Abstract

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Title of Thesis: Determination of the stoichiometry of the copper complexes with dehydrosilybin A

Silymarin, the standardized extract of the milk thistle (*Silybum marianum*), is a widely used approved over-the-counter drug that is recommended for a number of liver diseases. Silymarin contains as one of its components 2,3-dehydrosilybin A, which has an appropriate metal binding site in its structure. In general, flavonolignans, due to their structure, can interact with transition metals in the gastrointestinal tract by forming complexes. This property can be useful for the protection against excessive amounts of metals in the body. The aim of this *in vitro* study was to analyse the interaction of 2,3-dehydrosilybin A with copper, which plays a crucial role in the organism as a cofactor of many enzymes. Although being an essential element, it can, however, be toxic at elevated levels. Stoichiometry, as one of the most important characteristics of the complex, was determined by UV-Vis spectrophotometry in four (patho)physiological pH conditions (4.5; 5.5; 6.8; 7.5) using two non-competitive methods: the Job‘s Method and the Complementary method. Principles of the methods are different. In the Job‘s method the concentration of both components is changing, while total concentration is constant. During the complementary approach the molar concentration of chelator is changing while the concentration of metal is kept constant. 2,3-Dehydrosilybin A was found to be a moderately active Cu^{2+} chelator. The final stoichiometry was 2:1 or 3:1, flavonolignan:Cu^{2+}. The 2:1 stoichiometry was changing depending on the conditions due to the excess of the tested substance in the 3:1 stoichiometry. In contrast, Cu^{+} ions were not bound by 2,3-dehydrosilybin A in none of the tested conditions. These *in vitro* results could be used in the future as a basis for a more detailed characterization of the effect of 2,3-dehydrosilybin A on the absorption of copper in cell cultures at *in vitro* level and *in vivo* conditions.