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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ASSESSMENT OF TWO METHANOLIC 2% G. GLABRA L. HYDROGEL FORMULATION

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Introduction

Traditional herbal medicine has served as the foundation for many modern plant-based treatments. Presently, several clinical studies examine the effects of various phytochemicals on the management of various skin conditions. Gg has been extensively studied for its complex composition, which is linked to multiple benefits, including those for the skin.

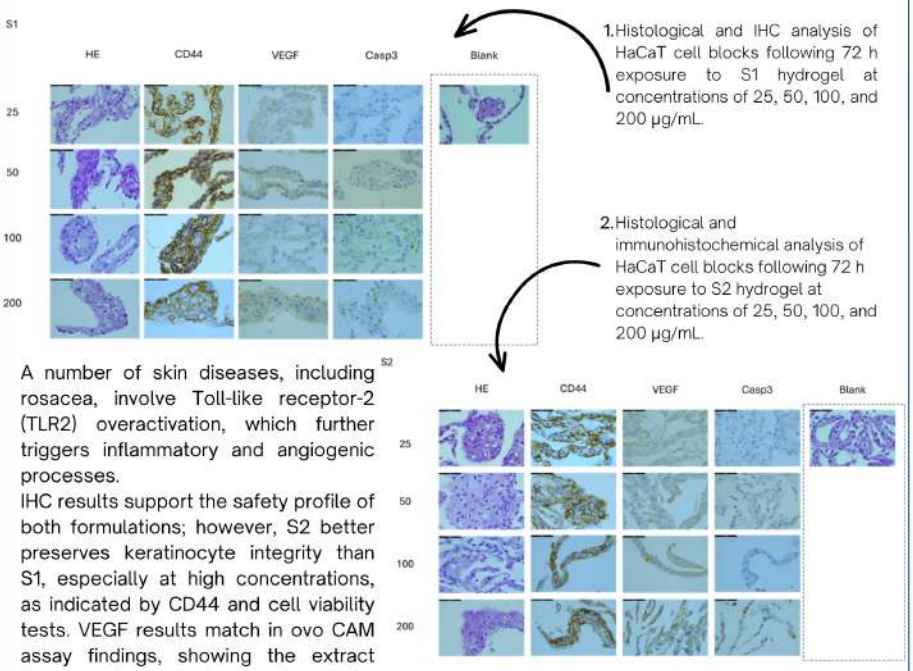
AIM: The present study aims to assess two methanolic 2% Gg hydrogel formulations (S1 and S2) for their histological and immunohistochemical properties.

Materials and Methods

For histological assessment, sections were stained with Hematoxylin-Eosin (HE) to visualize cellular architecture and cytoplasmic changes. Immunohistochemical (IHC) staining was performed to detect the expression of CD44 (cell adhesion), VEGF (angiogenesis), and Caspase-3 (apoptosis).
The following primary antibodies were used: Anti-CD44 Monoclonal Mouse Anti-Human (Clone 156-3C11, ready-to-use); Anti-VEGF Monoclonal Mouse Anti-Human (Clone VG1, ready-to-use); Monoclonal Mouse Anti-Human Anti-Caspase 3 (Polyclonal Rabbit, ready-to-use) from Agilent Technologies, CA, USA. The slides were counterstained with hematoxylin, dehydrated, and mounted for microscopic evaluation (performed by a board-certified pathologist: E.G.O).
Histological and IHC assessment was performed qualitatively, focusing on signal distribution and intensity across cell blocks. This aimed to provide a safety profile and identify potential dose-dependent trends.

Results & discussions

The HE analysis of HaCaT cytoblocks showed dose-dependent morphological changes between formulations. S1 preserved viable keratinocytes with normal morphology between 25–100 µg/mL but displayed cytoplasmic eosinophilia at 200 µg/mL, indicating acidification and apoptosis. While HE staining of the Blank controls appeared normal, the cell blocks were lost during processing, preventing IHC analysis of the controls. S2 showed a better safety profile, with viable cells at 200 µg/mL, allowing IHC. Immunostaining revealed that CD44 expression remained strong at lower doses for both formulations, but S1 showed reduced intensity at 200 µg/mL, whereas S2 maintained it. VEGF expression was moderate to low with no membrane intensity, indicating no pro-angiogenic effect. Caspase-3 was absent at low doses but appeared at 100 µg/mL and above, indicating controlled apoptosis.



A number of skin diseases, including rosacea, involve Toll-like receptor-2 (TLR2) overactivation, which further triggers inflammatory and angiogenic processes.
IHC results support the safety profile of both formulations; however, S2 better preserves keratinocyte integrity than S1, especially at high concentrations, as indicated by CD44 and cell viability tests. VEGF results match in ovo CAM assay findings, showing the extract does not promote angiogenesis, indicated by low VEGF expression

This histological assessment primarily focuses on qualitative analysis of morphological and molecular safety, not efficacy. The loss of Blank control cell blocks during processing limited direct comparison with treated samples. Therefore, interpretation relied on dose-dependent trends observed in the treated groups. Despite these limitations, the observed preservation of CD44 and delayed Caspase-3 expression at higher concentrations provide internal evidence of biocompatibility, depending on concentration. Detection of Caspase-3 at ⩾ 100 µg/mL clarifies the cell death mechanism, as its presence and cytoplasmic eosinophilia on HE staining indicate apoptosis rather than necrosis.

Conclusion

The IHC analysis offers important mechanistic insights, showing that the hydrogels—especially S2—preserve keratinocyte integrity by maintaining CD44 expression and promote a controlled apoptotic process via Caspase-3, rather than causing necrosis at higher concentrations. Additionally, the lack of VEGF upregulation at the cellular level matches the anti-angiogenic effects seen in ovo, indicating a profile that does not trigger excessive vascular responses.

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