ABSTRACT

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Title of Doctoral Thesis: Potential toxicity of terpenes and their effects in liver cells

The public interest in herbal products, supplements, extracts or isolated active compounds has been increasing during last decades. The aim of this doctoral thesis was to study selected compounds from the group of monoterpenes and sesquiterpenes and their interactions with liver cells: interactions with drug metabolising enzymes, their potential liver toxicity and looking for novel mechanisms of action.

The literature about hepatotoxic properties of several monoterpenes and sesquiterpenes, that showed liver toxicity, was summarized first. Then, the study was aimed toward a known hepatotoxicant (*R*)-pulegone and its presumed metabolite (*R*)-menthofuran. The toxicity results in precision-cut human liver slices have shown that (*R*)-menthofuran was less toxic to human liver and that the reason may be inter-species differences between human and mice.

Several sesquiterpenes (farnesol, cis-nerolidol, trans-nerolidol, α-humulene, β-caryophyllene, and caryophyllene oxide) have previously inhibited the activity of several cytochrome P450 (CYP) isoforms, especially CYP3A4. These compounds significantly induced CYP3A4 expression via pregnane X receptor interaction in transfected HepG2 cells. The intention was to verify this effect in precision-cut human liver slices, using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). For this reason, a validation study was first performed to check the stability of reference genes in human liver slices, required for normalisation of RT-qPCR data. In the end, no significant modulatory effect on the expression of studied drug metabolising enzymes in liver slices was observed under the effect of studied sesquiterpenes.

The toxicity of potential anticancer agents alantolactone (ALA) and germacrone (GER) was studied against a hepatocyte-like model, differentiated HepaRG cells. While

alantolactone has shown lesser toxicity towards HepaRG cells than highly proliferative cancer cell lines, both compounds have shown production of reactive oxygen species in considerably low concentrations. Using target prediction tool BATMANT-TCM, novel targets 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and aromatase (CYP19A1) were predicted for ALA and GER. While both compounds indeed targeted HMGCR, the effect was the most significant in toxic concentrations. They also influenced the aromatase mRNA expression, but each compound differently, showing that the mechanism will not be the same for both. The obtained results of this doctoral thesis extend the knowledge about acting of natural compounds on the human organism further.