薬物代謝工学分野 Section of Metabolic Engineering

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薬物代謝工学分野は和漢薬の薬効，毒性発現に関与する代謝系の分子生物学的研究を発展させることを設置目的とし，① 和漢薬の薬効発現に関与する腸内細菌の役割，② 薬物代謝に関する腸内細菌遺伝子の解明，③ 腎毒性物質産生機構の分子生物学的解明とその制御に関する研究を課題として取りあげ，和漢薬の薬効発現機構，生体へのレスポンスなどの基礎的研究を通じて，和漢薬の科学的評価や臨床応用をはかることを目指している。主な研究題目を以下に示す。

1．天然物のバイオトランスフォーメイション
2．和漢薬の薬効発現に関与する腸内細菌，酵素 および その遺伝子の解析
3．AIDS, C 型肝炎の予防 および 治療薬の開発
4．腎疾患，糖尿病性腎症の治療戦略
5．抗老化研究

本年度の主な研究を列挙すると：

1．*C*-配糖体マンギフェリンおよびプエラリンの C-C 結合の開裂に関与する腸内細菌を探索し *Bacteroides* 属の 2 菌種を同定した。またマンギフェリン *C*-配糖体開裂に関与する酵素を精製し，2種の蛋白よりなること，Mn^{2+} 要求性，補酵素を明らかにした。
2．台湾産樟芝菌糸体の抽出エキスおよびその分画がマウスに *Propionibacterium acnes* および LPS を投与することにより発症する劇症肝炎を顕著に抑制することを見出した。
3．中国少数民族薬物及びタイ薬用植物の C 型肝炎ウイルス由来の RNA ポリメラーゼ阻害活性を探索した。
4．腎疾患並びに糖尿病性腎症における新たな治療手段を探索するために，八味地黄丸，温脾湯，黄連，緑茶ポリフェノール，epicatechin 3-*O*-gallate，γ-aminobutyric acid を中心に検討した。
5．冠元顆粒 と ginsenoside-Rd の抗老化に及ぼす作用とその機序について検討した。

◇著書　Books

1）横澤隆子：「血管力をつければ病気は治る」，1-173，リヨン社，東京，2004.

2）中川孝子，横澤隆子：糖尿病性腎症における桂枝茯苓丸の有用性.「腎とフリーラジカル －第７集－」副島昭典，吉岡俊正監修，松澤直輝，青柳一正編，128-134，東京医学社，東京，2004.

3）中川孝子，横澤隆子：桂枝茯苓丸による糖尿病性腎症進展抑制作用 －aminoguanidine, butylated hydroxytoluene, captopril との比較－.「腎とフリーラジカル －第７集－」，副島昭典，吉岡俊正監修，松澤直輝，青柳一正編，135-140，東京医学社，東京，2004.

4）中川孝子，横澤隆子：温脾湯構成生薬ならびに大黄・甘草成分の advanced glycation end products（AGEs）形成抑制作用.「腎とフリーラジカル －第７集－」，副島昭典，吉岡俊正監修，松澤直輝，柳一正編，141-146，東京医学社，東京，2004.

◇原著　Original papers

1）**Ahn E., Akao T., Nakamura N., Komatsu K., Nishihara T., and Hattori M.: Screening of medicinal plant extracts for estrogenic activity in combination with a glycosidase treatment. *J. Trad. Med.*, 21: 81-86, 2004.**

Abstract: For the purpose of evaluating phytoestrogenic activity of medicinal plant extracts, a naringinase-pretreatment method was developed, monitoring with proliferation of MCF-7 human breast cancer cells and induction of β-galactosidase in a yeast two-hybrid assay system. Of various medicinal plant extracts examined, the extracts of *Alpinia katsumadai* (seeds), *Glycyrrhiza uralensis* (roots) and *Moghania philippinensis* (roots) showed higher estrogenic activity by pre-treatment with naringinase than the original extract themselves. The contents of liquiritigenin and isoliquiritigenin having potent estrogenic activity, appreciably increased after the naringinase treatment of the extract of *G. uralensis*. These findings suggested that orally administered crude drugs would increase their estrogenic activity, due to the hydrolysis of some glycosylated constituents by intestinal flora.

2）**Nakamura N., Hirakawa A., Gao J., Shiro M., Komatsu Y., Sheu C., and Hattori M.: Five new maleic and succinic acid derivatives from the mycelium of *Antrodia camphorata* and their cytotoxic effects on LLC tumor cell line. *J. Nat. Prod.*, 67: 46-48, 2004.**

Abstract: Five new maleic and succinic acid derivatives were isolated from the mycelium of *Antrodia camphorata*. Their structures were determined by various spectroscopic means. Maleimide derivatives **2** and **3** showed appreciable cytotoxic activity against LLC cells. (Chart 1 参照)

