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CENTRE FOR SCIENCE AND RESEARCH

Glycine soja extract boosts peptide regenerative properties - multidirectional assessment of *in vitro* and *ex vivo* efficacy in skin models

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INTRODUCTION & OBJECTIVES

Development of novel approaches to topical formulations targeted at skin rejuvenation is a perpetual challenge globally. Thoroughly chosen combinations of active ingredients, based on botanical origin solutions and engineering advances may be a crucial step forward in creation breakthrough innovation. Glycine soja is a well known botanical cosmetic ingredients with a large amount of scientific evidence of its efficacy in anti-aging skin care. However, little is known about its activity as an activator of regenerative properties of peptides.

The aim of the study was evaluation of *Glycine soja* extract (GSE) as a booster of peptide activity (Acetyl heptapeptide-9, Hexapeptide-9) in *in vitro* conditions. *Ex vivo* analyses of collagen, elastin and glycosaminoglycan (GAG) level in skin explants after exposure to a new cosmetic formulation (5555) containing aforementioned ingredients were also performed. Additionally, semi-quantification of the content of integrin β4 was performed.

MATERIALS & METHODS

In vitro studies were conducted in L929 fibroblast cell culture. Boosting effect of GSE on peptides at the concentrations contained in the final formulation, was evaluated using MTT method after a 24 h incubation. Cell migration and proliferation was assessed in a wound scratch assay. Cells were photographed before and after 24 h incubation with peptides alone and peptides with GSE to visualize injury healing. For *ex vivo* analysis, skin explants from 40-year-old female were used. Explants (6 mm diameter) were treated with 10 µL of 5555 for three consecutive days. Vaseline served as placebo. Fixed explants were stained: Mallory dye was used to reveal collagen content, for elastin and GAG, Verhoeff-Van Gieson and Alcian Blue stains, respectively, were used. Integrin β4 content was evaluated using immunofluorescent detection after topical application on normal human skin explants (donors 31 y.o. and 41 y.o.) twice daily for 48 hours.

RESULTS

Mean viability of cells after exposure to 0.5% Acetyl heptapeptide-9 and 1% Hexapeptide-9 (PEP) alone was 131.06 ± 6.27% compared to untreated control cells. An addition of 0.2% GSE resulted in an increase in mean cell viability to 168.74 ± 14.83% (in both cases $p<0.001$). Interestingly, GSE alone did not induce enhanced cell proliferation (mean viability was 98.08 ± 4.66%; **Fig. 1**).

Scratch assay showed a noticeable cell migration promotion induced by evaluated chemicals (**Fig. 2**). The average reduction in wound width observed in peptide only samples was by 43.45%. Addition of GSE caused a boost in cell migration and proliferation – scratch wound width was reduced by 74.87%. Reduction in scratch width in untreated control cells was by 32.44%. In *ex vivo* assessment after 72 h exposure (three applications of final serum formulation), an increase in the content of all evaluated structures (collagen, elastin, GAG) in comparison to placebo was observed (**Fig. 3**). A 48h incubation with 5555 and placebo resulted in a 46% increase in integrin β4 content ($p<0.001$) (**Fig. 4**).

