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# P e e r  R e v i e w

**of the doctoral thesis submitted to Scientific Council of the Faculty of the Veterinary Medicine, University of Life Sciences in Lublin, in form of dissertation entitled:**

**"Studies on the effect of bovine milk casein and whey proteins on cadmium toxicity in rats"**

The Author of the doctoral dissertation: **Hussein Baee Khudhur, PhD-Student**

The Promoter of the doctoral dissertation: **Jose Luis Valverde Piedra**, DVM, Ph.D. Assoc. Prof. Department of Preclinical Veterinary Science Sub Department of Pharmacology, Toxicology and Environmental Protection,

The Co-promoter: **Sylwia Szymańczyk**, PhD Eng. Department of Animal Physiology, Faculty of the Veterinary Medicine.

**General characteristics of the dissertation.** The subject matter of the performed works transfered onto the doctoral dissertation is situated in the field of experimental toxicology. Particular attention has been paid to the development of cell culture breeding method combined with electric cell-substrate impedance sensing (ECIS) as a tool for *in vitro* cytotoxicity testing. Whereas, the *in vivo* study was conducted on 72 male and female albino Wistar rats strain. The main goal of the studies was to recognize the protetive ability of bovine milk casein and whey proteins in course of cadmium induced cytotoxicity assayed in the cell cultures media and the threshold sub-chronic toxicity to the rats. As a starting point, the Author assumed that among the toxic elements: lead, mercury, arsen and cadmium, the last one needs special research due to serious problem of Cd-pollution in Iraq, when compared to neighbour's countries. Further, an interaction issue of cadmium with mineral elements (Zn, Fe, Cu) has been undertaken in order to extend the epidemiology scope, as a point of public health concern. In addition, toxicokinetic and toxicodynamic aspects of cadmium in the rats: blood and serum morphological and biochemical parameters, as well as histological changes in liver and kidney have been considered in respect to translate the data onto humans.

This elaboration was developed according to generally accepted principles for scientific dissertations. It contains 238 pages, divided into 8 chapters, supported with 60 figures photos included, 46 tables and 377 positions of literature, all in English. In addition, the dissertation was provided with a list of abbreviations and the summaries, both in English and Polish languages.

However, the text is not accurate from editorial point of view. Some acronyms are not followed by full wordings, part of informations are repeated in the subsequent sub-chapters, as well as misleading words are found. Also punctuation marks needs to be corrected, especially on the last parts of the dissertation.

**Detailed assessment of the dissertation.** The PhD candidate should paid the attention to the **title**: only the rats as the subject of an *in vivo* research, are covered. However, indicating fibroblasts (an *in vitro* assay) in the title would comprehensively pointed out the full range of performed research. Nonetheless, according to peer reviewer opinion, the choice of the subject of the doctoral dissertation in both its subjective and objective dimensions is accurate.

**Introduction** chapter touches on the topics related to the content of the research undertaken. It indicates the timeliness of the subject, that capture the history and the current topic data relevantly within the planned steps of the research. However, the data demonstratively are focused on sitaution in Iraq in respect to cadmium hazard to environment and public health. On the other hand, full commitment of the PhD candidate to select cadmium to realise the topic of the research project is ascertained.