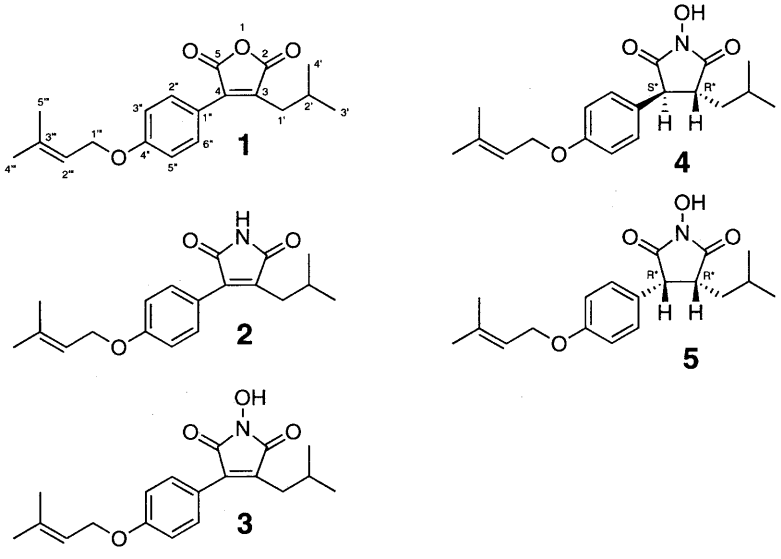
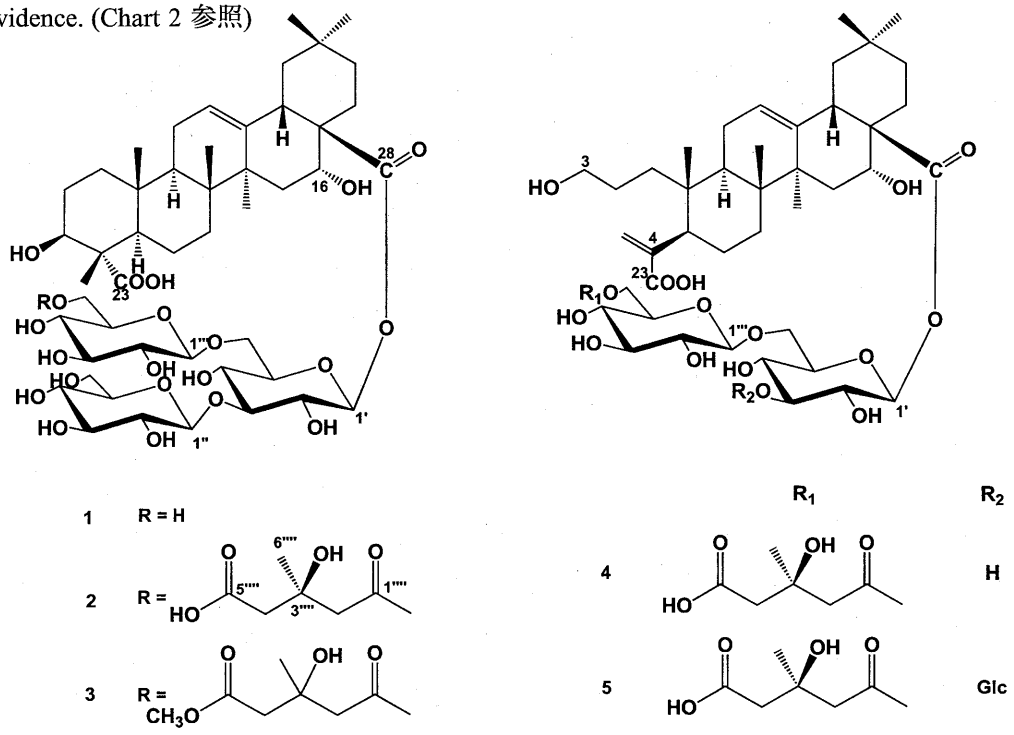


Chart 1

3）**Zhao J., Nakamura N., Hattori M., Yang X., Komatsu K., and Qiu M.: New triterpenoid saponin from the roots of *Sinocrassula asclepiadea*. *Chem. Pharm. Bull.*, 52: 230-237, 2004.**

Abstract: Five new triterpenoid monodesmosides (sinocrassulosides I-V, **1-5**) and six bisdesmosides (sinocrassulosides VI-XI, **6-11**), in which **2-11** possess different acyl groups in the glycosidic moieties, were isolated from the roots of *Sinocrassula asclepiadea* FRANCH. Sinocrassulosides VI (**4**) and V (**5**) also contained a novel A-*seco* aglycone in their structures. All of the structures were determined on the basis of spectroscopic and physicochemical evidence. (Chart 2 参照)



