**Antioxidant activity and cellular protection against oxidative stress damage of aqueous extracts from *Eucommia ulmoides* and *Cistanche deserticola*: an in vitro study**

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*Eucommia ulmoides* and *Cistanches deserticola* are two antioxidant herbs. Compounds extracted from *E.ulmoides* and *C.deserticola* have been shown to have anti-inflammatory and antioxidant properties[1], [2]. Oxidative stress plays an essential role in the process of wound healing[3]. Fibroblasts are known as key cells in the wound healing process, involved in extracellular matrix production and tissue repair, and their migration and proliferation rates are affected by the level of inflammation at the wound site[4]. In this study, we aimed to evaluate the antioxidant activity of aqueous extracts of *E.ulmoides* and *C.deserticola* in NIH/3T3 fibroblasts, expecting to obtain the optimal aqueous extract concentration for further wound healing studies.

0.5 mM H2O2 was used as a stressor to induce intracellular reactive oxygen species (ROS) production. Two cell exposure models were established: pretreatment with extracts for 24 hours, followed by exposure to 0.5 mM H2O2 for 15 minutes; simultaneous exposure to extracts and 0.5 mM H2O2 for 15 minutes. The antioxidant levels of the aqueous extracts were measured by the FRAP (iron reducing the capacity of plasma) method, and the cytotoxicity of different concentrations of aqueous extracts and their protective effect were determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The protective effect of low concentrations of aqueous extracts against cellular oxidative stress was further determined using DCFH-DA (2',7'-dichlorofluorescein) assay. The low concentration of aqueous extracts protected cells from oxidative stress damage under pretreatment conditions. The high concentration extracts could scavenge free radicals from the environment in a short period of time, but it was cytotoxic mainly due to its high water content and could not be used for cellular antioxidant protection for a long time.

The protective effect of *E.ulmoides* and *C.deserticola* on cellular oxidative stress may exist in two mechanisms due to different concentrations: a high concentration of aqueous extracts can directly scavenge free radicals from the environment, while a low concentration can activate intracellular antioxidant mechanisms and enhance cells' own antioxidant capacity after being absorbed. The latter may involve complex interactions with intracellular pathways and needs to be further investigated.

**References**

1.Y. Hu et al., Cistanche deserticola polysaccharide induces melanogenesis in melanocytes and reduces oxidative stress via activating NRF2/HO‐1 pathway // J Cell Mol Med, vol. 24, no. 7, p. 4023, Apr. 2020. doi: 10.1111/JCMM.15038.

2. N. B. Li, X. F. Luo, X. Yin, and X. Wei. Eucommia ulmoides Oliv polysaccharide reduces the injury of IL-1β-induced chondrocyte by inhibiting NF-κB pathway // Zhongguo Gu Shang. vol. 35, no. 7, pp. 661–668, Jul. 2022. doi: 10.12200/J.ISSN.1003-0034.2022.07.013.

3. M. Rojkind, J. A. Domínguez-Rosales, N. Nieto, and P. Greenwel. Role of hydrogen peroxide and oxidative stress in healing responses // Cell Mol Life Sci, vol. 59, no. 11, pp. 1872–1891, Nov. 2002. doi: 10.1007/PL00012511.

4. G. Wang, F. Yang, W. Zhou, N. Xiao, M. Luo, and Z. Tang. The initiation of oxidative stress and therapeutic strategies in wound healing // Biomed Pharmacother, vol. 157, Jan. 2023. doi: 10.1016/J.BIOPHA.2022.114004.