

results, we show that these flavanoids with other antioxidant substrates are increased antioxidant activity level.

[PC1-10] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Antioxidant effect of chitosan in the renal failure

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Oxidative stress has been implicated in a range of disease states, including end-stage renal failure treated with hemodialysis [Westhuyzen J. et al, 2003]. Free radicals react with biological molecules and destroy the structure of cells, which eventually causes free-radical induced disease such as cancer, renal failure, aging, etc. Exogenous or endogenously produced nitric oxide (NO) inhibits superoxide-stimulated urea permeability. In the inner medulla, superoxide generation by local oxidases may stimulate urea transport, and the role of endogenous NO may be to dampen this effect by decreasing superoxide levels [Zimpelmann J. et al, 2003 (Epub ahead of print)]. Xie W. et al (2001) reported that water-soluble chitosan derivatives had antioxidant activity. In the present series of experiments, we studied the amount of NO (nmol/mg protein) and the activity of superoxide dismutase (SOD, units/mg protein) in mouse kidney (in vivo) and porcine proximal tubules (in vitro) from normal and HgCl₂-induced renal failure models. In vitro, the amount of NO and the activity of SOD were increased in a concentration dependent manner (0.025, 0.05, 0.075, and 0.1%) of chitosan only or with HgCl₂ (20 μM). The production of NO by HgCl₂ (0.715±0.343) increased more than normal (0.525±0.192). But, HgCl₂ did not affect on the activity of SOD significantly. In vivo, the amount of NO and the activity of SOD were changed each time (24, 48, and 72 hour) after injection of HgCl₂ (1mg/kg); normal (NO: 0.104±0.069, 0.083±0.049, and 0.093±0.067; SOD: 6.366±2.011, 5.421±1.925, and 6.232±1.593), only HgCl₂ (NO: 0.145±0.048, 0.183±0.023, and 0.176±0.053; SOD: 7.017±1.203, 6.525±0.990, and 6.211±1.698), chitosan with HgCl₂ (NO: 0.209±0.074, 0.154±0.052, and 0.113±0.059; SOD: 9.964±0.824, 8.611±1.224, and 6.835±1.431). These data suggest that (a) HgCl₂ does not affect the activity of SOD and (b) chitosan may help to overcome oxidative stress caused by superoxide in the renal failure.

[PC1-11] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Dye-silver double staining method for proteins in SDS-polyacrylamide gels using a dye as a silver sensitizer

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We have developed a silver staining method using a dye as a silver sensitizer. Dye staining is performed in combination with silver nitrate staining. Dye-silver staining shortens the time of silver staining (~1 hr) and improves the sensitivity better than that of silver diamine stain (1-10 ng) or comparable to that of silver nitrate stain with glutaraldehyde as a silver sensitizer. In dye staining (silver sensitizing step), it has been proven that the sensitivity is at least 4 times comparing with that of CBBR stain and the staining time is about 45 min. It is convenient to succeed in silver staining following dye stain. The dye, an azo compound having polycyclic aromatic sulfonic acid, binds to both protein and silver ion. Sulfonic acid group of dye and chelation with silver produces binding sites for silver ion and so enhances silver nucleation. In addition, azo group of dye may contribute to silver ion reduction as a silver sensitizer when azo group breaks down to N₂ evolution in alkaline solution. These can enhance the sensitivity of the dye-silver staining up to 0.1-1 ng. This staining method can be applied to detect for the trace amount of protein in 1D and 2D-PAGE and compatible to MALDI-TOF MS (silver nitrate with glutaraldehyde is not MALDI-TOF MS compatible) for proteom research.

[PC1-12] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Agrobacterium-mediated transformation of *Eleutherococcus senticosus* with the