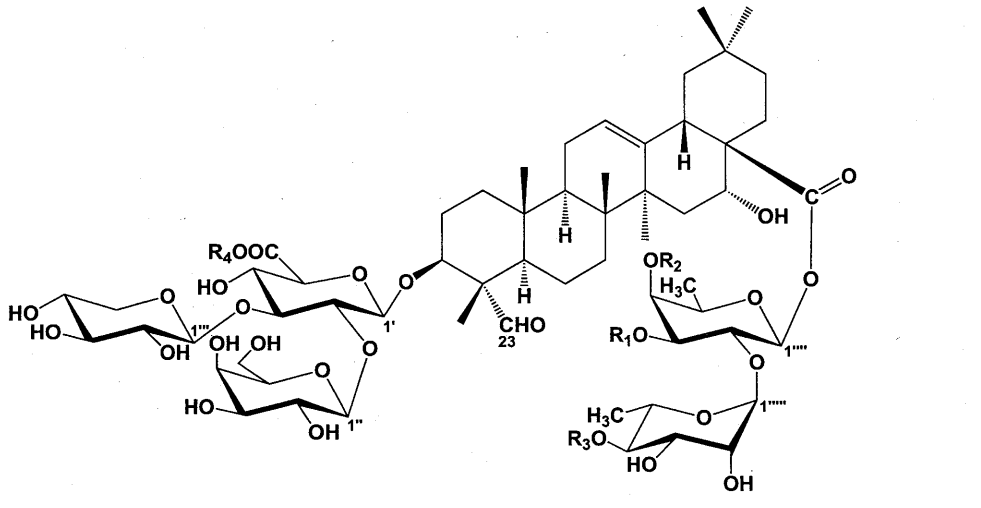


Chart 2

4) **Ahn E., Nakamura N., Akao T., Nishihara T., and Hattori M.: Estrogenic and anti-estrogenic activities of the roots of *Moghania philippinensis* and their constituents. *Biol. Pharm. Bull.*, 27: 548-553, 2004.**

Abstract: In the course of our search for natural estrogenic compounds from medicinal plants, we found that the methanolic extract from the roots of *Moghania philippinensis* (Fabaceae) showed significant effects on the proliferation of MCF-7 cells (human breast cancer) and induction of β-galactosidase activity in a yeast two-hybrid assay. Through estrogenic activity-guided fractionation, we isolated several active flavonoids including prenylated ones. The $CHCl_3$ fraction and its new constituent, 8-(1,1-dimethylallyl)genistein (**9**), appreciably increased the uterine weight in ovariectomized rats when administered orally for 14 consecutive days, in which compound **9** showed stronger estrogenic activity than genistein. Anti-estrogenic activities were also examined based on the inhibition of MCF-7 cell proliferation and β-galactosidase activity in the yeast two-hybrid assay, mediated by 17 β-estradiol. 5,7,3',4'-Tetrahydroxy-6,8-diprenylisoflavone (**6**) showed the strongest antiestrogenic activity.

5) **Zhang Y., Akao T., Nakamura N., Duan C., Hattori M., Yang X., and Liu J.: Extremly low bioavailability of magnesium lithospermate B, an active component from *Salvia miltiorrhiza*, in rat. *Planta Med.*, 70: 138-142, 2004.**

Abstract: We assessed the bioavailability of magnesium lithospermate B (MLB), a main polyphenolic component of *Salvia miltiorrhiza* and a potent antioxidant having various pharmacological activities, to evaluate its action *in vivo*. The plasma concentrations of lithospermic acid B (LSB) showed a biexponential decrease after intravenous administration of MLB to rats at doses of 4 and 20 mg/kg. The values of area under the concentration-time curve (AUC; 87.8 ± 10.9 and 1130 ± 329 μg·min/mL), total body clearance (CL_{tot}; 55.52 ± 7.07 and 23.51 ± 5.98 mL/min/kg), and distribution volume at steady state (V_{ss}; 7.60 ± 1.03 and 3.61 ± 1.16 L/kg) suggested non-linear pharmacokinetics between the two doses. After oral administration of MLB at a high dose of 100 mg/kg, The mean AUC was barely 1.26 ± 0.36 μg·min/mL. Absolute bioavailability of MLB was calculated to be 0.0002 from the AUC values after both intravenous dosing at 20 mg/kg and oral dosing at 100 mg/kg. The extremely low bioavailability was caused mainly by poor absorption from the rat gastrointestinal tract; about 65% of the dose was retained in the tract even 4 h after oral administration, and most of the dose was retained even 20 min after infusion in an *in situ* jejunal loop experiment. Urinary and biliary excretion of LSB were only 0.70% ± 0.26% and 5.10% ± 2.36%, respectively, over a 30 h time period after intravenous injection despite the large CL_{tot} and V_{ss} values, and were much less (0.010% ± 0.001% and 0.12% ± 0.04%) after oral dosing. These findings suggest that extensive metabolism, including a firstpass effect, and wide distribution of LSB besides the poor absorption contributed significantly to the extremely low systemic bioavailability.

