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Review of Ph.D. Thesis.

Ph.D Thesis: „**Some aspects of molecular mechanisms of xenobiotics’ hepatotoxicity and hepatoprotection**“.

Author: *MPharm. Nataša Lekić*, 1.LF UK Praha.

The manuscript of Ph.D thesis comprises 117 pages, contains 4 Tables and 30 Figures. Is divided to 2 parts – theoretical background and (27 pages) and study (methods, results, conclusions, discussion – 59 pages), list of references and necessary formalities.

Another part of thesis is an “Appendix” with 3 papers published in journals with impact factor and one manuscript. As to formalities, the thesis conforms to usual standards.

In the theoretical background, the processes involved in oxidative stress at liver injury and the role of some protective mechanisms (antioxidative enzymes) are described in detail. The process of apoptosis is also described. Moreover, the mechanisms of models of hepatotoxicity, used in an experiment are mentioned, as well as the theoretical background of hepatoprotection of polyphenols.

Theoretical background is detailed, based on good knowledge of literature. In the chapter focused on oxidative stress, actual knowledge raise the need for future research is described in detail. The up-to-dateness is emphasized. The references are relevant and up-to-date.

The aim of the study was to describe some molecular mechanisms of hepatotoxicity caused by Tert-butyl hydroperoxide (TBOOH) in vitro, eventually by Galactosamine lipopolysaccharide (D-GalN/LPS) in vivo. The author focused on mechanisms related to oxidative stress and apoptosis. Specific antioxidative enzymes (HO-1, SOD-1, GPx,

CAT), pro-oxidative enzymes (NOS-2), inflammatory cytokines (TNF- α) and apoptosis mediators (Bid, Bax, Casp3) were studied in this context. Another aim was to assess the hepatoprotective characteristic of polyphenols curcumin and quercetin.

In the methods, all examinations are described in detail including molecular methods. This indicates author's own work. The author also describes the models of hepatotoxicity – in vivo Wistar rats were used and in vitro hepatocyte culture was studied. The methods used in study conform to the newest knowledge.

Statistical methods are appropriate.

One of the most important results is related to the experimental model of TBOOH hepatotoxicity – the addition of quercetin led to the amelioration of hepatocytes injury. The addition of quercetin led also to the induction of HO-1, what could be the explanation for the favourable effect of this substance.

Other results concern the in vivo study: In the model of acute liver injury caused by D-GalN/LPS, the changes of enzymes involved in the oxidative stress as well as in parameters of apoptosis were found. This emphasizes the role of apoptosis in xenobiotic hepatotoxicity.

The addition of quercetin and curcumin to the rats with experimental caused liver failure led to the improvement of parameters of hepatocyte injury. Both substances induced HO-1, what is in concordance with the in vitro experiment.

The author concludes that both models of xenobiotic hepatotoxicity caused changes in some enzyme linked to cell oxidative stress. The main enzyme responsible for hepatoprotection is considered to be HO-1. The cytoprotective effect of curcumin and quercetin to hepatocyte toxicity was described. This effect is probably mediated by HO-1 induction.

The most important contribution of presented study consists of possible future clinical use of above mentioned favourable effects of two polyphenols.

Questions:

1. *Many antioxidants are under evaluation in hepatology. Why the author decided to examine curcumin and quercetin?*

2. *It seems that the preventive effect of both curcumin and quercetin could be mediated by HO-1 induction. How the author would explain fact that in vivo both substances induce HO-1 only in rats with experimental liver injury (D-GalN/LPS) and not in controls? The incubation with quercetin led to HO-1 induction also in controls at in vitro experiment.*
3. *Histology of liver tissue in animals with D-GalN/LPS liver injury is mentioned. Were the samples from animals treated with curcumin or quercetin examined? If yes, did the histology differ from animals with D-GalN/LPS liver injury?*
4. *Beside the apoptosis, toxic liver injury is characterized by "non-programmed cell death" – cell necrosis. Could the above mentioned polyphenols play a role also in the prevention of this type of liver injury?*
5. *Clinical use of any antioxidant is limited by the time frame – all substances could be usually used some time after the toxic injury. Are there any data evaluating the favourable effect of curcumin or quercetin at fully developed liver injury?*

On conclusion, the study is in accordance to the requirements for Ph.D Thesis. The aims were achieved, the problematic is up-to-date. The results indicate new information related to oxidative stress in xenobiotic hepatotoxicity and possible therapeutic consequences and were published in impacted journals.

I recommend accepting the study for Ph.D degree. Presented study demonstrates prerequisite of the author to the individual scientific work.

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