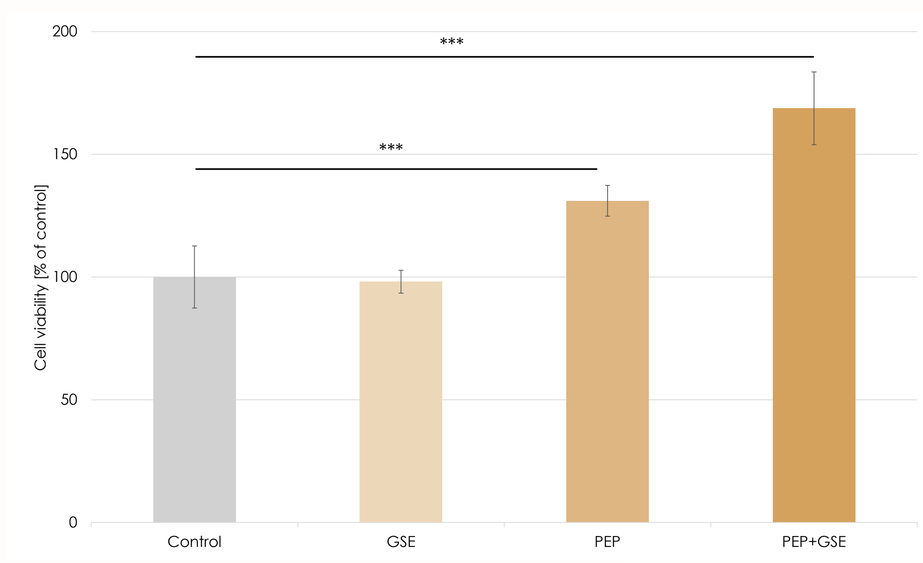

Figure 1. L929 cell viability after 24 h incubation with 0.5% Acetyl heptapeptide-9 and 1% Hexapeptide-9 (PEP) and Acetyl heptapeptide-9, Hexapeptide-9 with 0.2% *G. soja* extract (PEP + GSE). Values are presented as mean viability of control cells ± SD (n=6). Statistical analysis was performed using t-test for independent samples. Differences were statistically significant at *** ($p<0.001$).

