histological findings in the UVB+ group of the present study corroborate this mechanism, showing epidermal thinning and collagen loss.

Topical treatment with *C. moschata* seed extract cream improved dermal collagen architecture in a dose-dependent manner. The extract's bioactive constituents flavonoids, phenolics, sterols, and tocopherols likely contribute to these effects by scavenging ROS, downregulating MMP-1 expression, and promoting collagen synthesis via activation of the Nrf2/HO-1 and TGF-β signaling pathways[36]. The near-normal collagen density observed in the 10% group suggests substantial regenerative potential, consistent with the biomarker improvements in CPD, MDA, and MMP-1.

Our results demonstrate that topical *Cucurbita moschata* seed extract cream significantly reduced UVB-induced DNA damage (CPD), oxidative stress (MDA), and collagen degradation (MMP-1), while preserving dermal collagen density. These effects were most marked at the 10% extract concentration. In addition to our in vivo findings, relevant human and ex vivo studies reinforce the translational potential of pumpkin seed–derived formulations. For example, Endo et al. (2019) showed that red pumpkin seed extract activated Nrf2 signaling in human keratinocytes, reducing oxidative stress and melanosome transfer under UVB exposure[37], similarly, Bora et al. reported that a pumpkin seed oil–based dermal formulation with UV filters and melatonin enhanced UV protection in human or ex vivo skin models[38]. These findings parallel our rat-model outcomes, supporting the idea that *C. moschata* seed extract may be effective in human skin contexts. Nonetheless, interspecies differences in skin thickness, repair capacity, and formulation uptake mean that further human/ex vivo trials are needed to confirm efficacy and safety.

Overall, the visual and histological evidence supports the conclusion that UVB exposure causes marked dermal collagen atrophy, while *C. moschata* seed extract cream particularly at 10% concentration effectively mitigates this damage and restores dermal integrity. These findings reinforce its potential as an effective photoprotective and anti-photoaging topical formulation.

This study has several limitations that should be acknowledged. First, only male rats were used, which does not account for possible sex-related differences in photoaging responses or treatment efficacy. Second, the study was conducted over a relatively short period of four weeks; therefore, the long-term effects of *Cucurbita moschata* seed extract on chronic UVB exposure remain unclear. Third, the extract was not directly compared with established photoprotective agents or antioxidants, such as vitamin E or polyphenols, which would provide a stronger benchmark for its relative efficacy. Future studies should address these aspects to strengthen translational relevance.

CONCLUSION

This study demonstrates that *Cucurbita moschata* seed extract cream exerts significant photoprotective effects against UVB-induced skin damage in Wistar rats. UVB irradiation markedly increased CPD, MDA, and MMP-1 levels while reducing dermal collagen density. Topical application of the extract cream, particularly at 10% concentration, significantly reduced CPD and MDA levels ($P \leq 0.05$), suppressed MMP-1 expression to near-normal values, and improved dermal collagen density and organization. These effects are attributed to the extract's rich content of phenolics, flavonoids, sterols, and tocopherols, which provide antioxidant, anti-inflammatory, and collagen-preserving activities. Histological analysis confirmed a dose-dependent restoration of dermal structure, with the 10% extract group showing the greatest recovery. Collectively, these findings support the potential of *C. moschata* seed extract cream as an effective natural photoprotective and anti-photoaging agent.

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