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ASSOCIATION BETWEEN FERULIC ACID, HYALURONIC ACID AND RADIOFREQUENCY FOR SKIN MOISTURIZING, COLLAGEN SYNTHESIS AND LIPOLYTIC EFFECTS IN WISTAR RATS

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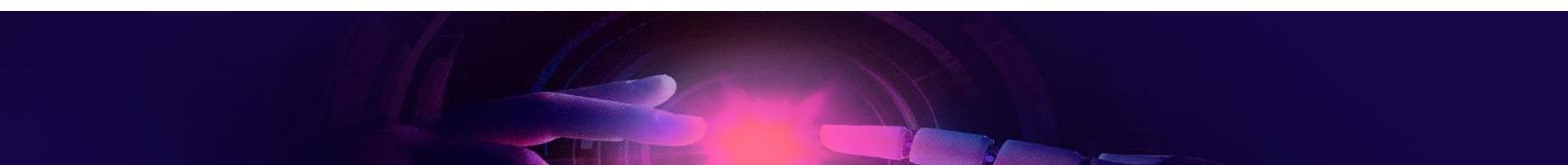
ABSTRACT

Radiofrequency (RF) induces the production of new collagen fibers and reorganizes existing collagen structures. Hyaluronic acid (HA) and ferulic acid (FA) are widely used in cosmetology due to their beneficial properties—HA for its moisturizing effect and FA for its role in stimulating collagen synthesis. This study aimed to evaluate whether RF combined with HA and FA exhibits lipolytic effects and influences collagen synthesis in rats. The animals were divided into six groups (n=5): G1 (control, no treatment); G2 (RF only); G3 (RF + FA cream); G4 (RF + HA cream); G5 (FA cream only); and G6 (HA cream only). RF was applied weekly (40°C for 2 minutes), while topical creams were used daily for four weeks. Biopsies were performed post-treatment for histological and immunohistochemical analysis. The results indicated that RF combined with FA cream led to a more pronounced reduction in adipose tissue, suggesting a stronger lipolytic effect. The RF with HA combination resulted in thickening of the papillary dermis, increased intercellular fluid, and enhanced skin turgor, with a 49% increase in type III collagen synthesis. Furthermore, a higher presence of mast cells in both the superficial and deep dermis was observed in groups G2 and G3. Overall, the findings suggest that combining RF with topical HA or FA enhances neocollagenesis, increases dermal thickness, and reduces adipose tissue more effectively than RF alone in Wistar rats.

Keywords: Radiofrequency; Hyaluronic acid; Ferulic acid.



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INTRODUCTION

Skin aging is characterized by a slower regeneration of its structure and functionality. As individuals age, they experience the effects of chronological skin aging as a result of changes in certain proteins and a reduction in cell proliferation. Consequently, there is a loss of tissue elasticity and a reduction in its ability to regulate water exchange (HO; DREESEN, 2021; LEE *et al*., 2021). Collagen and elastin are fundamental proteins in the extracellular matrix of connective tissue, providing resistance and elasticity. As the years pass by, collagen tends to become more rigid, compromising the elasticity and strength of its fibers. There is also a reduction in glycosaminoglycans and water, which hinders cell development and the maintenance of the subcutaneous fat layer, leading to the appearance of asymmetries. Moreover, the degeneration of elastic fibers combined with decreased tissue oxygenation results in dehydration (SWIFT, 2021; PARK, 2022).

The reversibility of these processes is based on the production of new collagen fibers and the reorganization of existing collagen structures, which can be achieved through therapeutic modalities, such as radiofrequency (RF). This technique involves the generation of energy through electromagnetic waves with high-frequency currents, which is capable of penetrating the epidermis, dermis, and hypodermis (MAJIDIAN *et al*., 2021; VASSÃO *et al*., 2022). This electromagnetic radiation induces a temperature increase in tissues, stimulating local circulation and producing a hyperemic, analgesic, antispasmodic, and anti-inflammatory effect. Consequently, local vasodilation improves tissue trophism, reabsorption of intercellular fluids, and stimulation of circulation, resulting in an increase in oxygen, nutrients, and trace elements for the tissues, as well as an enhanced system for the drainage of cellular waste (MONARETTI *et al*., 2022; GENTILE; HALAAS, 2024).

Radiofrequency promotes skin rejuvenation through two complementary mechanisms: (1) collagen denaturation followed by fiber contraction, resulting in an immediate tightening effect, and (2) fibroblast activation, which stimulates both neocollagenesis and remodeling of preexisting fibers. Together, these processes result in tissue reorganization and long-term improvement in skin structure (LYU; LIU, 2022). However, current research is exploring possible combinations of different therapies with the purpose of enhancing such results. In this context, cosmetic active ingredients have emerged as important allies in the prevention and treatment of skin aging through their antioxidant effect, increased skin hydration and even enhanced collagen production (SUNDER, 2019).

The use of cosmetic active ingredients, such as hyaluronic acid (HA), provides hydration and elasticity to the skin and can retain up to 100 times its molecular weight in water, contributing to improved skin structure and elasticity (PAVLOU *et al*., 2021; MIRTALEB *et al*., 2021; BRAVO *et al*., 2022). Another therapeutic alternative is the use of ferulic acid (FA), which is considered a functional substance with antioxidant and anti-inflammatory activities. Ferulic acid is regarded as an antioxidant compound with cytoprotective activity due to its ability to reduce free radicals and activate the cellular stress response, further enhancing collagen production (CAVALCANTI *et al*., 2021; HONG; YOON, 2022).

Based on the aforementioned rationale, we hypothesize that the combination of RF with topical HA or FA may amplify both neocollagenesis and remodeling of existing fibers, resulting in a more organized dermal architecture, a better hydration, and an enhanced lipolytic effect. Therefore, this study aims to evaluate the influence of topical application of HA or FA cream combined with RF, on collagen production and organization, as well as on skin hydration and lipolytic effects in Wistar rats.

MATERIALS AND METHODS

Preparation of Hyaluronic Acid and Ferulic Acid Creams

To assess the effect of topical application of ferulic acid or low molecular weight hyaluronic acid, alone or in combination with radiofrequency, creams containing 0.5% (w/w) of these active ingredients were prepared. The formulation was as follows:

- Ferulic or hyaluronic acid.....0.5%
- Self-emulsifying wax (CRODABASE CR2).25%
- Propylparaben.....0.05%
- Propylene glycol.....2.5%

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- Methylparaben.....0.15%
- Glycerin.....2.5%
- EDTA.....0.1%
- Distilled water.....qs.....50g

First, the base cream (without active ingredients) was prepared by melting Crodabase CR2® wax and propylparaben (oil phase) in a glass container in a water bath at 70°C. In another container, methylparaben and EDTA were dissolved in distilled water (aqueous phase), also heated to 70°C. After dissolution, the glycerin and propylene glycol were added to the latter container. The aqueous phase was then slowly incorporated into the oil phase under constant stirring. The container was removed from the water bath, and the mixture was continued stirring until a homogeneous and consistent cream was obtained.

