

## **Antiviral and antioxidant activity of a hydroalcoholic extract from *Humulus lupulus* L.**

1)Di Sotto A. 2)Celestino I. 3)Toniolo C. 4)Abete L. 5)Di Giacomo S. 6)Nicoletti M. 7)Mazzanti G. 8)Nencioni L.

Female strobilus inflorescences (hops or cones) from *Humulus lupulus* L., commonly named hop cones or hops, are widely used in the brewing industry as preservative and flavouring additives, as well as in traditional medicine as remedies for mild sleeping disorders and as bitter stomachic. Polyphenolic compounds, mainly prenylflavonoids (viz. xanthohumol), catechins and procyanidins represent some of the characteristic phytoconstituents [1]. Xanthohumol and its metabolites, the main hop components, seem to be responsible for a variety of interesting biological properties, including antioxidant, anti-inflammatory, chemopreventive, antimicrobial and antiviral [2,3]. On the other hand, proanthocyanidins and catechins have been described to possess antiviral properties, particularly against influenza A, which represents a major public health problem, with high rates of morbidity and mortality [4]. Although different strategies have been approached to prevent the disease and/or manage its complications, the development of mutant strains increases the need for further and more effective therapeutic options. Experimental evidences suggest that herbal medicines, likely due to their polyphenolic composition, can represent alternative or integrative strategies for fighting influenza. In this context, the present study was aimed at evaluating the ability of a hydroalcoholic extract from female inflorescences of *H. lupulus* (provided by EPO S.r.l.), standardized to contain 0.4 % of flavonoids, to inhibit the influenza virus infection. To this aim, Madin-Darby Canine Kidney (MDCK) epithelial cells, permissive to influenza virus replication, were infected with influenza A/Puerto Rico/8/34 H1N1 (A/PR8) strain. Cytotoxicity of the treatment was evaluated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay, while viral titer was measured by the hemagglutination assay [5]. The viral protein expression was evaluated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting analysis. The effect of extract on the intracellular GSH levels were also measured, being the virus infection associated to oxidative stress. In order to highlight the possible antioxidant mechanisms of the sample, the radical scavenging power and the inhibition of lipoperoxidation were assayed [6]. The polyphenolic composition of the *H. lupulus* extract was evaluated by chromatographic and colorimetric methods [6]. In the range of the concentrations tested (10-140 µg/ml), the *H. lupulus* extract exhibited no cytotoxicity on MDCK; in contrast, it induced a statistically significant and concentration dependent inhibition of the A/PR8 H1N1 virus replication, reaching about 80% inhibition at concentration of 140 µg/ml. A significant inhibition of viral proteins, mainly the late ones, was highlighted after treatment with the hop extract, along with an increased level of intracellular GSH into infected cells, so suggesting that the antiviral activity of the extract might be due to a restoration of the intracellular redox conditions. The sample was also able to scavenge different radicals and to interfere with lipoperoxidation, thus allowing to hypothesize possible protective effects on the infected cell. The phytochemical analysis of the extract highlighted the presence of different polyphenolic compounds, among which flavonoids, flavanols and tannins. In conclusion, the antiviral and antioxidant properties

here found, encourage further in vivo studies on *H. lupulus* extract, to better study its possible anti-influenza properties.

#### References

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