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"Novel effects of Paeonia lactiflora extract for modulation of inflammation process by bacterial invasion and hypersensitivity in skin and oral cavity: in silico, in vitro and stability tests."

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1. Introduction

Skin inflammation is a protective biological mechanism against tissue destruction, mediated by various inflammatory factors, such as transient receptor potential vaniloid (TRPV) channels, and matrix metalloproteinases (MMPs), and characterized by the dys-function of both immune and non-immune cells [1-3]. Advances in understanding in-flammation biology are rapidly being recognized as pharmacological opportunities for treatment of skin diseases especially, such as psoriasis, atopic dermatitis, eczema and other [3]. In this context, it is important that these inflammation-related targets are tightly regulated to alleviate the inflammatory reaction in skin and gingival cells.

TRPV1 channels play one of the main roles in pain signaling in the skin [4]. For example, TRPV1 has been shown to be involved in skin itching [5], rosacea [6] and apparently herpes zoster [7]. TRPV1 is also involved in inflammatory and pain processes in periodontitis and mediates pain signal transmission through the trigeminal nerve [8]. It is known that TRPV1 activity can be significantly increased under the action of inflammatory mediators, such as tumor necrosis factor (TNF-α) [9], interleukin-6 (IL-6) [10], interleukin-13 (IL-13) [11]. Bacterial lipopolysaccharides (LPS), being one of the major inducers of the innate immune response with associated inflammation, also contribute to increased TRPV1 expression [12]. Moreover, TRPV1 itself can mediate and modulate increase of MMP-8 and MMP-9 associated with inflammatory diseases and the degradation of collagen I type. That is why TRPV-1 channel and

MMPs were chosen as main targets for phytochemicals for treatment of skin and oral inflammation.

Phytochemicals are increasingly being used in cosmetics and medicine. *Paeonia lactiflora* Pall. has long been used in traditional Chinese, Korean and Japanese medicine for pain relief. P. lactiflora extract is rich in paeoniflorin, a water-soluble monoterpene glycoside. This compound is known for a wide range of anti-inflammatory and neuroprotective effects [13]. It is reported that paeoniflorin significantly reduced the degree of inflammation in the skin and had an anti-allergic effect in the treatment of contact dermatitis [14,15]. The total glycosides of *P. lactiflora* (which include paeoniflorin) have been reported to have analgesic, anti-inflammatory, immunomodulatory and antioxidant effects, which have applications in the treatment of autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus [16]. Thus, the research was aimed at investigation of *Paeonia lactiflora* extract for modulation of inflammation process by bacterial invasion and hypersensitivity in skin and oral cavity.

2. Materials and Methods

Dry *Paeonia lactiflora* Pall. root extract standardized for 50% paeoniflorin by HPLC was obtained from Organic Herb Inc., China in form of powder. The name of this extract is white paeony extract.

In silico research was conducted through Autodock 4.2 version. The protein data was retrieved in .pdb format from the Protein Data Bank (PDB). The structures of TRPV1 (PDB: 7LPE), MMP-8 (PDB: 1KBC), and MMP-9 (PDB: 8K5Y) were employed to construct the three-dimensional model of proteins and were docked with paeoniflorine using DiffDock [17], and the binding affinities were calculated with GNINA 1.0 [18] software. The model was prepared by removing water molecules, eliminating redundant chains, and adding polar hydrogens and charges. Finally, the processed struc-ture was saved in .pdb format for further in silico analysis. Initially, the native protein ligand was employed in the molecular docking process to validate the protocol, achieving a root mean square deviation (RMSD) of less than 2 Å. The docking grid was centered at coordinates (X, Y, Z) = (28.73, 58.834, 63.068) with dimensions of 40 × 40 × 40. During docking, the ligand was treated as flexible while the macromolecule remained rigid. The anal-ysis was conducted on a personal computer featuring an Intel Core i7-12700U CPU (2.3 GHz), an RTX 4090 GPU, and 64 GB of RAM, running a 64-bit version of Windows 11.

Keratinocytes cell viability was determined by a colorimetric method using MTT dye (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; Sigma, St. Lous, MO). For the assay the product was prepared in culture medium with 3% Tween 20 (Sigma) and added to the 96 well plate at a serial dilution in the range of 100.00 to 0.003 mg/mL using the dilution factor of 3.16. The human skin keratinocytes culture was incubated for a period of 48 hours. MTT was then added to the culture at a concentration of 0.5 mg/mL (30 µL/well) and incubated for additional 4 hours.