Next, ferulic acid or low molecular weight hyaluronic acid was ground in a mortar and pestle, dispersed in propylene glycol, and gradually incorporated into the base cream under continuous homogenization. The resulting products were stored in plastic syringes and kept at room temperature. In the case of hyaluronic acid, the low molecular weight form was chosen due to its greater skin penetration capacity compared to the high molecular weight forms, which tend to remain in the stratum corneum. Low molecular weight hyaluronic acid is capable of diffusing to deeper layers of the epidermis, promoting a better hydration and resulting in an enhanced interaction with dermal fibroblasts, therefore stimulating collagen synthesis. Furthermore, the final cream had a medium viscosity, ensuring good spreadability for topical application, while maintaining sufficient residence time on the skin surface, thus maximizing the active ingredient's bioavailability in the experimental model.

In Vivo Assays

This study aimed to evaluate the effects of combining radiofrequency with topical ferulic or hyaluronic acid (cream form) on collagen production, hydration, and lipolysis in Wistar rats. Biopsies of treated tissue were analyzed histopathologically.

Animal Housing Conditions

The study followed National Council for the Control of Animal Experimentation (CONCEA) guidelines and included 30 male Wistar rats (2 months old, 220-320g) from Potiguar University's (UnP) Bioterium. Ethics approval was granted by the Ethics Committee on the Use of Animals (CEUA) at UnP. Animals were housed in individual cages with pine shavings, in ventilated racks, under controlled conditions (20°C ± 2°C, <60dB noise, 45% ± 15% humidity, 12-hour light-dark cycle). They received Purina® feed and filtered water ad libitum.

Experimental Procedure

The animals were randomly divided into six groups (n=5). Group 1 (control) received no treatment. Group 2 received weekly radiofrequency for four weeks. Groups 3 and 4 received weekly radiofrequency plus daily 0.5 mL of 0.5% ferulic or hyaluronic acid cream, respectively. Groups 5 and 6 received only daily applications of the same creams. After four weeks, all animals were sacrificed.

Animals were weighed and anesthetized (Tiletamine/Zolazepam, 50 mg/kg, intramuscularly). A 6 cm x 3.5 cm dorsal area was shaved and disinfected with 2% chlorhexidine gluconate. Radiofrequency (VIP Eletromedicina, Tecatherapy, 40°C) was applied for 2 minutes. Creams (0.5 mL) were spread evenly for 1 minute.

Sample Preparation for Histopathological and Immunohistochemical Analysis

On day 29, animals were sacrificed and treated tissue biopsies were fixed in 10% formalin for 24 hours. Samples were dehydrated in ethanol, cleared with xylene, and embedded in paraffin at 65°C. Sections (3-5 µm) were obtained, mounted on slides, and stained with Hematoxylin & Eosin (H&E), Masson's Trichrome, and Picrosirius Red. Toluidine blue staining assessed mast cell count, and immunohistochemical analysis followed standard protocols.

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Qualitative Analysis

The tissue samples were examined qualitatively by three blinded examiners using a binocular optical microscope (Model: CX31; Brand: OLYMPUS/JAPAN, 2011) equipped with a digital camera (Model: PL-1; Brand: OLYMPUS/JAPAN, 2011). Microphotographs were taken at magnifications of 40x, 100x, and 400x. The following parameters were assessed:

a) Collagen fiber thickness: normal or increased.

b) Collagen fiber organization: ordered or disordered.

c) Epidermal thickness: up to 4 layers or more than 4 layers.

Quantitative Analysis

Photomicrographs (5x-40x) were taken using a Leica DMR microscope and DFC450 camera. Histomorphometric measurements (dermal/adipose tissue areas, hair follicle count) were conducted using Leica Application Suite (LAS). Five fields per sample (5x magnification) were analyzed. Picrosirius-stained sections under polarized light quantified type I (red/orange) and type III (green) collagen in ten 20x magnification fields.

Mast cells were counted in twenty high-magnification fields (ten superficial, ten deep dermis) following Beer *et al*. (1998). Scar tissue areas were assessed in H&E and immunohistochemically stained sections. Degranulated mast cells were identified by distinct brown cytoplasmic staining. A Graticules Ltd. reticle ensured accurate counts. Ten fields (250x) were analyzed for mast cell density (per mm^2) in the scar and adjacent dermis.

Statistical Analysis

Normality tests (Shapiro-Wilk, Kolmogorov-Smirnov) evaluated Gaussian distribution of dermal/adipose areas, hair follicles, and collagen type. Two-way ANOVA compared group means. Significance was set at $P \leq 0.05$; highly significant results at $P < 0.0001$.

RESULTS

Histological Analysis

The descriptive histological analysis was conducted by comparing the evaluated groups. Figure 1 compares the control group (G1, untreated) and the group treated with weekly application of radiofrequency (G2). The radiofrequency-treated group (Figure 1b) showed a greater reduction in adipose tissue, as well as an increased presence of blood vessels and fibroblasts compared to the control group (Figure 1a). This indicates that weekly applications of radiofrequency over a total of 4 weeks resulted in satisfactory lipolytic, angiogenic, and fibroblast-inducing effects.

Figure 1. Histological results of the control group (A) and the group treated with a weekly application (for 4 weeks) of radiofrequency (B); HE 40X.

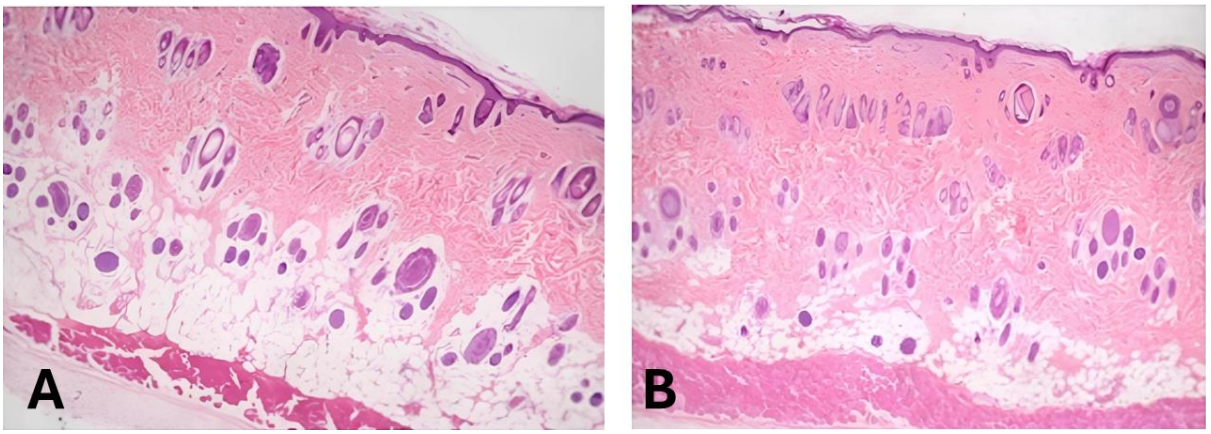

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Figure 2 provides the same comparison with a different staining technique (Masson's Trichrome) between the G1 and G2 groups. Figure 2b highlights the presence of fluid in the intercellular space of the papillary dermis, which is not observed in the control group (2a), indicating a moisturizing effect on the skin resulting from the treatment.

