Glutathione Reductase

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Peroxynitrite, a potent cytotoxic oxidant formed by the reaction of nitric oxide(NO) with the superoxide anion radical(O2.-), reacts rapidly to cause of highly toxic oxidizing and nitrating process. The importance of the regulation of ONOO- has been recently recognized because of lack of specific endogenous anti-oxidative defense enzyme against ONOO-. This lack of defense necessitates search for the exogenous source of effective scavengers against ONOO-. Coffee is a complex mixture containing a variety of compounds. The mutagenic effects of instant coffee have been reported and its constituents are known free radical generators, damaging DNA, lipid and protein. In the present study, we report on the anti-oxidative effect of coffee ingredients. Our major effort was on the scavenging effect on peroxynitrite. At present, there is no information available on peroxynitrite scavenging activity of coffee ingredients. We investigated the protective effect of coffee ingredients against peroxynitrite using GSH reductase whose activity is dependent on the integrity of tyrosine. The effectiveness of protection was monitored by the prevention of protein nitration by peroxynitrite. In the study, we focussed on two major coffee components: hydroquinone and 3-methyl-1, 2-cyclopentadione. The scavenging activity of two coffee ingredients was assessed by following three methods: 1) the quantitation of the oxidation of dihydrorhodamine 123 to rhodamine 123. 2) inactivation of GSH reductase activity and 3) tyrosine nitration of GSH reductase by Western blot analysis.
Results showed that hydroquinone and 3-methyl-1, 2-cyclopentadione effectively suppressed peroxynitrite-mediated tyrosine nitration of glutathione reductase in a dose-dependent manner. The extent of prevention of nitration was reflected on GSH reductase activity, indicating the protection of tyrosine moiety in the enzyme. Data are further confirmed by level of tyrosine nitration in GSH reductase analyzed by Western blot analysis.
To our knowledge, this is the first report on the peroxynitrite scavenging action by hydroquinone and 3-methyl-1, 2-cyclopentadione in coffee. Reaction mechanisms of these active ingredients against peroxynitrite need further elucidation.

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Hamamelitannin as a peroxynitrite scavenger

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Peroxynitrite (ONOO-), formed in vivo from the reaction of superoxide ('O2-) with nitric oxide ('NO), is a cytotoxic species that can oxidize several cellular components such as proteins, lipids, and DNA and nitrate many amino acids, including tyrosine. The purpose of our present study was to investigate the protective effect of hamamelitannin, the major active component isolated from witch hazel (Hamamelis virginiana L.) bark, against damages induced by ONOO-. Hamamelitannin (IC50=1.05μM) exhibited very potent ONOO- scavenging activity measured by oxidation of dihydrorhodamine 123 with fluorescence method. Our data suggest that hamamelitannin led to decrease ONOO--mediated nitration of tyrosine by its electron donation in spectrophotometric analysis. Using immunoassay, hamamelitannin showed the significant inhibition on nitration of bovine serum albumin (BSA) and low-density lipoprotein (LDL) by ONOO- in a dose dependent manner and the relative inhibitory effect on oxidation of BSA and LDL by ONOO-. Hamamelitannin also provided protection against cell damage mediated by ONOO-. In conclusion, our results suggest that hamamelitannin can be developed as an effective supplementary antioxidant in peroxynitrite toxicity.

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