6) **Gao J., Nakamura N., Min B., Hirakawa A., Zuo F., and Hattori M.: Quantitative determination of bitter principles in specimens of *Ganoderma lucidum* using high-performance liquid chromatography and its application to the evaluation of ganoderma products. *Chem. Pharm. Bull.*, 52: 688-695, 2004.**

Abstract: For quantitative determination of 19 triterpene constituents, including six ganoderma alcohols (**1-6**) and 13 ganoderma acids (**7-19**), in the products of *Ganoderma lucidum,* an analytical system was developed using high-performance liquid chromatography with an ODS column. The mobile phase was a linear gradient of 1% AcOH/H_2O-CH_3CN and 2% AcOH/H_2O-CH_3CN, and the elution profile was monitored at 243 and 250 nm for ganoderma alcohols and acids, respectively. The relative standard deviations of this method were less than 2.35% and 2.18% (n=5) for intraday and interday assays, and the recoveries were 90.9-100.8% and 93.4-103.9% for constituents of alcohol and acid groups, respectively, This system was applied to a quantitative determination of the constituents in 10 different products of *G. lucidum*: six usual umbrella forms of the fruiting bodies, three antlered forms of the

fruiting bodies and spores, and eight specimens from the same *G. lucidum* strain, which was parasitized on logs from different plants or different fungus beds. The analytical results indicated that the quantity and composition of these triterpenes differed appreciably among various specimens, but the relative ratio of the alcohols and acids was not significantly different when the same strain of *G. lucidum* was used.

7) **Min B., Gao J., Lee Y., Nakamura N., and Hattori M.: Chemical and biological evaluation of germinated and mature antler-shaped fruiting bodies of *Ganoderma lucidum. Natural Medicines,* 58: 91-97, 2004.**

Abstract: For the purpose of evaluating germinated (**I**) and mature (**II**) antler-shaped fruiting bodies of *Ganoderma lucidum,* aqueous extracts of both crude drugs were compared from the point of view of chemical constituents and antitumor activity. In both aqueous extracts, ganodermanontriol and ganoderic acid A were major components of *Ganoderma* alcohols and acids, respectively. However, the total lanostane-type triterpene content of **II** was 6 times greater than that of **I**. The contents and compositions of the respective triterpenes were different from each other. However, the total polysaccharide contents of aqueous extracts of **I** and **II** were not significantly different, as indicated by 13.4 and 11.7%, respectively.

The aqueous extracts of **I** and **II** showed inhibitory effects on the growth of s.c. transplanted Lewis lung carcinoma (LLC) in BDF-1 mice by intraperitoneal administration; an aqueous extract of **I** gave T/C values of 80.8 and 73.0% at doses of 100 and 500 mg/kg/d i.p., and that of **II**, T/C values of 76.5 and 61.5% at the same doses, respectively, indicating the extracts **I** and **II** were similar antitumor activity.

8) **Min B., Kwon O., Park B., Kim Y., Hattori M., Joung H., and Lee H.: Apoptosis-inducing activity of galloylglucose from *Juglans mandshurica* in human promyeloid leukemic HL-60 cells. *Nat. Prod. Sci.,* 10: 48-53, 2004.**

Abstract: Two galloyl monosaccharides, 1,2,6-trigalloylglucose (**1**, TRgG) and 1,2,3,6-tetragalloylglucose (**2**, TEgG), were isolated from the stem-bark of *Juglans mandshurica.* Two galloylglucoses showed cytotoxic effects on human promyelocytic leukemia HL-60 cells. In order to elucidate their mechanism of action, we have investigated the flow cytometric analysis after Annexin V-FITC and PI staining, caspase-3 activity, and internucleosomal DNA fragmentation in HL-60 cells. HL-60 cells treated with both compounds **1** and **2** at 150 and 100 μM, respectively, led to a morphological features of apoptosis, such as plasma membrane blebbing and cell shrinkage. TRgG (**1**) and TEgG (**2**) increased the percentage of $FITC^+$ and $FITC^+PI^+$ cell in flow cytometry after Annexin V-FITC and PI staining. The increase of apoptotic cells was preceded by the activation of caspase-3 reported to play a central role in apoptotic process and inducing internucleosomal DNA fragmentation. TEgG (**2**) showed to have stronger apoptosis inducing activity in HL-60 cell lines as compared with TRgG (**1**).

9) **Zhang Y., Akao T., Nakamura N., Hattori M., Yang X., Duan C., and Liu J.: Magnesium lithospermate B is excreted rapidly into rat bile mostly as methylated metabolites, which are potent antioxidants. *Drug Metabolism and Disposition,* 32: 752-757, 2004.**

Abstract: To elucidate the *in vivo* pharmacological activities of magnesium lithospermate B (MLB), an active constituent of Radix Salviae Miltiorrhizae, in the rat, its metabolic fate both *in vivo* and *in vitro* was investigated. High-performance liquid chromatography revealed that four major metabolites with lower polarity were excreted into bile after intravenous and oral administration of MLB. The metabolites present in combined samples of bile from rats after intravenous injection were isolated and purified by column chromatography and identified as four *meta-O*-methylated products, namely 3-monomethyl- (M1), 3,3'''-dimethyl- (M2), 3,3''-dimethyl-, and 3,3'',3''' -trimethyl-lithospermic acid B according to their spectroscopic characteristics (^1H, ^{13}C NMR, ^1H-^1H correlation spectroscopy, ^1H-detected multiple quantum coherence, and heteronuclear multiple bond coherence combined with positive ion fast atom bombardment-mass spectroscopy). After administration of MLB at an intravenous dose of 4 mg/kg or an oral

dose of 100 mg/kg, the total biliary recovery of the four metabolites after 30 h reached 95.5 ± 2.4% (with approximately 90% recovered within 2 h) or 5.5 ± 0.7%, respectively. The metabolic pathway was proposed to involve sequential formation of the four methylated metabolites. Incubation of MLB, M1, M2, or M4 in rat hepatic cytosol in the presence of S-adenosyl-L-methionine demonstrated the formation of all four metabolites, which indicated that the enzyme responsible for the biotransformation is catechol *O*-methyltransferase. MLB and its main metabolites M1 and M2 showed potent 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activities, the activity of M1 being stronger than those of caffeic acid (the monomer form of MLB) and α-tocopherol (a representative antioxidant) but weaker than that of MLB. The rapid and high biliary excretion levels of these metabolites suggested that they could undergo enterohepatic circulation in rats and that they might thereby be largely responsible for the pharmacological effects of MLB.