Figure 2. Histological analysis of the control group (A) and group treated with a weekly application (for 4 weeks) of radiofrequency (B); Masson's Trichrome, 100X.

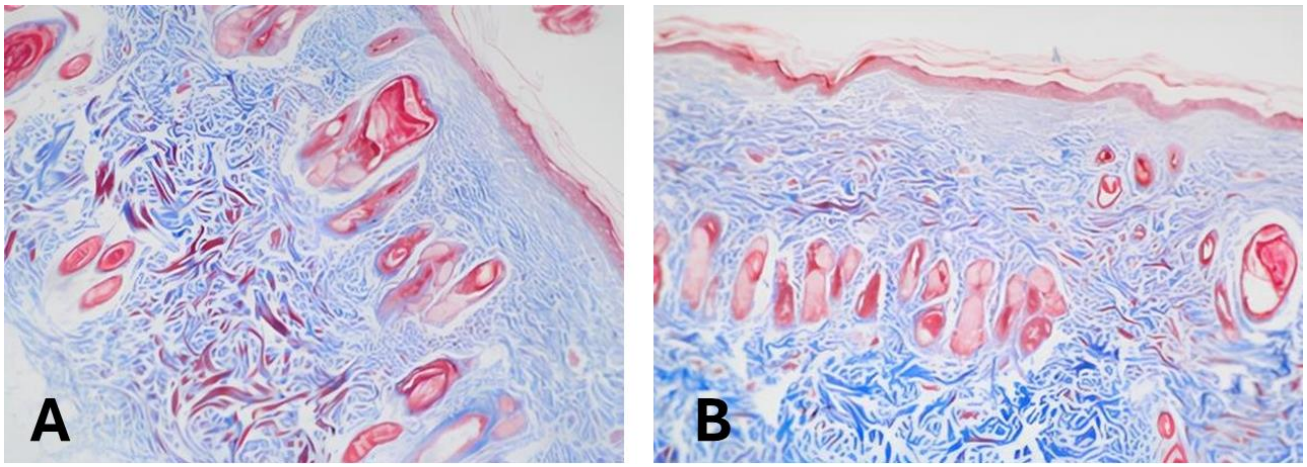
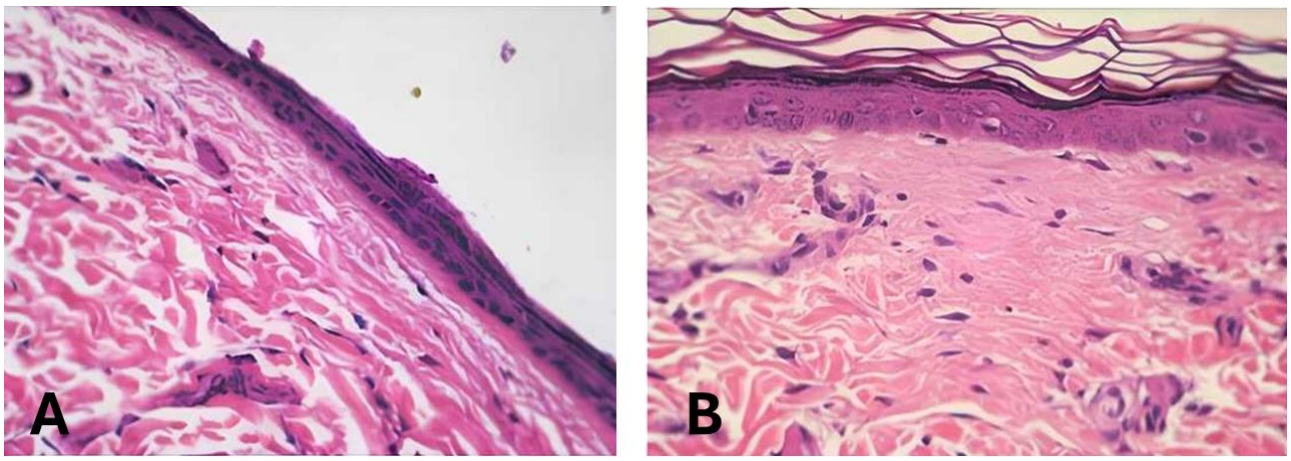


Figure 3 illustrates the effects of radiofrequency on the increase and organization of collagen fibers. The control group exhibits more delicate subepithelial collagen fibers with horizontal orientation (Figure 3a), while the radiofrequency-treated group (Figure 3b) displays thicker subepithelial collagen (papillary dermis).

Figure 3. Histological results of the control group (A) and the group treated with a weekly application (for 4 weeks) of radiofrequency (B), showing the arrangement and presence of collagen fibers; HE 400x.



A comparison of the histopathological results between the group treated with radiofrequency alone (RF) and the group treated with radiofrequency combined with daily applications of ferulic acid cream (RF + ferulic acid) is shown in Figures 4a and 4B, respectively. The figures reveal that the papillary dermis in the RF + ferulic acid

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group (Figure 4b) is thinner than that in the RF group (Figure 4b).

Figure 4. Histological results of the groups treated with radiofrequency alone (weekly application for 4 weeks) (A) and with a combination of radiofrequency and daily applications of ferulic acid cream (B); Masson's Trichrome, 400x.