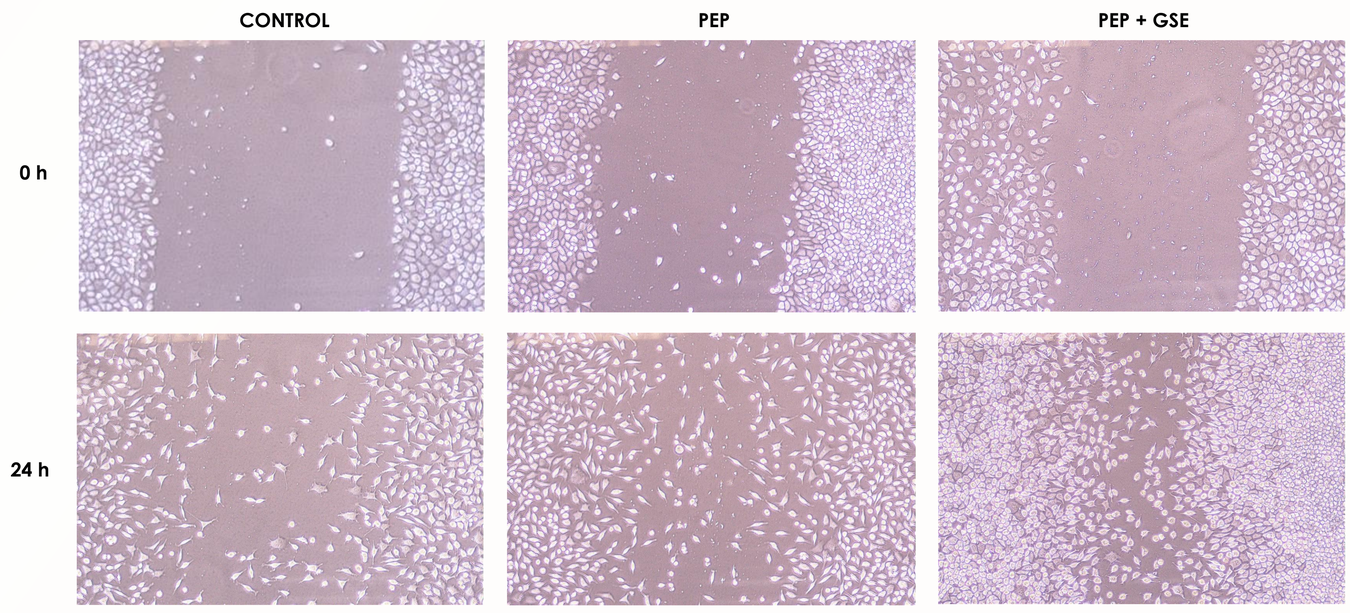

Figure 2. Scratch assay results in L929 fibroblast cell line. Photographs were taken at 20x magnification, directly before the exposure and after 24h of incubation with Acetyl heptapeptide-9 and Hexapeptide-9 (PEP), and Acetyl heptapeptide-9, Hexapeptide-9 with *G. soja* extract (PEP + GSE). GSE caused a notable reduction in scratch width, compared to both PEP and untreated control cells.

