results, we show that these flavanoids with other antioxidant substrates are incresed 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 $HgCl_2$-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 $HgCl_2$ (20μM). The production of NO by $HgCl_2$ (0.715±0.343) increased more than normal (0.525±0.192). But, $HgCl_2$ 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 $HgCl_2$ (1㎎/㎏); 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 $HgCl_2$ (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 $HgCl_2$ (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) $HgCl_2$ 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

## squalene synthases gene derived from panax ginseng

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Transgenic Eleutherococcus senticosus plants were prepared by introducing the genes for squalene synthase (SQS), hygromycin phosphotransferase (HPT) and green fluorescent Protein (GFP) through Agrobacterium–mediated transformation. The enzyme, SQS, represents a putative branch point in the isoprenoid pathway capable of diverting carbon flow specifically to the biosynthesis of phytosterol and oleanolic acid. The full SQS gene was isolated from P. ginseng roots. Early globular embryo clusters developed from embryogenic callus were used as the explant source. Following infection, selection was achieved on hormone-free MS medium containing 300-mg/l cefotaxime and 25-mg/l hygromycin at 2-week intervals. Somatic embryos were germinated and converted into plantlets after the cotyledonary embryos were pretreated with 14.4 mM $GA_3$. Establishment of transgenic somatic embryos was confirmed by presence of SQS and HPT genes, green fluorescence of GFP, and increased SQS enzymatic activity. The SQS enzyme activity of transgenic plant was 3 times higher than the wild type. In addition, a gas chromatographic analysis revealed that phytosterol (b-sitosterol and stigmasterol) levels in transgenic E. senticosus were increased remarkably. These results suggest that the SQS gene may play a regulatory role for phytosterol synthesis, and the produced transgenic E. senticosus plantlet can be used as the sources of medicinal raw materials.

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

## Antioxidant Activity of major protein from Panax Ginseng C.A. Meyer.

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A major protein was isolated from ginseng root (Panax ginseng C.A. Meyer) using a combination of ammonium sulfate fractionation, gel filtration chromatography, ion-exchange FPLC. Electrophoretic and gel permeation chromatographic studies revealed that the major protein, GMP, is composed of two subunits of approximately 28 kDa. In this study, investigated the ability of GMP to inhibit the oxidation of low-density lipoprotein (LDL). GMP inhibited $Cu^{2+}$ (5 μM)-promoted oxidation of LDL (125 μg protein/mL) in a dose-dependent mamer (0~5 μM), with a maximal inhibitor at GMP/copper ratio of 1:10 and an $IC_{50}$ value of 0.2 μM, as determined by measurement TBARS. Meanwhile, bovine serum albumin (BSA) and histidine showed $IC_{50}$ value of 2.4 μM and 8.0 μM, respectively. In related experiment, GMP was more effective than histidine in preventry against $Cu^{2+}$-induced formation of carbonyl in BSA, but the reverse in preventry $Cu^{2+}$-induced oxidation of ascorbic acid. However 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)(1mM)-induced oxidation of LDL was not inhibited by ether GMP or histidine. These data suggest that the antioxidant action of GMP against $Cu^{2+}$-promoted LDL oxidation might the mainly due to the selective interaction with $Cu^{2+}$ associated with LDL.

**[PC1-14] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]**

## Inhibitory effect of benzoxathiol LYR-71 compound on inflammatory enzymes and cytokines

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The benzoxathiol LYR-71 compound was discovered as an inhibitor of NF-kB transcriptional activity with an IC50 value of 5.4 uM. Furthermore, benzoxathiol LYR-71 compound inhibited the NF-kB binding activity to DNA in a dose-dependent manner, which was identified by EMSA with oligonucleotide corresponding to NF-kB consensus sequence. It is well known that NF-kB is an important transcription factor to regulate the expression of inflammatory enzymes (iNOS and COX-2) and cytokines (TNF, IL-1 and IL-6). The benzoxathiol LYR-71 compound suppressed all expressions of iNOS, COX-2, TNF, IL-1 and IL-6 transcripts in LPS-stimulated murine