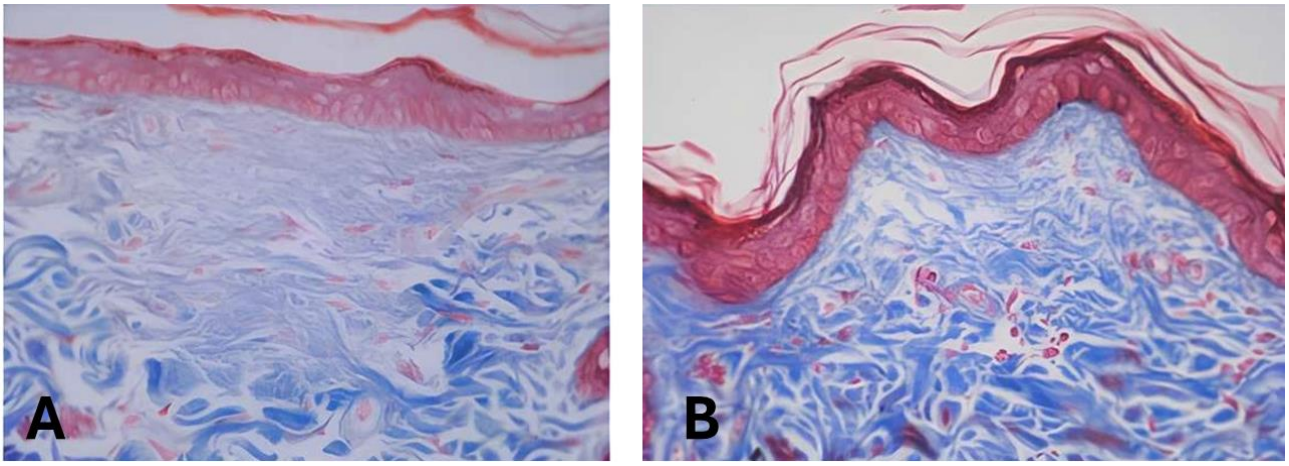
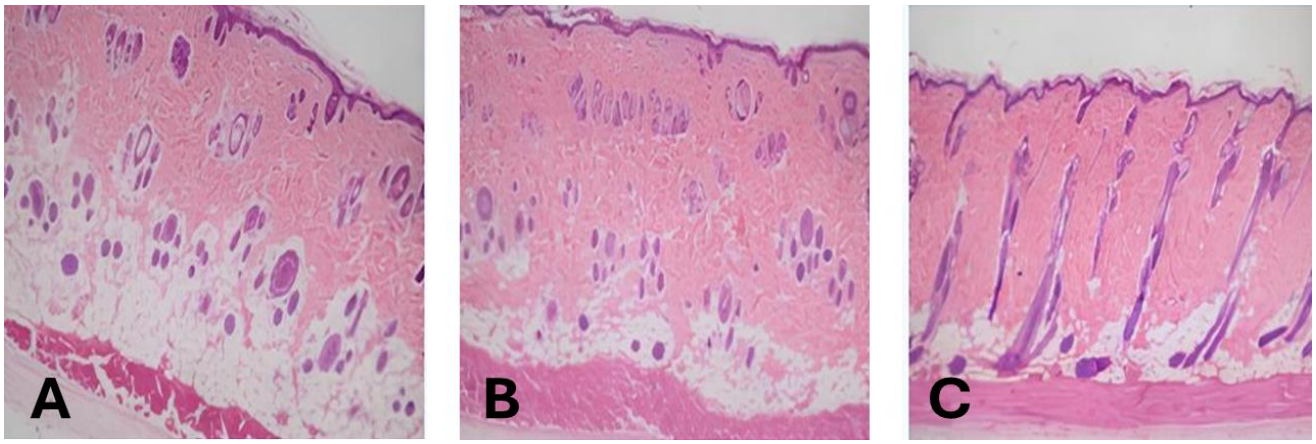


Figure 5 compares the groups with no treatment (control), treated with radiofrequency alone (RF), and treated with the combination of radiofrequency and ferulic acid cream (RF + ferulic acid) concerning the area of adipose tissue (Figure 5a, Figure 5b and Figure 5c, respectively).

Figure 5. Histopathological analysis of the control (A), RF (B) and RF + ferulic acid (C) groups, showing the effect of these treatments on the reduction of adipose tissue; HE 40X.



In Figure 6, the comparison between groups treated with radiofrequency combined with topical applications of ferulic acid (RF + ferulic acid) and hyaluronic acid (RF + hyaluronic acid) is shown. Figure 6b illustrates that the group treated with RF + hyaluronic acid cream has a loose and disorganized collagen fibers in the papillary dermis than the group treated with RF + ferulic acid cream (Figure 6a), which shows a more organized papillary dermis with parallel collagen fibers,

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suggesting a potential advantage of ferulic acid over hyaluronic acid in collagen organization

Figure 6. Histopathological analysis of the groups treated with RF + ferulic acid cream (A) and RF + hyaluronic acid cream (B), showing the effect of these treatments on the organization of collagen fibers. Trichrome 400X.

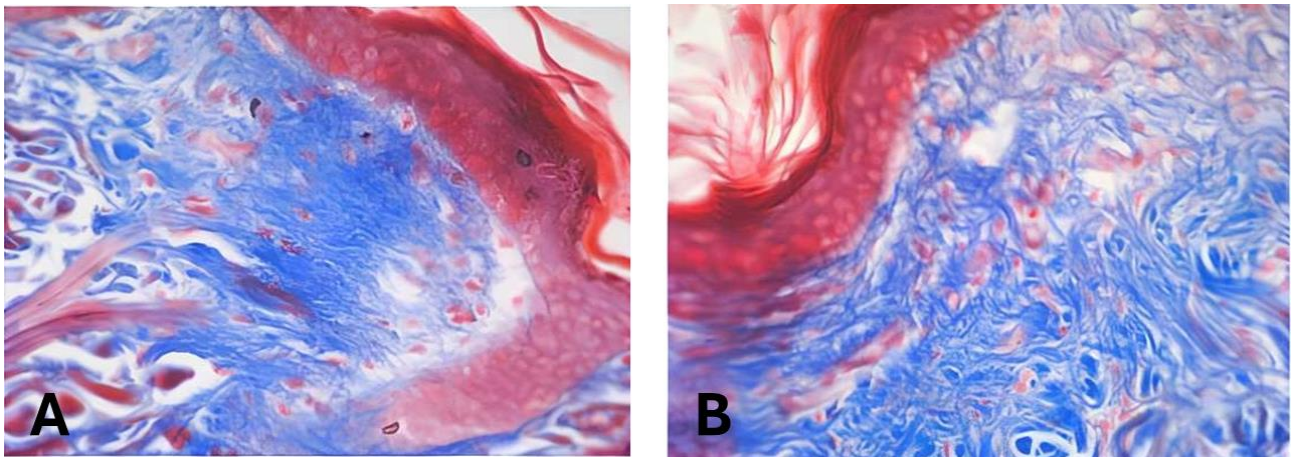

Figure 7 shows a comparison between the group treated with radiofrequency alone (RF) and the group treated with radiofrequency combined with daily applications of hyaluronic acid cream (RF + hyaluronic acid), highlighting a thicker papillary dermis in the latter group (Figure 7b) with a greater amount of intercellular fluid.

Figure 7. Histopathological analysis of the groups treated with RF alone (A) and RF + hyaluronic acid cream (B), showing the presence of thicker papillary dermis with intercellular fluid in the latter group (B). Trichrome 400X.

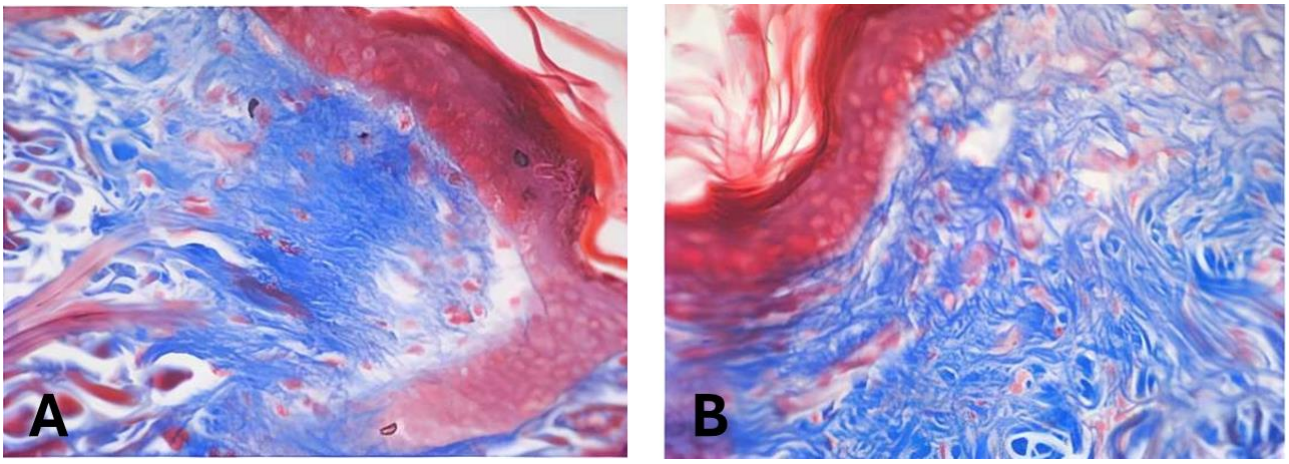

Figure 8 presents the histopathological analyses of the following groups: RF alone (A), RF + ferulic acid cream (B), RF + hyaluronic acid cream (C), ferulic acid cream alone (D), and hyaluronic acid cream alone (E). It is evident that the group treated with daily applications of ferulic acid cream alone exhibited the thinnest papillary