The contents of the well were removed and 100 µl of isopropanol was added for the purpose of solubilizing the formazan crystals formed by viable cells. The absorbance of each well was determined at 570 nm in Multiskan GO monochromator (Thermo Scientific, Finland).
Upon reaching confluence, the cells were seeded in 96-well plate (Corning, USA) to determine the non-cytotoxic concentrations of the evaluated product. Cells were treated for 4 days with the *P. lactiflora* extract at three non-cytotoxic concentrations together with inflammatory stress as LPS in a concentration of 50 µg/mL. *S. aureus* LPS (Sigma-Aldrich, Cat. No. L2515) was used to model the inflammatory process induced by the presence of lipopolysaccharides of the bacterial cell wall. After treatment, cell lysate and supernatants were collected for TNF-α, IL-6 and IL-13 quantification and the TRPV-1 was measured on the cell lysate. TNF-α, IL-6, TRPV-1 and IL-13 concentrations were measured in the cell lysate and supernatant of the cultures by means of the sandwich ELISA assay, using commercially purchased kits (TRPV-1: Biorbyt; TNF-α: R&D systems; IL-6: R&D systems; IL-13: R&D systems). The absorbance reading was carried out at 450 nm in a Multiskan GO monochromator (Thermo Scientific).
To evaluate the data obtained, the ANOVA test was used, which also allowed measuring the variation in results, comparing data between groups. The Bonferroni post-test was then applied, which reinforced and made the result presented in the ANOVA test even more precise.

3. Results

Skin inflammation is a protective biological mechanism against tissue destruction, mediated by various inflammatory factors, such as transient receptor potential vaniloid (TRPV) channels, and matrix metalloproteinases (MMPs), and characterized by the dysfunction of both immune and non-immune cells [1-3]. Therefore TRPV-1 channel and pro-inflammatory cytokines, such as IL-6, IL-13 and TNF-α, were chosen as main targets for paeoniflorin-enriched *P. lactiflora* extract for treatment of skin and oral inflammation.
Firstly, the affinity of peoniflorin to targets was discovered in silico. Using DiffDock for pose generation and GNINA 1.0 for affinity calculations, the minimal score of Gibbs free energy was predicted for paeoniflorin's binding to TRPV-1, MMP-8, and MMP-9 active sites. The results indicated a strong affinity of peoniflorin toward the active sites of TRPV1 (-10.79 kcal/mol) and MMP-9 (-9.99 kcal/mol), while it showed a relatively low affinity to MMP-8 (-6.82 kcal/mol).
For in vitro research human keratinocytes cultures were treated with the *P. lactiflora* exract at three non-cytotoxic concentrations, determined by the MTT assay. After subsequent exposure to inflammatory stress, TRPV-1, TNF-α, IL-6, and IL-13 amount were quantified by ELISA immunoassay. Through MTT assay it was established that 100% of the cell survival rate was observed at the *P. lactiflora* extract concentration of 0.1 mg/mL (equal to 0.01 weight %) and maximum tolerated concentration was evaluated on keratinocytes as 0.5 mg/mL.

Exposure of the cell culture with 50 µg/mL LPS promoted an increase of 144.45% ($p<0.001$) in TRPV1 amount on human keratinocytes. Meanwhile, *P. lactiflora* extract showed a protective effect, modulating TRPV1 overproduced amount by 113.47%, 88.40% and 64.41% at percentages of 0.05%, 0.01% and 0.003%, respectively, in comparison wih value between LPS-induced control and basal control ($p<0.001$) (Figure 1). These results highlighted the role of *P. lactiflora* extract as targeted substance in action to TRPV1 channels related to itch, increased sensitivity, and pain sensation.

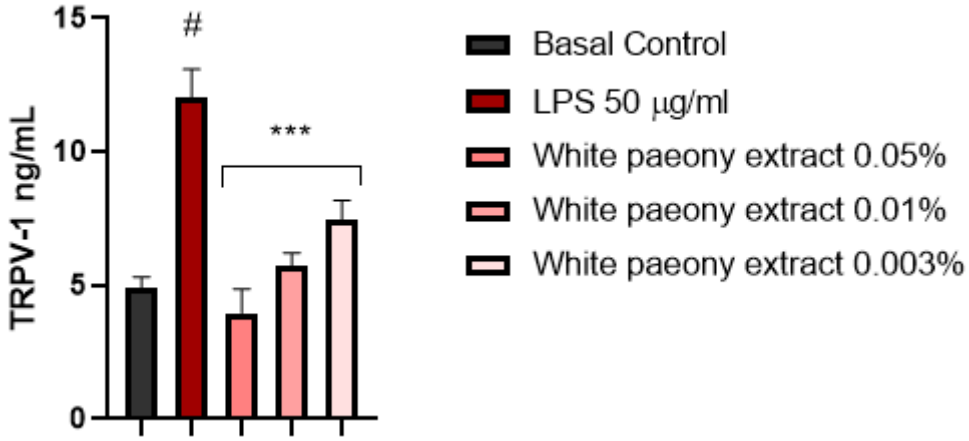

Figure 1. TRPV1 modulation in skin keratinocytes by *P. lactiflora* extract.