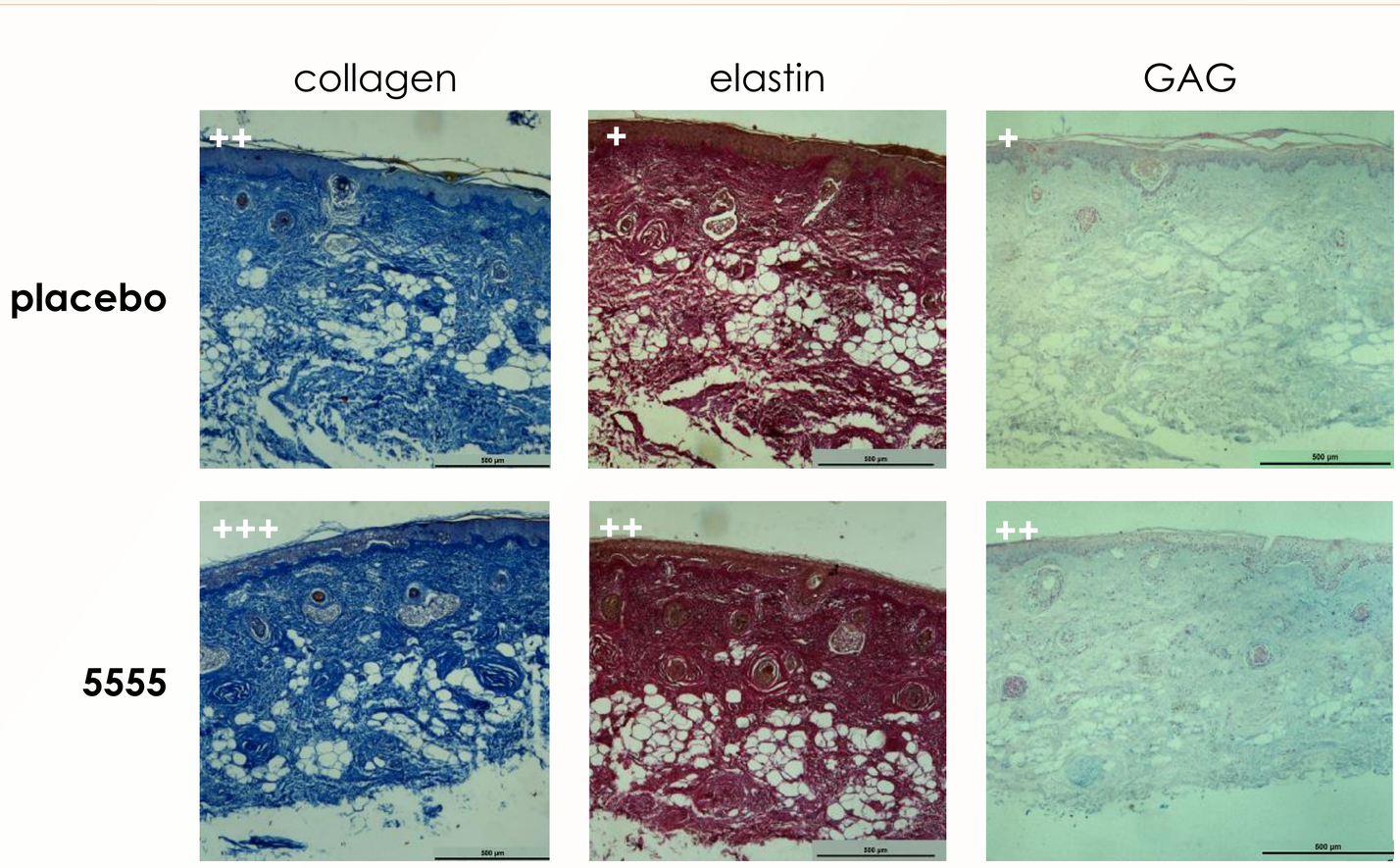

Figure 3. Influence of 5555 on the content of collage, elastin, and GAG in skin explants obtained. Photographs were taken after 72 h of exposure (once daily) to placebo (vaseline) and 5555. A notable increase in the content of all evaluated skin structures was observed, in comparison with placebo. Semi-quantification was expressed as: + - low content; ++ - moderate content; +++ - high content.

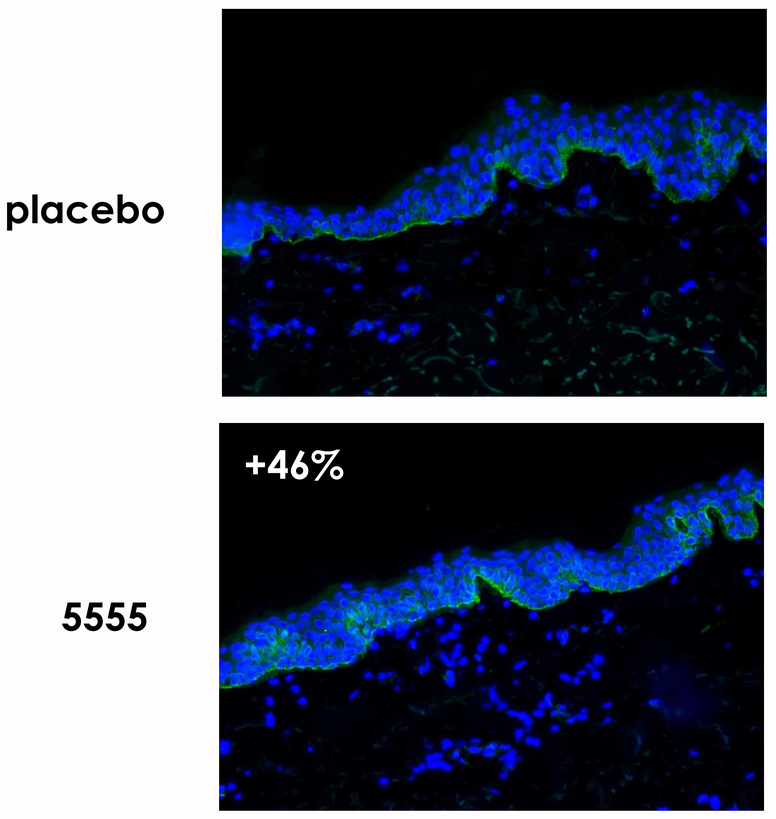

Figure 4. Immunofluorecent detection of Integrin β4 content. Photographs were taken at 20x magnification, after 48h of exposure (twice daily) to placebo (formulation of 5555 without active ingredients) and 5555 (formulation with PEP and GSE). A 46% increase in the content of the evaluated protein was observed, suggesting strong enhancement in the integrity in dermo-epidermal junction (DEJ).

CONCLUSIONS

The study proved that GSE was a booster for peptide regenerative potency. A combination of GSE, Hepta-, and Hexapeptide exhibits promising anti-aging results in *in vitro and ex vivo* assays. Treatment with a new cosmetic formulation containing those ingredients increased the content of skin structural molecules, suggesting enhancement in DEJ and notable rejuvenating properties.