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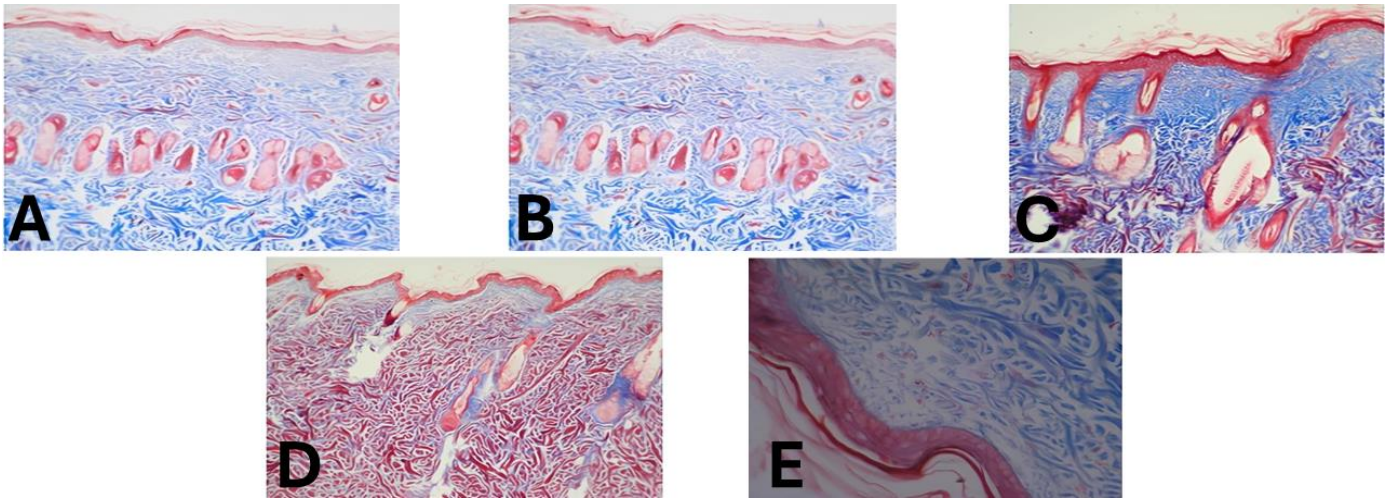
dermis. Additionally, the group treated with daily applications of hyaluronic acid cream alone shows prominent interstitial spaces, loose collagen, edema with a large amount of fluid, and greater spacing between collagen fibers, indicating higher skin turgor compared to the other groups.

Figure 8. Histopathological analyses of groups RF (A), RF + ferulic acid cream (B), RF + hyaluronic acid cream (C), ferulic acid cream alone (D) and hyaluronic acid cream alone (E); Trichrome 100x.



Histomorphometry of the Dermis and Subcutaneous Adipose Tissue Thickness

The previously presented histological analyses were subjected to histomorphometric analysis to confirm the results (Figure 9). Representative photomicrographs of the control group (A) show a normal distribution of hair follicles in the dermis and a considerable amount of subcutaneous adipose tissue. In contrast, the group treated with weekly radiofrequency applications (B) shows a reduction in subcutaneous adipose tissue with an increase in the number of hair follicles and dermal area, whereas the RF + ferulic acid group (C) shows areas of neocollagenesis, an increase in dermal area and the number of hair follicles, along with a slight amount of subcutaneous adipose tissue, similar to the events observed in the other treated groups (D, E, and F).

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Figure 9. Photomicrographs of the control group (A) and the groups treated with radiofrequency alone (B), with the combination of RF + ferulic acid cream (C), with RF + hyaluronic acid cream (D), as well as with topical applications of ferulic acid cream (E) and hyaluronic acid cream (F). HE, 50x.

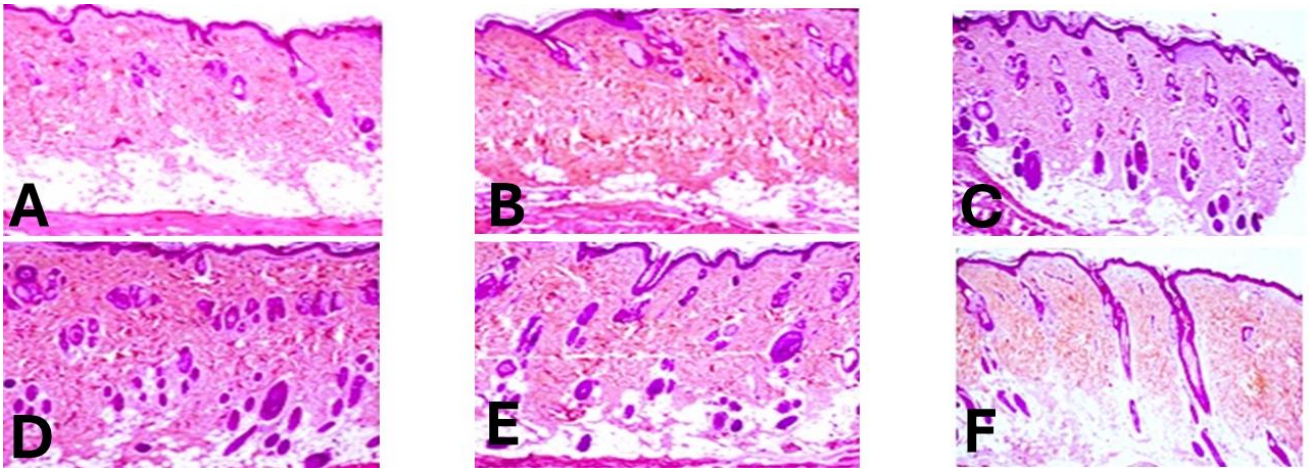

Figure 10 shows the results of the quantitative analyses of dermal area (Figure 10a), number of hair follicles (Figure 10b), and adipose tissue area (Figure 10c). Statistical analysis shows significant differences between the groups ($p<0.0001$) concerning the three quantified parameters. The results indicate that the dermal area was larger in the group treated only with weekly radiofrequency applications (Figure 10a). Moreover, the number of hair follicles remained the same between the control group and those treated with daily applications of ferulic acid cream and hyaluronic acid cream (Figure 10b). On the other hand, the combination of these creams with radiofrequency resulted in a significant increase in the number of hair follicles. The results also show that the combination of radiofrequency with topical applications of ferulic acid cream and hyaluronic acid cream was advantageous concerning the lipolytic effect, given the greater reduction in adipose tissue area in the RF + ferulic acid cream and RF + hyaluronic acid cream groups (Figure 10c).