The effect of the *P. lactiflora* extract on the production of proinflammatory IL-6 in cultured keratinocytes was also evaluated. As expected, exposure of the cell culture with 50 µg/mL LPS promoted an increase of 194.20% in IL-6 synthesis ($p<0.001$). Remarkably, *P. lactiflora* extract showed a protective effect, decreasing IL-6 overproduction by 37.78% and 28.70% at percentages of 0.05% ($p<0.01$) and 0.01% ($p<0.05$), respectively, considering the baseline condition without exposure to LPS.

The same protective efffect was showed in a model of proinflammatory IL-13 related to adaptive immunity in case of atopic dermatitis, eczema and other dermaological diseases. *P. lactiflora* extract showed significant anti-inflammatory effect, decreasing IL-13 overproduction in keratinocytes by 68.63% and 32.61% at percentages of 0.05% and 0.01%, respectively ($p<0.001$). This action is due to the varied composition of glucosides like peoniflorin, flavonoids and complexes of other biologically active molecules. As expected, exposure of the cell culture with 50 µg/mL LPS promoted an increase of 409.75% ($p<0.001$) in IL-13 synthesis in comparison with basal control in skin keratinocytes, revealing immune response to bacteria cell wall compounds.

The immediate anti-inflammatory effect of the *P. lactiflora* extract on the production of TNF-α in cultured keratinocytes was also investigated (Figure 2). As expected, exposure of the cell culture with 50 µg/mL LPS promoted an increase of 25.64% ($p<0.01$) in TNF-α production as marker of immediate immune response. *P. lactiflora* extract enriched by paeoniflorin reduced

TNF-α overproduction by 148.82% (p<0.001), 105.49% (p<0.01) and 100.00% (p<0.01) at percentages of 0.05%, 0.01% and 0.003%, respectively. This normalisation of TNF-α level has beneficial effect for wound healing and management of inflammation and pain sensation.

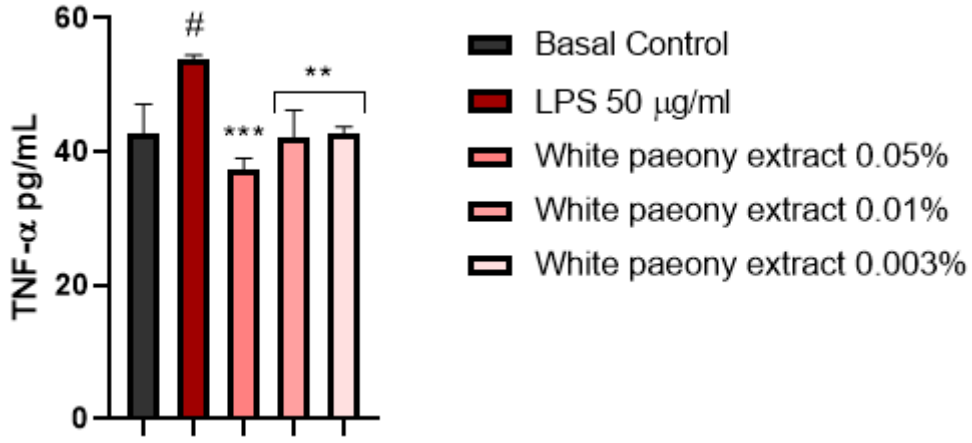

Figure 2. Regulation of TNF-α production in skin keratinocytes by *P. lactiflora* extract.

In the presence of *P. lactiflora* extract, a decrease in the overproduced amount of TRPV1 channels and then pro-inflammatory cytokines like IL-6, IL-13 and TNF-α was observed, which clearly showed the dual anti-inflammatory effects of this compound on LPS-induced skin inflammatory reaction.

