



CHARLES UNIVERSITY  
Faculty of Pharmacy  
in Hradec Králové

## ASSESSMENT OF DOCTORAL THESIS

**Effects of isoflavonoids and their metabolites on vascular smooth muscles *in vitro* and *in vivo***

**by Thomas Migkos, MSc.**

### Characteristics of the thesis

The subject of the evaluated doctoral dissertation was an experimental systematic *in vitro* and *in vivo* study on impact a large spectrum of isoflavonoids on vascular system including explanation of the involved molecular mechanisms. The submitted thesis presents relative broad complex study with several related aims. The first aim was to examine potential vasorelaxant effects of more than 20 isoflavonoids and their metabolites using rat aorta preparation *ex vivo*. This study was followed by a detail exploration of molecular mechanisms of the found vasodilation effects in the most potent compounds under study on porcine coronary arteries. The final step of the presented work was a confirmation of the previous findings in a study on blood pressure in rats *in vivo* performed with selected isoflavones.

The evaluated thesis represents a classical version of dissertation. Therefore, it is divided into standard sections typical for the presented type of scientific report: abstracts, a theoretical background, aims of the studies, a description of the used experimental methods, discussion on the obtained results, conclusions. An appendix containing a published article is attached as the additional part of the thesis. The presented paper was published in a scientific journal with higher impact factor. The applicant is the first author of the published article and, according to the declared share on the study, his contribution was decisive. In addition, several experimental studies with the candidate's contribution focused on other aims were published in impacted scientific journals.

### Evaluation of the dissertation

The area of scientific topics that the author dealt with in his work fully corresponds to the field focus. The topic of the dissertation aimed at pharmacological testing vasorelaxation effects of isoflavonoids can be considered highly topical, because it is associated with real clinical issue.

In the theoretical part of the thesis, the author briefly characterizes the studied isoflavones and their pharmacokinetics and pharmacodynamic effects. Epidemiological studies aimed at isoflavonoid effects are also summarized in the introductory part of the dissertation. The review is written in a comprehensive way, without serious content or formal shortcomings. Although the opponent would welcome more detailed information on some effects of isoflavonoids, such as estrogenic action, the theoretical introduction brings sufficient information to the

understanding of the matter and interpretation of results provided further. The following part of the dissertation brings a detailed description of the methods applied for solution of the study aims. The reviewer especially appreciates the involved pictures showing illustratively process of preparation samples used in *ex vivo* studies. Discussion section of the thesis thoroughly compares the obtained results to those found by previous relevant published studies focused on isoflavone affection of arterial tonus.

In particular, the complexity of the presented research should be appreciated. *In vitro*, *ex vivo* and *in vivo* models were employed to study isoflavone vascular effects, therefore, the achieved results led to creation of a very complex set of preclinical data on isoflavone effect on arteries. A significant contribution of the presented work to recent knowledge lies in a head-to-head comparison of the large spectrum of isoflavones and their metabolites at the same experimental conditions. To bring more light into information on cardiovascular effects of isoflavonoids is very desirable as only partial and inconsistent data are available.

The employment of three experimental preparates, which represent different parts of the arterial system, enabled to detect potential differences of the vasoactive effect within the circulation. Another significant contribution of the work is exploration and determination of molecular mechanisms involved in isoflavone effects on the arteries. *In vivo* confirmation of the found vasodilation effect of a selected isoflavone seems to be only preliminary as only one compound was evaluated. Although there are significant limitations to perform *in vivo* experiments a more thorough study on this issue can be recommended. A combinatory effect of flavonoids on arterial tonus is only suggested as a good idea for further studies but, unfortunately, even a preliminary experiment has not been performed *in vitro* or *in vivo*. This topic could present another interesting area open for further studies.

Furthermore, the identification of relatively potent vasodilation compounds among the studied isoflavones should be appreciated, as these findings may have not only clinical significance, but the tested compounds may be potentially used in further experimental studies as comparative agents.

The level of achieved scientific results contained in the attached publication could be considered to be adequate, as evidenced by the fact that the obtained experimental data were published in peer-reviewed scientific journal with an IF over 3.

### **Comments and questions:**

Taken together, the thesis reaches a scientific quality needed for dissertations of this type. The publication of a paper in quality scientific journal documents considerable achievements of the candidate and supervisor's team. The results represent a significant advancement in scientific knowledge and, after further investigation, may become clinically interesting. The published paper was reviewed by experts in the appropriate area. Thus, the following questions are focused on some details of the submitted dissertation and, in addition, they are focused on more general aspects of the issue:

1. Did you consider or evaluate potential toxicity of the isoflavones investigated in *in vitro* and *ex vivo* studies?
2. Would it be possible to include positive controls in your *ex vivo* experiments?
3. You used rat and porcine arterial preparations as experimental models to study vasodilatory effects. Are these models similar in terms of vascular physiological functions or are there significant interspecies differences? For example, is the structure and function of the relevant receptors in the used vessels identical?

4. Although isoflavones have good absorption following oral administration (p. 17) a low blood levels are generally observed. Are there developments how to increase bioavailability of isoflavones and other flavonoids?

**Conclusion:**

Based on the above-mentioned facts, I state that the dissertation submitted by Thomas Migkos "Effects of isoflavonoids and their metabolites on vascular smooth muscles in vitro and in vivo" meets the requirements for a doctoral dissertation in the given scientific field and documents the author's ability to independently work scientifically at an appropriate level. I therefore recommend the afore-mentioned dissertation for defense, on the basis of which the applicant would be awarded the scientific degree of Ph.D.

In Hradec Králové, 25th November 2023

Prof. PharmDr. František Trejtnar, Ph.D.