Figure 10. Quantitative analysis of the dermis area (A), number of hair follicles (B) and adipose tissue area (C). Columns with different letters are those that presented statistically different results (for each graph separately).

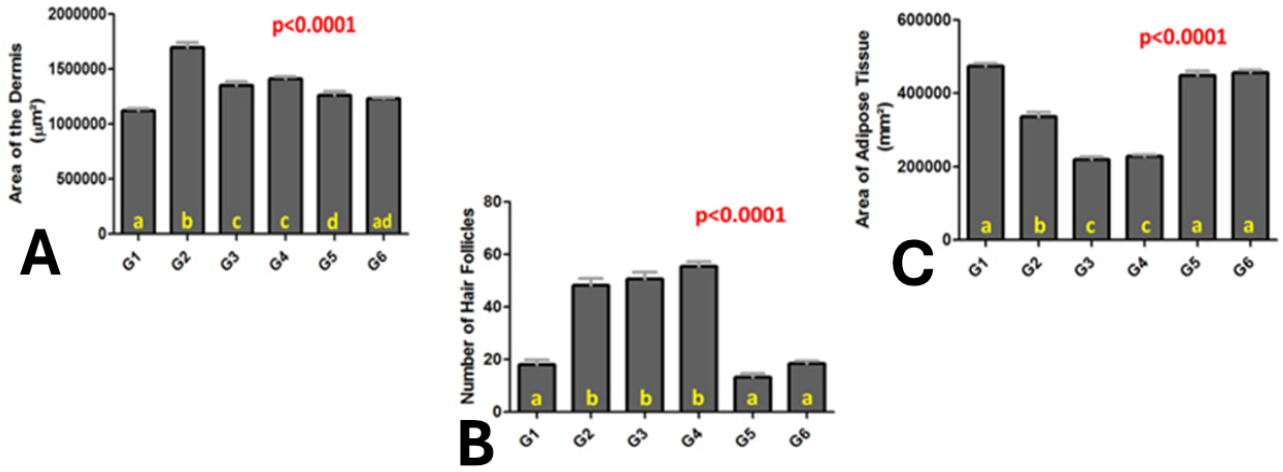

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Figure 11 depicts representative photomicrographs of all groups, showing the predominance of red-polarized fibers (Type I collagen) in the untreated group (Figure 11a), while the presence of red and green fibers, with a predominance of Type III collagen in deeper areas, is observed in the group treated with weekly applications of radiofrequency alone (Figure 11b). In contrast, the combination of radiofrequency with topical applications of ferulic acid cream (Figure 11c) and hyaluronic acid cream (Figure 11d) resulted in an increase in Type III collagen, whereas the groups treated with topical applications of these creams alone, without radiofrequency, showed an absence of this collagen type.

Figure 11. Photomicrographs of the groups without treatment (A) and treated with radiofrequency alone (B), as well as in combination with daily applications of ferulic acid cream (C) and hyaluronic acid (D), in addition to applications of ferulic acid cream (E) and hyaluronic acid cream (F), without the use of radiofrequency, showing the presence of type I (red) and type III (green) collagen fibers. Picrosirius red, 200x.

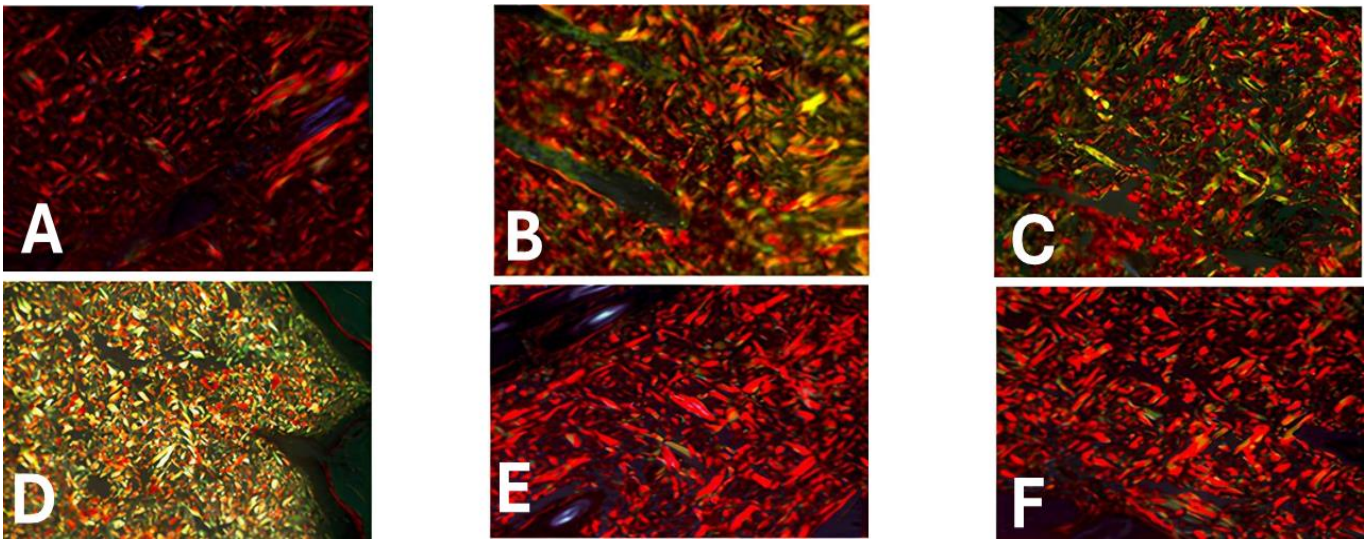

Figure 12 represents the percentage of type I and type III collagen in each group, demonstrating the superiority of the group treated with radiofrequency combined with daily topical applications of hyaluronic acid cream, corroborating the qualitative histological results.

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Figure 12. Percentage of type I and type III collagen in the groups without treatment (G1) and treated with weekly application of radiofrequency alone (G2), and associated with daily applications of ferulic acid cream (G3) and hyaluronic acid crem (G4), in addition to applications of ferulic acid cream (G5) and hyaluronic acid cream (G6) without radiofrequency.