The potential protective effect of *P. lactiflora* extract on periodontal extracellular matrix structures was evaluated by quantifying MMP-8 in human gingival fibroblast cultures. Exposure of cell culture to LPS promoted an increase in MMP-8 production by 69.87% (P<0.001) compared to the basal control group. However, *P. lactiflora* extract showed a protective effect, reducing MMP-8 production by 95.59% (p<0.001), 94.94% (p<0.001), and 111.22% (p<0.001) at concentrations of 0.50, 0.10, and 0.03 mg/mL, respectively, compared with the value between LPS-induced control and basal control. These are completely new data on the protective effect of paeoniflorin in composition of *P. lactiflora* extract on oral gingival cells in case of inflammatory response induced by oral bacterial LPS and mediated by TRPV1 channels. The exposure of *P. lactiflora* extract promotes the restoration of MMP-8 level and ensures the maintenance of its amount in case of inflammatory response.

In this experiment of stability in cosmetic formulation, high-performance liquid chromatography (HPLC) was used to investigate the paeoniflorin content in the oral mouhwash after prolonged storage in accelerated stability test. In theory, the developed product can be used to reduce inflammation in the oral cavity and on the skin, so the stability of its active ingredients is a very important parameter. The chromatographic analysis showed very high stability of paeoniflorin and its isomers. Even in samples stored for a long time at a temperature of above 40°C, the degradation of peoniflorin was not more than 13%. At the same time, the content of paeoniflorin and its isomers remained constant under thermal conditions during 84 days. It should be noted

that 0.05% concentration of P. lactiflora *extract* was used in the composition of the cosmetic product for oral care. Despite this, this component significantly did not degrade, which indicates its excellent stability and makes it a promising component for cosmetics.

4. Discussion

In this research, the complex anti-inflammatory effect of *P. lactiflora* extract on LPS-induced inflammation in skin on skin and gingival tissue cells was demonstrated for the first time. Paeoniflorin in the extract is not only able to bind to TRPV1, modulating their activity (as evidenced by our *in silico* model), but also to promote a reduction in the number of TRPV1 on the cell surface. In addition, a decrease in IL-6, IL-13 and TNF-α was observed under the influence of *P. lactiflora* root extract. It was also demonstrated that in addition to the above properties, paeoniflorin-rich *P. lactiflora* extract has a noticeable protective effect on gingival cells and skin cells. In the presence of *P. lactiflora* extract, there was a decrease in the activity of metalloproteinases of MMP-8 in oral gingival cells. Thus, due to its chemical properties as well as its complex effects on TRPV1, pro-inflammatory cytokines and metalloproteinases, *P. lactiflora* extract standardized for paeoniflorin is a very promising phytochemical for the control of pain sensation and LPS-induced inflammation in the skin and oral cavity cells.

5. Conclusion

The novel *Paeonia lactiflora* root extract works as a potent anti-inflammatory phytochemical in a model of LPS-induced inflammation in skin and gingival cells. The obtained results confirmed that the *P. laciflora* extract enriched peoniflorin glucoside has a beneficial effect to modulate amount of TRPV1 channels and level of pro-inflammatory cytokines, such as IL-6, IL-13 and TNF-α. This effect has a beneficial impact on cell and humoral immunity reaction in soft tissues like dermis and gingival cavities. Moreover, the *P. laciflora* extract normalizes level of MMP-8 in oral gingival fibroblasts, creating conditions for wound healing and protection of extracellular matrix providing the gums health. Nevertheless, additional studies in skin explants in comparison with clinically recommended antiinflammaory drugs like glucocorticoseroids, dermatological tolerance, comprehensive toxicological evaliuation, and clinical research of dual anti-inflammatory effect are needed to fully confirm the beneficial effects.

6. References

[1] Bagood, M.D.; Isseroff, R.R. TRPV1: Role in Skin and Skin Diseases and Potential Target for Improving Wound Healing. Int. J. Mol. Sci. 2021, 22, 6135.

[2] Lee, H.S.; Kim, W.J. The Role of Matrix Metalloproteinase in Inflammation with a Focus on Infectious Diseases. Int. J. Mol. Sci. 2022, 23, 10546.

[3] Shirley, S.N.; Watson, A.E.; Yusuf, N. Pathogenesis of Inflammation in Skin Disease: From Molecular Mechanisms to Pa-thology. Int. J. Mol. Sci. 2024, 25, 10152.

[4] Iftinca, M.; Defaye, M.; Altier, C. TRPV1-Targeted Drugs in Development for Human Pain Conditions. Drugs 2021, 81, 7–27.

