

# Abstract

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Title of Doctoral Thesis: Screening of new iron- and copper-chelating substances  
– *in vivo* and *in vitro* studies

Iron and copper are essential trace elements which play a role in a series of physiological processes in human organism. Homeostasis of these transition metals is meticulously regulated since free or loosely bound iron and copper can catalyse the production of free radicals. Hereditary hemochromatosis, transfusion hemosiderosis and Wilson's disease are associated with absolute iron and copper overload in the organism. Transition metal chelators have crucial significance for the treatment of these states. There are several other diseases with documented involvement of iron and/or copper in their pathophysiology. Examples are primarily neurodegenerative diseases, cardiovascular diseases, tumours and diabetes mellitus. Various chelating compounds are examined in these possible indications.

The aim of this doctoral thesis was to perform a screening of iron- and copper-chelating substances and to study their properties in detail using *in vitro* and *in vivo* experiments.

Copper-chelating properties of synthetic as well as natural substances were investigated *in vitro*, by a developed spectrophotometric method using

bathocuproinedisulphonate or hematoxylin. In contrast to other compounds, the clinically used copper chelator D-penicillamine was shown as a relatively weak copper-chelator, which possesses marked reducing properties. Many flavonoids were able to bind copper; however, potent chelators were especially 3-hydroxyflavone, kaempferol and baicalein. Iron-reducing properties and influence on the iron-catalysed Fenton reaction were also investigated in a series of flavonoids. These compounds reduced ferric ions only under acidic conditions and pro-oxidant activity was found particularly in flavonols. Novel approach aimed at the determination of transition metal-chelator complex stoichiometry based on mathematical calculations was developed. In comparison with the standard Job's method, the approach appeared to be advantageous especially in the cases of moderately active chelators.

An *in vivo* study concerning oral administration of quercetin for seven days in rats has been published. This approach was unable to protect cardiovascular system against acute catecholamine cardiotoxicity. However, quercetin affected some haemodynamic parameters and decreased the responsiveness of aorta to vasoconstriction in control group. Other data concerning the effect of D-penicillamine are currently being prepared for publication and a similar experiment with rutin is in a peer-review process.

In conclusion, a spectrum of substances was tested for copper-chelating effects. Other important properties of chelators were investigated, such as reducing and pro-oxidant activities or the complex stoichiometry. Moreover, the developed *in vitro* methods may promote research in this field. Potent chelators were observed among flavonoids which showed numerous interactions with both metals. Quercetin represents such an example. It was unable to protect against complex catecholamine cardiotoxic effects and it was even pro-oxidant in some concentrations in an *in vitro* study.