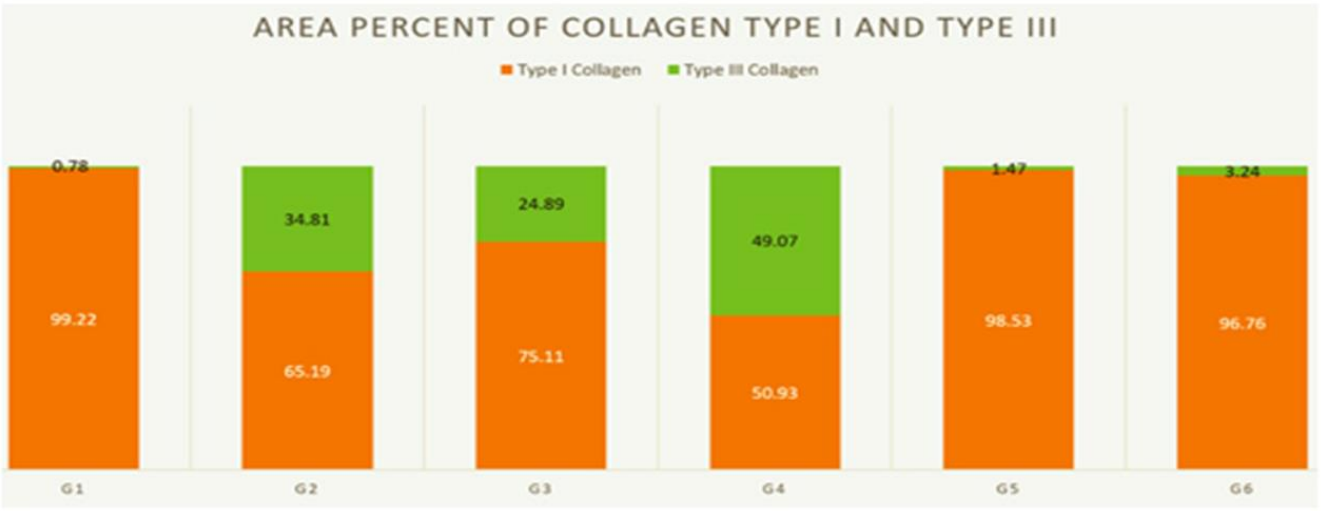

Figure 13 shows the presence of mast cells in the dermis of untreated groups and those with isolated and combined treatments. In the control group (Figure 13a), a few mast cells were found adjacent to blood vessels, located only in the deep dermis, whereas the radiofrequency-treated group (Figure 13b) presented mast cells distributed in both the deep and superficial dermis (adjacent to the epithelium). The group treated with RF + ferulic acid cream (Figure 13c) exhibited an increase in mast cells adjacent to hair follicles, while the RF + hyaluronic acid cream group (Figure 13d) showed mast cells contiguous with subcutaneous adipose tissue. Similar to the control group, the groups treated with topical applications of ferulic acid (Figure 13e) and hyaluronic acid (Figure 13f) without radiofrequency presented few mast cells, found near sebaceous glands and blood vessels.

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Figure 13. Analysis of the presence of mast cells in the dermis of the groups without treatment (G1) and treated with weekly application of radiofrequency alone (G2), as well as associated with daily applications of ferulic acid cream (G3) and hyaluronic acid cream (G4), in addition to applications of ferulic acid cream (G5) and hyaluronic acid cream (G6) without radiofrequency. Toluidine blue staining, 200x.

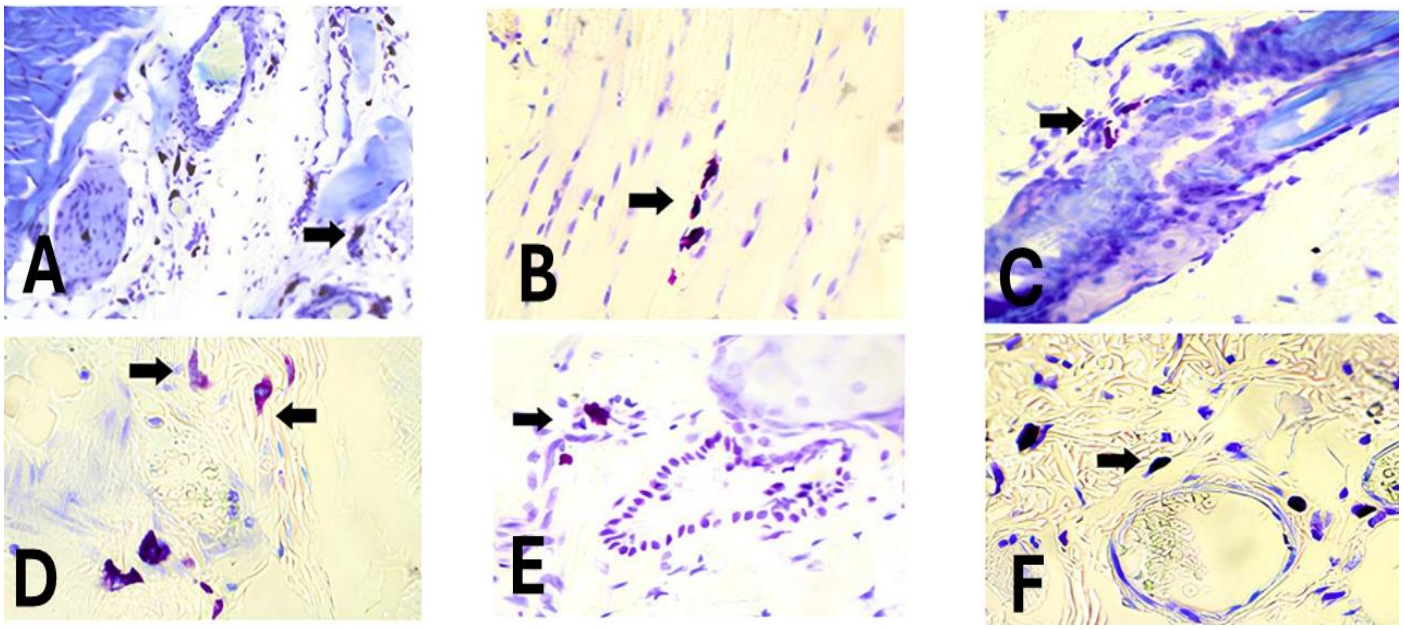

Figure 14 shows the quantitative results of mast cell counts in the superficial and deep dermis, demonstrating a predominance of mast cells in the deep dermis in the groups treated with RF (G2) and RF + ferulic acid cream (G3), once again indicating the superiority of the combined treatment over isolated treatments (RF alone or ferulic acid or hyaluronic acid cream alone).