[5] Feng, J.; Yang, P.; Mack, M.R.; Dryn, D.; Luo, J.; Gong, X.; Liu, S.; Oetjen, L.K.; Zholos, A.V.; Mei, Z.; et al. Sensory TRP Channels Contribute Differentially to Skin Inflammation and Persistent Itch. Nat. Commun. 2017, 8, 980.

[6] Lee, S.G.; Kim, J.; Lee, Y.I.; Kim, J.; Choi, Y.S.; Ham, S.; Lee, J.H. Cutaneous Neurogenic Inflammation Mediated by TRPV1-NGF-TRKA Pathway Activation in Rosacea Is Exacerbated by the Presence of Demodex Mites. J. Eur. Acad. Dermatol. Venereol. 2023, 37, 2589–2600.

[7] Han, S.B.; Kim, H.; Cho, S.H.; Lee, J.D.; Chung, J.H.; Kim, H.S. Transient Receptor Potential Vanilloid-1 in Epidermal Keratinocytes May Contribute to Acute Pain in Herpes Zoster. Acta Derm. Venereol. 2016, 96, 319–322.

[8] Evangeliou, E.; Plemmenos, G.; Chalazias, A.; Piperi, C. Impact of TRP Channels in Oral Pathology and Therapeutic Targeting Options: A Narrative Review. Curr. Top. Med. Chem. 2023, 23, 1559–1573.

[9] Wang, Y.; Feng, C.; He, H.; He, J.; Wang, J.; Li, X.; Wang, S.; Li, W.; Hou, J.; Liu, T.; et al. Sensitization of TRPV1 Receptors by TNF-α Orchestrates the Development of Vincristine-Induced Pain. Oncol. Lett. 2018, 15, 5013–5019.

[10] Fang, D.; Kong, L.-Y.; Cai, J.; Li, S.; Liu, X.-D.; Han, J.-S.; Xing, G.-G. Interleukin-6-Mediated Functional Upregulation of TRPV1 Receptors in Dorsal Root Ganglion Neurons through the Activation of JAK/PI3K Signaling Pathway: Roles in the Development of Bone Cancer Pain in a Rat Model: Roles in the Development of Bone Cancer Pain in a Rat Model. Pain 2015, 156, 1124–1144.

[11] Rehman, R.; Bhat, Y.A.; Panda, L.; Mabalirajan, U. TRPV1 Inhibition Attenuates IL-13 Mediated Asthma Features in Mice by Reducing Airway Epithelial Injury. Int. Immunopharmacol. 2013, 15, 597–605.

[12] Luo, Y.; Suttle, A.; Zhang, Q.; Wang, P.; Chen, Y. Transient Receptor Potential (TRP) Ion Channels in Orofacial Pain. Mol. Neurobiol. 2021, 58, 2836–2850.

[13] Gong, P.; Long, H.; Guo, Y.; Wang, Z.; Yao, W.; Wang, J.; Yang, W.; Li, N.; Xie, J.; Chen, F. Chinese Herbal Medicines: The Modulator of Nonalcoholic Fatty Liver Disease Targeting Oxidative Stress. J. Ethnopharmacol. 2024, 318, 116927.

[14] Wu, X.; Qi, X.; Wang, J.; Zhang, Y.; Xiao, Y.; Tu, C.; Wang, A. Paeoniflorin Attenuates the Allergic Contact Dermatitis Response via Inhibiting the IFN-γ Production and the NF-κB/IκBα Signaling Pathway in T Lymphocytes. Int. Immunopharmacol. 2021, 96, 107687.

[15] Chen, J.; He, Q.; Jin, J. Targeting Dendritic Cell Activation: The Therapeutic Impact of Paeoniflorin in Cortosteroid-Dependent Dermatitis Management. Arch. Derm. Res. 2024, 316, 348.

[16] He, D.-Y.; Dai, S.-M. Anti-Inflammatory and Immunomodulatory Effects of Paeonia Lactiflora Pall., a Traditional Chinese Herbal Medicine. Front. Pharmacol. 2011, 2, 10.

[17] Corso, G.; Stärk, H.,; Jing, B.; Barzilay, R.; Jaakkola, T. DiffDock: Diffusion Steps, Twists, and Turns for Molecular Docking. ArXiv. 2022. V. abs/2210.01776.

[18] McNutt, A.T.; Francoeur, P.; Aggarwal, R.; Masuda, T.; Meli, R.; Ragoza, M.; Sunseri, J.; Koes, D.R. GNINA 1.0: molecular docking with deep learning. Journal of Cheminformatics. 2021, 13(1), 43.