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THE EFFECT OF LEMON *(Citrus Limon)* ETHANOL EXTRACT CREAM ON COLLAGEN DENSITTY IN MALE WISTAR RATS *(Rattus Norvegicus)* AFTER EXPOSURE TO ULTRAVIOLET RAYS

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ABSTRACT

Background: Exposure to ultraviolet B (UVB) radiation causes premature aging characterized by collagen fiber degradation due to the formation of Reactive Oxygen Species (ROS). Lemon (Citrus limon) contains high levels of vitamin C and flavonoids, which have antioxidant properties and therefore have the potential to inhibit collagen degradation.
Objective: This study aimed to evaluate the effect of lemon ethanol extract cream on collagen density in the skin of male Wistar rats (Rattus norvegicus) exposed to UVB radiation.
Methods: An experimental study with a post-test only control group design was conducted on 28 male Wistar rats. The animals were divided into four groups: Control (K, base cream + UVB), and three treatment groups receiving lemon ethanol extract cream at concentrations of 6% (P1), 10% (P2), and 14% (P3). All groups were exposed to UVB (840 mJ/cm²) three times a week for four weeks. Collagen density was measured histologically using ImageJ software. Data were analyzed using One-Way ANOVA.
Results: Histological images showed a trend of increased collagen density in the treatment group compared to the control group. The highest average collagen density was observed in group P2 (10% concentration). However, statistical analysis showed no significant difference in collagen density between groups ($p > 0.05$).
Conclusion: Application of lemon ethanol extract cream at doses of 6%, 10%, and 14% did not inhibit UVB-induced collagen degradation in this experimental model.

Keywords: Citrus limon, collagen density, premature aging due to sunlight, ultraviolet B, antioxidants.

INTRODUCTION

Chronic exposure to ultraviolet B (UVB) radiation is a primary extrinsic factor accelerating skin aging, known as photoaging. UVB radiation penetrates the epidermis and dermis, triggering the generation of Reactive Oxygen Species (ROS). Components of the extracellular matrix, specifically collagen types I and III, undergo proteolytic degradation by Matrix Metalloproteinases (MMPs), which are upregulated by oxidative stress resulting from the excessive accumulation of Reactive Oxygen Species (ROS)[1,2]. The reduction in collagen density directly correlates with the formation of wrinkles and loss of skin elasticity[3,4].

To counteract these deleterious effects, the use of topical antioxidants has gained prominence. Lemon (*Citrus limon*) is a potent source of natural antioxidants, specifically ascorbic acid (Vitamin C) and flavonoids[5,6]. Vitamin C functions as a cofactor for prolyl and lysyl hydroxylase enzymes, which are crucial for stabilizing the collagen triple helix, whereas flavonoids neutralize free radicals and suppress the formation of reactive oxygen species[7,8].

Previous studies have demonstrated the efficacy of various plant extracts in protecting skin collagen[9,10]. However, the specific potential of a cream formulation containing the ethanol extract of whole lemon fruit (peel and flesh) in mitigating UVB-induced collagen loss remains under-researched. This study investigates the effect of

varying concentrations of lemon ethanol extract cream on the collagen density of UVB-exposed Wistar rats to determine its potential as a photoprotective agent.

However, the specific potential of a cream formulation containing the ethanol extract of whole lemon fruit (peel and flesh) in mitigating UVB-induced collagen loss remains under-researched. Based on the background description above, this study aims to evaluate the effect of lemon ethanol extract cream on collagen density in the skin of male Wistar rats (*Rattus norvegicus*) exposed to UVB radiation.

MATERIAL AND METHODS

Study Design and Animals

This true experimental study utilized a post-test only control group design. The sample consisted of twenty-eight male Wistar rats (*Rattus norvegicus*), aged 8–12 weeks, with a body mass ranging from 150–200 g. The inclusion criteria were healthy male Wistar rats exhibiting active movement, clear eyes, and no physical anatomical abnormalities. Rats that appeared sick, had physical defects, or died during the acclimatization period were excluded from the study. The animals were obtained and acclimatized for seven days prior to the experiment to ensure phenotypic homogeneity.

Ethical Clearance The study protocol and all experimental procedures involving animals were approved by the Ethics Committee of the Faculty of Medicine, Udayana University (Ethical Clearance Number: B/262/UN14.2.2.V.1/PT.01.01/2025).

Formulation of Lemon Extract Cream

Whole lemons (*Citrus limon*) were extracted using the maceration method with 96% ethanol solvent. The viscous extract was formulated into a cream base (oil-in-water emulsion) at concentrations of 6%, 10%, and 14%.

Experimental Procedure

The rats were randomly divided into four groups (7 per group). UVB irradiation was performed using a PL-S9W/01/2P lamp at a dose of 840 mJ/cm², three times weekly for 28 days. The distance between the light source and the dorsal skin was maintained at ± 5 cm. Creams were applied topically 15 minutes prior to irradiation and 4 hours after irradiation. The cream is applied twice a day.