10) **Ma C., Nakamura N., Nawawi A., Hattori M., and Cai S.: A novel protoilludane sesquiterpene from the wood of *Xanthoceras sorbifolia*. *Chinese Chem. Lett.*, 15: 65-67, 2004.**

Abstract: A protoilludane sesquiterpene (named xanthocerapene) was isolated from the wood of *Xanthoceras sorbifolia* Bunge. Its structure, including the relative configuration was established by spectroscopic and chemical methods.

11) **Basu N. K., Kubota S., Meselhy M. R., Ciotti M., Chowdhury B., Hattori M., and Owens I. S.: Gastrointestinally distributed UDP-glucuronosyltransferase 1A10, which metabolizes estrogens and nonsteroidal anti-inflammatory drugs, depends upon phosphorylation. *J. Biol. Chem.*, 270: 28320-28329, 2004.**

Abstract: Among gastrointestinal distributed isozymes encoded at the UGT1 locus, UDP-glucuronosyltransferase 1A10 (UGT1A10) metabolizes a number of important chemicals. Similar to broad conversion of phytoestrogens (Base, N. K., Ciotti, M., Hwang, M. S., Kole, L., Mitra, P. S., Cho, J. W., and Owens, I. S. (2004) *J. Biol. Chem.* 279, 1429-1441), UGT1A10 metabolized estrogens and their derivatives, whereas UGT1A1, -1A3, -1A7, and -1A8 differentially exhibited reduced activity toward the same. UGT1A10 compared with UGT1A7, -1A8, and -1A3 generally exhibited high activity toward acidic nonsteroidal ant-inflammatory drugs and natural benzaldehyde derivatives, while UGT1A3, metabolized most efficiently aromatic transcinnamic acids known to be generated from flavonoid glycosides by microflora in the lower gastrointestinal tract. Finally UGT1A10, -1A7, -1A8, and -1A3 converted plant-based salicylic acids; methylsalicylic acid was transformed at high levels, and acetylsalicylic (aspirin) and salicylic acid were transformed at moderate to low levels. Atypically UGT1A10 transformed estrogens between pH 6 and 8 but acidic structures preferentially at pH 6.4. Furthermore evidence indicates UGT1A10 expressed in COS-1 cell depends upon phosphorylation; UGT1A10 *versus* its single, double, and triple mutants at three predicted protein kinase C phosphorylation sites incorporated [^{33}P]-orthophosphate and showed a progressive decrease with no detectable label or activity for the triple T73A/T202A/S432G-1A10 mutant. Single and double mutants revealed either null/full activity or null/additive activity, respectively. Additionally UGT1A10-expressing cultures glucuronidated 17β- [^{14}C] estradiol, whereas cultures containing null mutants at protein kinase C sites showed no estrogen conversion. Importantly UGT1A10 in cells supported 10-fold higher glucuronidation of 17β-estradiol than UGT1A1. In summary, our results suggest gastrointestinally distributed UGT1A10 is important for detoxifying estrogens/phytoestrogens and aromatic acids with complementary activity by UGT1A7, -1A8, -1A3, and/or -1A1 evidently dependent upon phosphorylation.

12) **Ahn E. M., Nakamura N., Fushimi H., Komatsu K., Batkhuu J., and Hattori M.: Constituents of the seeds of *Glycyrrhiza uralensis*. *Natural Medicines*, 58: 311, 2004.**

13) **Yokozawa T., Satoh A., and Cho E.J.: Ginsenoside-Rd attenuates oxidative damage related to aging in senescence-accelerated mice. *J. Pharm. Pharmacol.*, 56: 107-113, 2004.**