A peer reviewer comments are involved with the lapse, that some information from introduction are re-called in next pages. Namely, **literature review** chapter repeats the same sequences, e.g. (...)'141 patients pmp of chronic renal failure(...) on p. 27, formerly on p. 21. *Nota bene* acronym 'pmp' is not explained elsewhere. Nonetheless, the chapter detaily citting an abundant literature, appropriately selected by the Author, properly ranks and keeps proportion towards the intended steps of research purposes. It contains 8 sub-chapters focused on: cadmium effects on ecosystem, cadmium pollution in Iraq, cadmium toxicity and epidemiology, cadmium toxicokinetic, mechanism of toxic effects, cadmium toxicity effects, cadmium histopathological effects and milk proteins issues. However, according to toxicological principles the data of sub-chapter 2.3: **Cadmium Toxicity** are rather pertain to toxicodynamics, which entities are covering mainly by 2.6. and partly by 2.5 or 2.7 sub-chapters. Thus, the same themed informations are not repeated in the next parts of the text. Additional comments deal with toxicokinetics and toxicodynamics concerns in order to find out the information on NO(A)EL, LO(A)EL and ADI. These threshold levels for cadmium compounds, mainly for rats, should be extracted from the literature, since above indices might be evidently related to choosen dosage, both in performed *in vivo* and *in vitro* studies. Especially in the case when the Author assumed to adapt the experiment conditions to practical exposure of mankind, which is of threshold level. Hence, the above mentioned data: NO(A)EL, LO(A)EL and ADI should be considered for projecting cadmium dosage in both *in vitro* an *in vivo* studies.

Sub-chapter 2.7 sufficenty outlines cadmium histopathological data found in the literature and combining them within the planned scale of investigations in respect to subchronic level of rat's exposure. The text is comprehensively and precisely edited.

Disclosure of milk proteins, casein and whey proteins selection to the study procedures do not raise doubts as to their proper choice in terms of presumed preventive properties against toxicants that expose rat's organisms.

**The aim of the study** submitted to peer evaluation in the edited form should be re-arranged. Skiping majority of the descriptive text of this chapter (lines 1-23 p. 47), and certainly omitting the citation, would allow for the synthetic formulation of research objectives. In addition, the sentence regarding procedure I: "(...)peptides on cells in '*in vitro*'studies throw(...)", (whether through ?). Nevertheless, the presented concepts, assumptions and objectives of the work are consistent and sufficiently justify the commencement and implementation of subsequent stages of well-planned research. Their implementation was supposed to enable the Author to bring his own and original data in a novelty dimension to the current state of knowledge.

**Material and methods** are blocked-up in two sets: in vitro and in vivo experiments, which are measuring into achieve the main goal of the study, i.e.: whether the bovine milk proteins might protect against cytotoxicity and toxicity induced by cadmium.

The sophisticated procedure of ECIS coupled with cell culture media is clearly presented, although some signs, e.g.: ZΘ, are enigmatic, as well as the dots in the middle of the sentences. The last ones and other type of errors [e.g. how to interpret the record: Cn**.**V/(V+1000)?] are found also in a number of the other sentences of the manuscript. The graphic schedule (Fig.1) of an *in vitro* experiment using mouse fibroblast line L929 is well illustrative for the designed procedures .

Also description of the animal experimental model clearly explains subsequently, step by step the procedures, including helpful tables (Tab. 3 and 4) positioned within the text. However, the information on ethical norms on animal experimentation needs correction: instead of "(...)Bioethic commission(...)", the resolution nr 83/2015 was delivered by Local Ethical Comission affiliated at University of Life Sciences in Lublin. Furthermore, the notation: "(...)the PHS Policy on Humane Care and Use of Laboratory Animals(...)" is inappropriate and enigmatic; the acronym, PHS, is undecipherable.

The both experimental models were properly designed, which enabled to measure a number of parameters relevantly to the planned aims: an impedance of ECIS and cultured cell morphology within the *in vitro* studies. Additionally, the parameters of the *in vivo* experiment: rat's body weight, weekly feed consumption, daily water intake, picture of blood morphology, serum biochemical indices, values from metals determination in the tissues, histopathological

pictures were obtained. Each of the experimental step was subjected to statistical analysis by means of methods accepted in model biological studies.

From the reviewer standpoint: it would be advisable to standardize description of cadmium chloride concentration units, both *in vitro* and in *vivo* studies. Instead of molar units, i.e. 19,4 mM/L, let use weight units (w/v: mg/L). When following this recomendation, e.g. the sentence from 4.2.3 sub-chapter: "(...)water containing 19.4 mM/L of CdCl2 (it should provide the daily dose of 4±0.5 mg/kg body weight(...)" would operate within the same units. In many parts of the manuscript such an unification is profitable, because of easy comparison with literature data operating in majority presented at w/v units.

