ABSTRACT

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Title of diploma thesis: Effect of monoterpene citral on the expression of detoxification enzymes in HepaRG cells

Citral is an acyclic monoterpene, which is mainly produced by higher plants. It is one of the many secondary metabolites, which serve the plant as detoxifying factors, morphoregulators and attractants for pollinators or defense substances. Besides other things, it has numerou positive effects such as antibacterial, anti-inflammatory or antitumor, but it may be toxic, especially for the liver, if used incorrectly. The main goal of this thesis was to find out the effect of citral on selected biotransformation and antioxidant enzymes in HepaRG cells. Differentiated HepaRG cells were incubated with 10 μM rifampicin, 10 μM β-naphthoflavone, 30 μM oltipraz, 10 μM citral, 30 μM citral and 100 μM citral for mRNA expression detection for 12 and 24 hours and for protein expression evaluation for 24 and 48 hours. At the mRNA level, rifampicin induced CYP3A4 at 12- and 24-h incubation, β-naphthoflavone induced CYP1A2 at 12- and 24-h incubation and aldo-ketoreductase (AKR1C) at 24-h incubation, oltipraz induced glutathione peroxidase (GPX1) at 12- and 24-h incubation. Following citral treatment, gene expression of some enzymes was changed as well, 30 μM citral inhibited carbonyl reductase (CBR1 at 12-h) incubation, 100 μM citral induced GPX1 and glutathione reductase (GR) at 12-hour incubation, 100 μM citral induced CYP3A4, CYP2B6 and glutathione-S-transferase (GSTA) at 24-h incubation. At the protein level, rifampicin induced CYP3A4 at 24- and 48-h incubation and 10 μM citral inhibited CYP3A4 at 24-h incubations. Morevover, 100 μM citral induced CYP2C9 and significantly inhibited carbonyl reductase (CBR1) and aldo-ketoreductase (AKR1C3) at 48-h incubations. Furthermore, the cytotoxicity of citral was studied using Neutral Red Uptake (NRU) assay. The IC_{50} value was 0.892 mM at 48-h incubation and 0.708 mM at 72-h incubation.