Abstract: Among the various theories of the aging process, the free radical theory, which proposes that deleterious actions of free radicals are responsible for the functional deterioration associated with aging, has received widespread attention. The theory suggests that enhancement of the antioxidative defense system to attenuate free radical-induced damage will counteract the aging process. We used senescence-accelerated mice (SAM) to investigate the relationship between aging and the antioxidative defense system and evaluated the effects of ginsenoside-Rd, the saponin from ginseng, by measuring antioxidative defense system parameters, including the glutathione (GSH)/glutathione disulfide (GSSG) redox status, antioxidative enzyme activities and level of lipid peroxidation. SAM at 11 months of age (old SAM) showed a significantly lower hepatic GSH/GSSG ratio, due to decreased GSH and increased GSSG levels, than SAM at 5 weeks of age (young SAM). However, the administration of ginsenoside-Rd at a dose of 1 or 5 mg/kg body weight/day for 30 days to 10-month-old SAM significantly increased GSH, but decreased GSSG, resulting in elevation of the GSH/GSSG ratio. In addition, ginsenoside-Rd increased the activities of glutathione peroxidase (GSH-Px) and glutathione reductase that were both significantly lower in old than young SAM. This suggests that ginsenoside-Rd could play a crucial role in enhancing the defense system through regulation of the GSH/GSSG redox status. Moreover, decreases in the superoxide dismutase (SOD) and catalase activities in old SAM compared with young SAM were also revealed, indicating that the aging process resulted in suppression of the antioxidative defense system. However, ginsenoside-Rd did not affect SOD and catalase activities. As catalase is localized in peroxisome granules and GSH-Px is present in the cytoplasm and mitochondrial matrix, the site of ginsenoside-Rd action may be the cytoplasm and mitochondrial matrix. Furthermore, the serum and liver malondialdehyde levels, indicators of lipid peroxidation, were elevated with aging, while ginsenoside-Rd inhibited lipid peroxidation. The present study indicates that the aging process leads to suppression of the antioxidative defense system and accumulation of lipid peroxidation products, while ginsenoside-Rd attenuates the oxidative damage, which may be responsible for the ntervention of GSH/GSSG redox status.

14) **Yokozawa T., Rhyu D.Y., and Cho E.J.: (-)-Epicatechin 3-*O*-gallate ameliorates the damages related to peroxynitrite production by mechanisms distinct from those of other free radical inhibitors. *J. Pharm. Pharmacol.*, 56: 231-239, 2004.**

Abstract: This study was carried out to elucidate whether the protective activity of (-)-epicatechin 3-*O*-gallate (ECg) against excessive peroxynitrite ($ONOO^-$) production, is distinct from the activities of several well-known free radical inhibitors, the $ONOO^-$ inhibitors ebselen and uric acid, the superoxide anion (O_2^-) scavenger copper zinc superoxide dismutase (CuZnSOD) and the selective inducible nitric oxide synthase inhibitor L-N^6-(1-iminoethyl)lysine hydrochloride (L-NIL). To generate $ONOO^-$, male Wistar rats (n=6/group) were subjected to ischemia-reperfusion process together with lipopolysaccharide (LPS) injection. Although ECg did not scavenge the $ONOO^-$ precursors nitric oxide (NO) and O_2^-, it reduced the 3-nitrotyrosine level, a property similar to that of uric acid, but distinct from L-NIL. In addition, the elevation in myeloperoxidase activity was reversed by the administration of ECg, uric acid and SOD, but not by that of L-NIL. Furthermore, ECg was the more potent scavenger of the $ONOO^-$ decomposition product the hydroxyl radical (·OH) than any other free radical inhibitor tested. The LPS plus ischemia-reperfusion process resulted in renal dysfunction, estimated by measuring the parameters of renal functions of serum urea nitrogen and creatinine levels. However, administration of ECg ameliorated renal dysfunction more than that of the other free radical inhibitors. Moreover, ECg reduced the excessive uric acid level, while the others did not, suggesting a property of ECg distinct from the others. Furthermore, proteinuria, which was demonstrated by the low- and high-molecular weight (LMW and HMW) protein bands of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern, caused by LPS plus ischemia-reperfusion was attenuated by administration of ECg and L-NIL, after which the HMW band intensities decreased and LMW protein bands were absent Please check. This study indicates that,

in an *in vivo* model of $ONOO^-$ generation, ECg, L-NIL and uric acid exert stronger protective activity against $ONOO^-$-induced oxidative damage than SOD and ebselen, and that the mechanism whereby ECg protects against $ONOO^-$ is distinct from that of L-NIL or uric acid.

15) **Yokozawa T., Ishida A., Kashiwada Y., Cho E.J., Kim H.Y., and Ikeshiro Y.: Coptidis Rhizoma: protective effects against peroxynitrite-induced oxidative damage and elucidation of its active components. *J. Pharm. Pharmacol.*, 56: 547-556, 2004.**

