



**A review of the doctorate thesis entitled „Screening of novel chelators of microbiogenic metals” written by MSc Maria Carmen Catapano under supervision of Prof Premysl Mladenka**

Essential trace elements, regardless their name, are well known and established elements of mineral origin that are crucial for the homeostasis of living organisms, especially humans. Unfortunately our knowledge in this matter is far from satisfactory, which trigger continuous research. One of the most important aspects of the trace elements' biochemistry and homeostasis is their dual impact on health. Although these elements are present only in tiny amounts in our bodies and often needs to be supplemented from daily food intake, they are able to express severe toxicity in concentrations beyond homeostatic levels. Indeed several genetic or metabolic disorders, that are connected with the improper intake or storage of the trace elements, results in inferior health condition and decrease the quality of life.

Similarly, the supplementation due to medical procedures or individual decision may lead to serious health problems. Particularly the latter situation seems important when considered increasingly high popularity of the mineral supplements that are available on the



OTC and cosmetic market. Therefore, more investigations are needed to understand mechanisms of the trace elements' toxicity and elaborate better treatment. There is a visible trend of growing interest in trace elements supplementation also in numbers of scientific papers available in this area. For the last decade roughly 500 new reports have been published. Contrarily, the toxicity of trace elements is investigated in far less extent and the overall number of papers does not exceed one thousand. In this regards research undertaken in esteemed group of professor Mladenka are especially important and valuable for broader community. The same is true for the thesis of MSc Catapano, who devoted herself to chelating of trace elements, its biochemical and medical aspects.

The aim of this work was to investigate several compounds for their chelation of the iron, copper and zinc. Another task that has naturally evolved from the first one was to compare a various methods suitable for these efforts, including the novel methodology. As a compounds of interest several dietary substances were selected. Their interaction with metals has been investigated at patho-physiologically relevant pH. As such the work is established as an evaluation and substantial extension of limited knowledge in this field. The selection of substance naturally present in human diet as flavonoids and particularly sylimarin flavonolignans accentuate the applicatory aspect of this research. On the other hand study with an isoquercitrin led to the conclusion that the methods that are available for determination of the metal chelation should not be over-esteemed. The same point was arisen by author in discussion of the results obtained with silymarin. In my opinion another intriguing conclusion can be drawn from results obtained with chelators that, as for example coumarin derivatives, possess some reducing properties. Reducing chelators may be responsible for artifacts and errors in some assays. Moreover the environmental reducing ability is one of the prerequisites for Fenton and Haber-Weiss reactions in biological systems. Generation of reactive oxygen species has been proposed as anti-proliferative mechanism of action for several cytotoxic agents. In combination with fact that reducing potency may affect bioavailability of the metal in the digestive tract this phenomena may help to explain various effects e.g. contradictory results from supplementation of antioxidants.



The dissertation is composed as a monothematic series of publications associated with commentary, including background, fate and pathophysiology of iron, copper and zinc that were chosen by author. It is perfectly understandable that selection of elements of interest is more or less individual decision and right of the author. Moreover such selection is necessary to keep work readable and, colloquially speaking: put together. Nevertheless I think that more discussion on how those particular elements were chosen would be beneficial. For sure these three can be considered as most important or abundant, but author's personal opinion included in introduction is always well seen. Another chapters describe chelators that are typically used for treatment the overload by an element. Additionally the list of papers published by author has been equipped with commentary including contribution and conclusions. The whole thesis is well composed and perfectly meets standards for similar elaborations. Number of publications that are presented in this cycle is definitely exceeding typical values and the overall list of publications of Ms Catapano is even larger. What is more noticeable the articles were published in esteemed journals with good impact factors for example *Nutrients* or *Bioorganic Chemistry*. The mean IF for publication exceeds 3.9. It is also worth of mention that the author's contribution in those publications declare at least participation or performing experiments and also partially design of study and writing the manuscripts. In four out of seven publications the candidate is a first author. With all above, it could be stated that Ms Maria Carmen Catapano is well developed young scientist with experience and ability to conduct research and report findings and fully deserves the admission of a doctorate degree. Although it is the reviewer duty to point out the weak or underdeveloped points, not by malice but to help in further development of the candidate.

As mentioned the thesis is devoted to chelation of trace elements and possible toxicity that is connected with this phenomena. Personally I am not fully convinced to term "microbiogenic elements". Typically biogenic refers to element derived from living organisms or more rarely present in such matter. Microbiogenic is somewhat delusive.



Nevertheless I understand that its presence in literature may encourage to use it as an alternative to all time repeating “essential trace elements”.

On page 18 one can read that “iron can redox cycle, which in fact is one of its biological features”. To be strict, a copper also can redox cycle and this has been described as an important aspect of amyloid proteins functionality in a brain, which in turn is important to understand the copper hypothesis in the neurodegenerative disorders.

Author is rather temperate in citing literature references. For example the first page of “metal toxicity” provides several facts but only three times the reader is able to follow the original information. Later, one can find a statement “About 70% of iron in the body...”. It is not sure where are these information from. Probably ref 20.

The editorial work that has been done on this thesis in my opinion deserves complements. I was able to find only few misspelled words or odd fragments, that arisen probably during re-edition of the text. Below are examples of them.

Theme for figure 2 should be “by hepcidin”

Page 26 “complexed with c ceruloplasmin”

Page 39 However I can perfectly imagine myself the “oral viability” I think the “availability” is correct word here.

Page 124 contribution for 3: “finishing of the manuscript/ whole manuscript”

Page 126 “Previous research on was performed on”

Page 130 “since it absorbs in the visible area” certainly the nature of this area should be explained

Ref 30 is incomplete

Ref 53 is in Japanese, does author read in this language or used only an abstract?

Ref 108 is fully capitalized.



I would like to stress out that such clear form is rather uncommon nowadays, when everything is done on deadlines with great belief in computers.

In conclusion it should be stressed out that the dissertation written by MSc Maria Catapano is well prepared example of individual research. The experimental methodology is well designed and managed in this work, even if not fully discussed. For more detailed information reader have to check papers that are supplemented. Results are well described and conclusions are correct with full coverage in facts. Number of experimental articles that have been published during this study highlights the merit and scientific soundness of the research. In my opinion the scientific achievements of MSc Maria Catapano fully confirm her development and attitude as young devoted scientists. Therefore it is my great pleasure to request the Scientific Council of Pharmaceutical Faculty of Charles University to award her with the PhD degree.

Your Sincerely

Robert Musioł

