

ABSTRACT

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Biochemical Sciences

Candidate: Terézia Kúdelová

Supervisor: Doc. Ing. Barbora Szotáková, Ph.D.

Consultant: Prof. RNDr. Rudolf Štětina, CSc.

Title of diploma thesis: Detection of the oxidative damage of DNA by the Comet Assay

The reparative mechanisms have a very important role in organisms. Imbalance develops due to disruption of these mechanisms, which can lead to induction of many disorders. The aim of this diploma thesis was to study protection of DNA against oxidative damage. The ability of A549 cell line to cope with this damage was studied *in vitro*. Oxidative damage was induced by hydrogen peroxide (H₂O₂), which initiated induction of single strand breaks (SSBs) and at the same time oxidation of pyrimidine and purine bases. Mechanism of damage and reparation was detected by two methods. In the first case, cells were treated with antioxidant and then influenced by H₂O₂. In the second case, cells were influenced by H₂O₂ and then let to repair in the presence of antioxidants. Tested antioxidants were epigallocatechin gallate (EGCG), glutathione (GSH) and chemical modifications of GSH – S-allyldithioglutathione (GSH-A) and S-allyltrithioglutathione (GSH-B). The extent of damage was assessed using by alkaline version of Comet Assay, which allowed the disintegration of alkali labile sites induced by oxidative damage. Specific enzymes, the endonuclease III (EndoIII) and formamidopyrimidine-DNA-glykosylase (Fpg) were used for detection of oxidized bases. The results suggest a significant therapeutic effect of EGCG which inhibits the induction of oxidative damage of DNA and has a positive effect to reparation of the damaged DNA. GSH, GSH-A and GSH-B at low concentrations inhibit the induction of DNA damage as well. The results of this research would be base for next research, dealing with the serious disorders connected with oxidative damage of DNA in humans.