Abstract: The aim of this study was to investigate the protective effects of Coptidis Rhizoma against peroxynitrite ($ONOO^-$)-induced oxidative damage and elucidate the active components of this preparation. In an *in vitro* system, Coptidis Rhizoma extract scavenged $ONOO^-$ and its precursors, nitric oxide (NO) and superoxide anion (O_2^-), and this scavenging activity was more marked for $ONOO^-$ than its precursors. In addition, against 3-morpholinosydnonimine-induced cellular damage, this extract significantly reduced cellular $ONOO^-$ formation and increased cell viability. In an *in vivo* lipopolysaccharide plus ischemia-reperfusion system that generates $ONOO^-$, the administration of Coptidis Rhizoma extract at 50 and 100 mg/kg body weight/day for 30 days exerted greater inhibition of $ONOO^-$ than NO and O_2^-, suggesting that it acts as a direct scavenger of $ONOO^-$, rather than as a scavenger of its precursors. Moreover, the suppression of the activities of the antioxidative enzymes superoxide dismutase, catalase and glutathione peroxidase was significantly attenuated by the administration of Coptidis Rhizoma extract. Furthermore, the extract ameliorated renal dysfunction judged by decreasing serum urea nitrogen and creatinine levels. To elucidate the active components of Coptidis Rhizoma extract, we evaluated and compared the effects of the phenol plus alkaloid and alkaloid fractions on $ONOO^-$-induced damage. We found that the alkaloid fraction consisting of berberine, palmatine and coptisine was the most effective at protecting against $ONOO^-$. We also confirmed that berberine (10 and 20 mg/kg body weight/day for 10 days), the main and most active alkaloid in Coptidis Rhizoma extract, was also protective, exerting NO-, O_2^-- and $ONOO^-$-scavenging activities. This study suggests that Coptidis Rhizoma could protect against $ONOO^-$-induced oxidative damage and that this effect is mainly attributable to the constituent alkaloids, especially berberine. This study is the first to demonstrate an antioxidative effect of alkaloids, including berberine, against $ONOO^-$-induced damage.

16) **Satoh A., Yokozawa T., Cho E.J., Okamoto T., and Sei Y.: Antioxidative effects related to the potential anti-aging properties of the Chinese prescription Kangen-karyu and Carthami Flos in senescence-accelerated mice. *Arch. Gerontol. Geriatr.*, 39: 69-82, 2004.**

Abstract: The popular oxidative stress theory predicts that enhancement of the antioxidative defense system to attenuate free radical-induced damage counteracts the aging process. We used senescence-accelerated mice (SAM) because SAM has been shown to suppress the antioxidative defense system and mitochondrial dysfunction induced by oxidative stress. We investigated the antioxidative effects of the Chinese prescription Kangen-karyu and its crude drug component Carthami Flos. The administration of Kangen-karyu extract at 100 mg/kg body weight/day for 10 weeks inhibited generation of nitric oxide, superoxide and the hydroxyl radical (·OH), while Carthami Flos extract showed only ·OH-scavenging activity. Diet supplemented with Kangen-karyu and Carthami Flos extracts enhanced the activities of the antioxidative enzymes superoxide dismutase in hepatic tissue and glutathione peroxidase in renal tissue, and reduced the hepatic lipid peroxidation level which increased with aging, indicating the protective action against oxidative stress by enhancing the antioxidative status. Hepatic and renal dysfunction with aging was also ameliorated by the administration of Kangen-karyu and Carthami Flos supplements. Furthermore, the observed antioxidative properties of the Chinese prescription Kangen-karyu were more evident than those of Carthami Flos. These findings suggest that the protective activity of Kangen-karyu against the oxidative tissue damages during aging may be due partly to synergistic and/or additive effects of its crude preparation. The present study strongly indicates that Kangen-karyu counteract the oxidative stress and ameliorating tissue damage possibly associated with aging in SAM.

17) **Yokozawa T., and Nakagawa T.: Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. *Food Chem. Toxicol.*, 42: 975-981, 2004.**

Abstract: Luobuma tea, prepared from the leaves of *Apocynum venetum* L., is a popular beverage in China. In this study, the activity of Luobuma leaf extract and its components against the formation of advanced glycation endproducts (AGEs), which are largely involved in the pathogenesis of diabetic vascular complications, was examined using the *in vitro* glycation reaction. Strong inhibitory activity against the formation of AGEs was shown by Luobuma aqueous extract. Following further fractionation of this extract, seven polyphenolic compounds, i.e. (±)-gallocatechin, (-)-epigallocatechin, (±)-catechin, (-)-epicatechin, epicatechin-(4β-8)-gallocatechin, epigallocatechin-(4β-8)-epicatechin and procyanidin B-2, were isolated by Sephadex LH-20 column chromatography. These purified compounds also exerted inhibitory activities that were more potent than the positive control, aminoguanidine. Our findings may help to explain the beneficial effects of this plant against atherosclerosis.

18) **Nakagawa T., Yokozawa T., Sano M., Takeuchi S., Kim M., and Minamoto S.: Activity of (-)-Epigallocatechin 3-*O*-gallate against oxidative stress in rats with adenine-induced renal failure. *J. Agric. Food Chem.*, 52: 2103-2107, 2004.**

Abstract: Methylguanidine (MG) is widely recognized as a strong uremic toxin. The hydroxyl radical (·OH) specifically plays an important role in the pathway of MG production from creatinine (Cr). In this study, we investigated whether oral administration of (-)-epigallocatechin 3-*O*-gallate (EGCg) suppresses MG production in rats with chronic renal failure after intraperitoneal Cr injection. MG production from Cr was significantly increased in rats with adenine-induced renal failure, which was more vulnerable to oxidative stress, compared with that in normal rats. However, oral administration of EGCg 30 min before and after Cr injection effectively inhibited MG production. Our findings suggest that EGCg, an excellent antioxidant from green tea, exerts protective activity in rats with chronic renal failure, resulting in suppression of Cr oxidation influenced by ·OH.