Histological Analysis

After 28 days, skin tissue samples were stained with Masson's Trichrome to visualize collagen fibers. Collagen density was quantified digitally using ImageJ software on images captured with an Optilab Pro camera.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), with the threshold for statistical significance set at a p-value<0.05.

RESULT

The data analyzed came from 28 rat samples that had been divided into four groups (K, P1, P2, and P3) with seven samples in each group (N = 7).

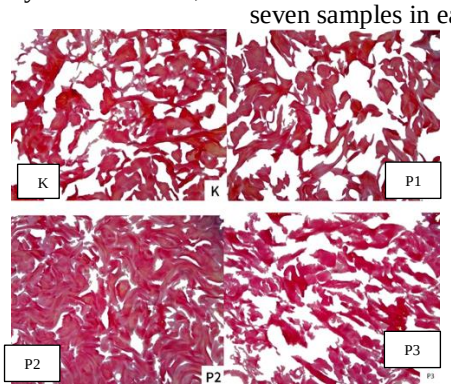


Figure 1. Collagen Density at 400x Magnification

Histological Observations

Histopathological examination revealed varying degrees of collagen density across the groups. The control group (P0), exposed to UVB without active treatment, exhibited fragmented and loosely packed collagen fibers, indicative of photodamage. In contrast, the treatment groups (P1, P2, P3) showed relatively denser and more organized collagen bundles. Visually, the group treated with 10% extract (P2) appeared to have the most compact collagen arrangement compared to the 6% (P1) and 14% (P3) groups based on **Figure 1.**

Quantitative Analysis of Collagen Density

Quantitative analysis using ImageJ supported the histological observations. The P2 group (10% extract) demonstrated the highest mean collagen density among all groups. However, the statistical analysis using One-Way ANOVA yielded a p-value of 0.595 (p>0.05).

Table 1. Mean Collagen Density

Group	Treatment	N	Mean Collagen Density (%) ± SD	P-Value
K	Base Cream + UV B (Control)	7	71.82 ± 9.40	*0.595
P1	6% Lemon Extract Cream + UV B	7	69.20 ± 7.50	
P2	10% Lemon Extract Cream + UV B	7	75.80 ± 9.90	
P3	14% Lemon Extract Cream + UV B	7	71.78 ± 9.20	

P-value: significance value of One-Way ANOVA test to compare the mean collagen density among the four groups simultaneously.
*: normally distributed data (p-value>0.05)

Based on **Table 1.** the highest average collagen density was found in group P2 (10% Concentration) at 75.80 ± 9.90%, followed by group P0 (Control) at 71.82 ± 9.40%. The lowest average was found in group P1 (6% Concentration) at 69.20 ± 7.50%. The results of the One-Way ANOVA test showed a p-value = 0.595 (p>0.05), which means there was no statistically significant difference in collagen density between the four treatment groups.

DISCUSSION

The present study aimed to assess the protective effect of lemon ethanol extract cream against UVB-induced collagen degradation. Our findings showed that while the 10% extract concentration (P2) resulted in the highest collagen density visually, the difference was not statistically significant compared to the control group.

The lack of statistical significance (P=0.595) suggests that under the experimental conditions employed, the lemon extract cream did not fully prevent UVB-induced damage. Several factors may account for this outcome. Firstly, the bioavailability and stability of the active compounds (vitamin C and flavonoids) in the cream formulation were not assessed. Vitamin C is highly unstable and susceptible to oxidation; without specific stabilization or encapsulation, its potency may have diminished during the 4-week storage and application period[11].

Secondly, the permeability of the extract into the dermis is critical. The stratum corneum acts as a lipophilic barrier, while vitamin C is hydrophilic. Without a penetration enhancer or specific delivery system, the active ingredients may not have reached the dermal fibroblasts effectively to stimulate collagen synthesis or scavenge ROS[12].

Interestingly, the 10% concentration (P2) showed better visual results than the 14% concentration (P3). This non-linear dose-response relationship, often seen in antioxidant studies, could imply a "pro-oxidant" effect at higher concentrations or saturation of absorption pathways, although the differences here remained non-significant[13].

This study did not include stability testing of the cream formulation or in vitro permeation studies. Additionally, markers of oxidative stress (e.g., MDA) or specific collagen gene expression were not measured, which could have provided molecular insights beyond histological density.

CONCLUSION AND SUGGESTION

Application of lemon ethanol extract cream at doses of 6%, 10%, and 14% did not inhibit UVB-induced collagen degradation in this experimental model. Future research should focus on optimizing the formulation to enhance stability and skin penetration and verifying efficacy with molecular markers of collagen synthesis.

Future studies should investigate wider extract concentration ranges and extended treatment durations to better evaluate long-term collagen remodeling.

FUNDING, CONFLICT OF INTEREST, AND ETHICAL CONSIDERATION

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Ethical Consideration

This research was conducted in compliance with the ethical standards for animal welfare as approved by the Ethics Committee of the Faculty of Medicine, Udayana University.

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