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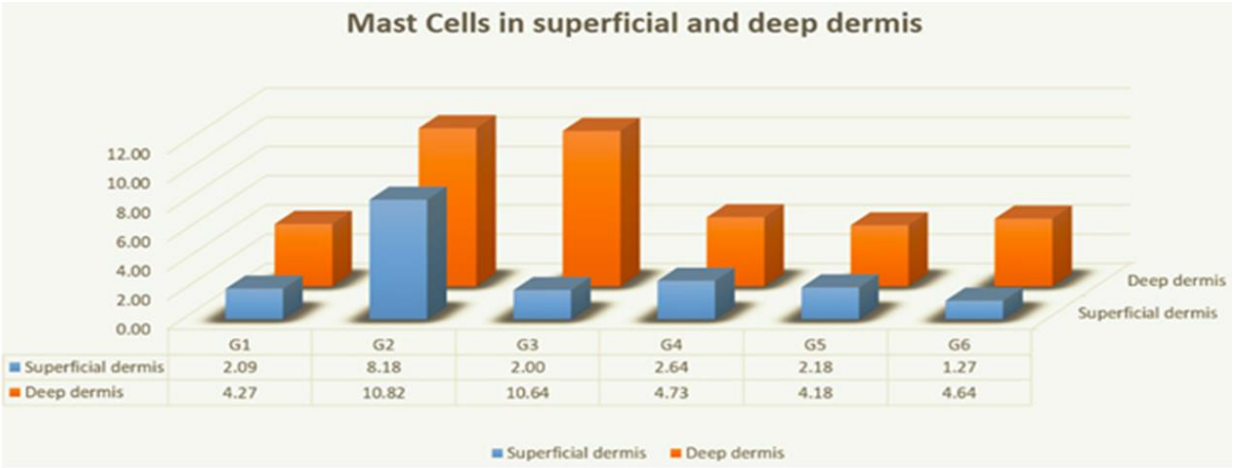
Figure 14. Graphical representation of the number of mast cells in the dermis of the groups without treatment (G1) and treated with weekly application of radiofrequency alone (G2) or associated with daily applications of ferulic acid cream (G3) or hyaluronic acid cream (G4), in addition to daily applications of ferulic acid cream (G5) and hyaluronic acid cream (G6) without radiofrequency.



DISCUSSION

The findings from this histological and histomorphometric analysis provide insights into the effects of radiofrequency (RF) alone and in combination with ferulic acid and hyaluronic acid on skin tissue, particularly focusing on adipose reduction, collagen organization, and dermal hydration. The RF treatment alone induced a notable lipolytic effect in Wistar rats, evidenced by reduced adipose tissue, increased vascularization and fibroblast presence. These outcomes corroborate with recent literature data indicating that RF stimulates fibroblast activity and adipocyte reduction, thus enhancing dermal firmness and elasticity due to the remodeling of the extracellular matrix (ECM) (HERNÁNDEZ-BULE *et al*., 2021; SANTOS *et al*., 2024).

The combination of RF with ferulic acid cream demonstrated significant benefits in collagen organization, where the papillary dermis presented a more structured alignment of collagen fibers compared to RF alone. This synergistic effect may be attributed to the antioxidant properties of ferulic acid, which supports collagen stabilization and inhibits ECM degradation caused by oxidative stress, as documented in recent studies (ZDUNSKA *et al*., 2018; LI *et al*., 2021). The RF + hyaluronic acid group also showed increased dermal hydration, marked by prominent interstitial spaces and edema, which are consistent with hyaluronic acid's role in enhancing moisture retention within the dermal matrix (YASIN *et al*., 2022; MATALQAH *et al*., 2024). Thus, while FA primarily promotes structural organization, HA enhances dermal expansion through hydration, and these distinct mechanisms may translate into complementary therapeutic outcomes.

The qualitative findings are further corroborated by quantitative collagen analysis (Figure 12). Notably, the RF + HA group (G4) showed a 49% increase in type III collagen, a subtype associated with elasticity and tissue repair, while the RF + FA group (G3) showed a smaller, albeit significant, increase. This difference suggests that HA not only improves hydration but may also promote a regenerative dermal microenvironment that stimulates type III collagen deposition. On the other hand, FA appears to play a greater role in maintaining organized and stable collagen networks, which may contribute to a firmer dermal architecture rather than pronounced elasticity.

Although the present study focused on histological and histomorphometric results, it is plausible that the observed effects may be mediated by molecular pathways involving oxidative stress modulation, extracellular matrix remodeling, and hydration signaling.

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Ferulic acid, through its antioxidant activity, can attenuate ROS-induced collagen degradation, thus increasing the organizational impact of RF-induced neocollagenesis (CAVALCANTI *et al*., 2021; ZDUŃSKA *et al*., 2018). On the other hand, hyaluronic acid can activate CD44-mediated signaling pathways, promoting fibroblast proliferation and matrix remodeling, while simultaneously retaining water molecules (FUKUI *et al*., 2000). The combination of RF with these topical agents can, therefore, act synergistically: RF initiating controlled thermal stress and neocollagenesis, while FA and HA modulating the oxidative and hydration microenvironment to enhance tissue repair (HERNÁNDEZ-BULE *et al*., 2021).

Furthermore, the combination treatment of RF with ferulic or hyaluronic acid resulted in an increased number of hair follicles and a shift towards Type III collagen synthesis, which is a collagen subtype associated with skin elasticity and repair. The increase in Type III collagen in deeper dermal layers in the RF-treated groups suggests that RF may initiate a regenerative response, further supported by previous studies that show that RF induces neocollagenesis and promotes skin rejuvenation (KACZMAREK *et al*., 2020; NATARI *et al*., 2020). These cellular and structural modifications underscore the potential of RF combined with specific topical agents to address age-related changes in skin elasticity and hydration.

A notable aspect of this study is the differential distribution and increased density of mast cells in the treated groups. The increase in mast cells, especially near hair follicles and adipose tissue in the RF-treated and combined groups, aligns with recent findings that suggest mast cells may play a role in wound healing and tissue remodeling by releasing mediators that recruit fibroblasts and influence collagen synthesis (FUKUI *et al*., 2000; PASTWINSKA *et al*., 2022). This mast cell proliferation and strategic localization may enhance skin regeneration and dermal repair mechanisms, which might contribute to the therapeutic effects observed in the combined treatments.

Taken together, these findings show that the RF + FA combination is more effective in promoting collagen organization and stability, while the RF + HA combination improves hydration, dermal turgor, and type III collagen synthesis. These complementary mechanisms highlight the therapeutic potential of associating RF with topical application of HA or FA cream to achieve distinct clinical objectives in skin rejuvenation.

As an important limitation of this study is the lack of a long-term follow-up, which prevents us from determining whether the observed dermal changes are transient or sustained over time. Considering that radiofrequency-induced collagen remodeling and HA-mediated hydration may follow distinct temporal dynamics, future studies with longer observation periods are needed to clarify the durability and clinical relevance of these effects. Another limitation is the lack of mechanistic exploration of the interactions between RF and topical agents at the molecular level, which would be beneficial to better understand the pathways driving the observed results. Regarding clinical translation, although the results in Wistar rats provide robust evidence of structural and functional changes in the skin, caution is necessary when extrapolating to humans. Differences in dermal thickness, collagen turnover, and topical absorption between species may require adjustments to RF parameters and formulation vehicles. However, the combination of RF with topical HA or FA presents a promising therapeutic alternative that must be validated in controlled clinical trials to assess safety, efficacy, and long-term outcomes.

CONCLUSION

The results suggest that weekly application of RF, particularly when combined with daily application of ferulic acid cream or hyaluronic acid cream, enhanced collagen organization, adipose tissue reduction, and skin hydration compared to RF alone in Wistar rats. The combination treatments seemed to be advantageous in promoting dermal regeneration, supported by structural and cellular changes observed histologically. These findings may serve as a basis for future skin rejuvenation strategies and warrant further investigation into the molecular mechanisms involved. Clinical studies are needed to confirm whether the same effects occur on human skin.

Conflicts of Interest

The authors declare no conflicts of interest.

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