**Results** constitute the most extensive chapter of the manuscript, covers 80 pages of text, which is illustrated by 59 figures and 12 tables. They are complementary to research procedures descripted in Material and Methods and in the Discussion chapter.

The results allow the reader to perceive the substantive side of the manuscript. At the same time, to notice the impressive amount of work put into the implementation of the research plan. It is also easy to appreciate the original work achievements that the Author emphasizes in his dissertation. Basing on quantitative and qualitative data, the Candidate proved to find out the right path to determine the scale of the protective effect of bovine milk proteins in course of the cadmium toxicity. The mentioned elements enabled to draw a conclusions both of pracical value (casein and whey proteins supplementation of the diet) as well as in the cognitive aspects: novel data on protective substances intermediating within toxicokinetic and toxicodynamic action of cadmium in the mammal's body and within the cell cultures. Hence, peer reviewer states, that the documentation of the results obtained, in an insightful, proper and even exemplary way are representing the considerable achievements of the doctoral project. Moreover, the statistical and mathematical dimension, in addition to illustrative figures, are worth emphasized.

In the **Discussion** chapter, the proper role of the PhD student was revealed. Starting from the analysis of elaborated data, he proceeds to the synthesis and evaluations, summarizing the subject taken up by himself. This is interesting part of the work, in which confrontation of his results with the literature data, at the same time outlining the most important new trends and scientific achievements in the studied topic were evidenced. These results are quantitatively and quantitatively in the range of data found in both domestic (Iraqi and Polish) and global scientific sources. Analyzing the results obtained, he perceives them through the prism of the methods that were used in the research project. The differences of the obtained data in comparison with other authors' findings are confirmatory in majority of cases, when pathophysiological effects recorded for cadmium exposure were related.

Starting from the thorough knowledge of biomedical sciences and basing on the obtained results, the Author discusses the mechanisms of milk proteins cytoprotective and *in vivo* protecive effects. Relevance in the selection of arguments allow for the recognition and the inference on target sites for cadmium detoxication. According to the reviewer, this aspect is the most creative element of the dissertation crowning the doctoral research project. The success in this respect was possible due to the high discipline in solving the research tasks undertaken: from accepting the thesis to drawing the right conclusions.

The **conclusions** are valid, correctly reported in the context of the obtained results and are reflected in the logically conducted discussion, corresponding to the scope of the planned research.

**Summarizing** the peer reviewed manuscript showing achievements both in application and cognitive aspects, I conclude, that the doctoral dissertation of Hussein Baee Khudhur, entitled: ”Studies on the effect of bovine milk casein and whey proteins on cadmium toxicity in rats” is based on the original results of the research. It corresponds to the conditions specified in art. 13 of the Act of 14 March 2003 on academic degrees and academic title, and on degrees and title in the field of art. and the enactment of the Ministry of Science and Higher Education of 26 September 2016 with latter changes. Therefore, I am bringing to the High Scientific Council of the Faculty of the Veterinary Medicine, University of Life Sciences in Lublin, for the admission of a PhD student Hussein Baee Khudhur to further stages of the doctoral dissertation proceeding.

The undeniable advantages of the reviewed work allow to apply for its award with an appropriate prize. I justify the application with the most important creative achievements of the outcomes from the study, which contribute the science in international scale.

was ground into bread and tragically consumed by Iraqis tragically exposing over 40,000 people of all ages.

Jednakże forma redakcyjna pozostawia sporo do życzenia, zarówno jeżeli chodzi o styl jak i błędy gramatyczne

oceniał zawartość 7. pierwiastków (Pb, Cd, Hg, As,)

Reviewed doctoral dissertation situate it in the public health protection in respect to chemical safety, a superior strategy both to the contemporary human and veterinary medicine.