19) **Satoh A., Yokozawa T., Tanaka T., Okamoto T., and Sei Y.: The antioxidative activity of Kangen-karyu extract delays senescence of human lung fibroblasts. *J. Trad. Med.*, 21: 87-93, 2004.**

Abstract: Replicative senescence (RS) of human diploid fibroblasts (HDFs) has become a classical model of aging and HDFs, such as WI-38 cells, display increased cellular oxidant production associated with RS. Several phenomena associated with RS are also observed in stress-induced replicative Should this be premature, as stated below? senescence (SIPS). In particular, SIPS of WI-38 cells caused by hydrogen peroxide (H_2O_2) is a useful and reasonable cellular aging model for evaluating the effects of potential anti-aging agent against oxidative stress. We used this well-established model to evaluate the anti-aging effect of Kangen-karyu, focusing on its antioxidant activity. Treatment of WI-38 cells undergoing SIPS caused by H_2O_2 with Kangen-karyu extract significantly reduced reactive oxygen species (ROS) generation and lipid peroxidation levels. In addition, the intracellular GSH levels, reflecting cellular ROS generation, were reduced by treatment with Kangen-karyu extract. These results suggest that Kangen-karyu attenuated the age-associated increase of cellular oxidative damage. Moreover, Kangen-karyu extract normalized the G_0/G_1 phase arrest and reversed the diminished cell viability resulting from exposure to H_2O_2. Furthermore, the extract prolonged the lifespan of WI-38 cells undergoing SIPS. This study suggests that Kangen-karyu may delay the aging process in cells undergoing SIPS by attenuating oxidative damage.

20) **Yokozawa T., Yamabe N., Cho E.J., Nakagawa T., and Oowada S.: A Study on the effects to diabetic nephropathy of Hachimi-jio-gan in rats. *Nephron Exp. Nephrol.*, 97: e38-e48, 2004.**

Abstract: To investigate the effects of Hachimi-jio-gan on diabetic nephropathy, we employed an animal model, rats subjected to sub-total nephrectomy followed by streptozotocin injection, and administered Hachimi-jio-gan orally at

a dose of 50, 100 or 200 mg/kg body weight/day for 15 weeks. The administration of Hachimi-jio-gan reduced dose-dependently the elevated blood glucose and urinary protein excretion levels in rats with diabetic nephropathy over the experimental period, whereas it increased creatinine clearance significantly, suggesting that Hachimi-jio-gan would prevent or delay the progression of diabetic nephropathy. In addition, the serum glycosylated protein and urea nitrogen levels were markedly elevated in rats with diabetic nephropathy compared with normal rats, and were significantly reduced by the administration of Hachimi-jio-gan, whereas Hachimi-jio-gan reversed the decrease in the serum albumin level. The serum triglyceride and total cholesterol concentrations were reduced by Hachimi-jio-gan, implying that Hachimi-jio-gan would improve the metabolic disorder of lipids caused by diabetic nephropathy. Moreover, Hachimi-jio-gan inhibited lipid peroxidation in the serum and kidney, which suggests that Hachimi-jio-gan would ameliorate oxidative stress associated with diabetic nephropathy. Furthermore, the disorders of the glucose-dependent metabolic pathway due to this pathological condition were also normalized by the administration of Hachimi-jio-gan through decreases in advanced glycation end-product formation and sorbitol levels in the kidney. Hachimi-jio-gan protected against the development of renal lesions, glomerular sclerosis, tubulointerstitial lesions, mesangial matrix expansion and arteriolar sclerosis, estimated by histopathological evaluation and scoring. This study suggests that Hachimi-jio-gan may be a novel therapeutic approach to improving diabetic nephropathy.

21) **Kitani K., Yokozawa T., and Osawa T.: Interventions in aging and age-associated pathologies by means of nutritional approaches.** ***Ann. N.Y. Acad. Sci.,*** **1019: 424-426, 2004.**

Abstract: So-called antioxidant strategies have not been shown convincingly to be effective in increasing life spans of animals. Thus, the general consensus of experimental gerontology in the last century was that the only reproducible means of prolonging survivals of animals is the calorie restriction paradigm. As a challenge against this dogma, we attempted to examine the effect of two potent antioxidants, one tetrahydrocurcumin (a biotransformed metabolite of curcumin contained in turmeric of Indian curry) and the other green tea polyphenols.

22) **Cho E.J., Yokozawa T., Kim H.Y., Shibahara N., and Park J.C.:** ***Rosa rugosa*** **attenuates diabetic oxidative stress in rats with streptozotocin-induced diabetes.** ***Am. J. Chin. Med.,*** **32: 487-496, 2004.**

Abstract: The effects of Rosa rugosa on diabetic oxidative stress were investigated using rats with streptozotocin (STZ)-induced diabetes. The diabetic rats showed less body weight gain and heavier kidney and liver weights than normal rats, while the oral administration of Rosa rugosa at a dose of 100 or 200 mg/kg body weight/day for 20 days attenuated the physiological changes induced by diabetes. In addition, giving Rosa rugosa to diabetic rats resulted in significant and dose-dependent decreases in the serum glucose and glycosylated protein levels, implying that Rosa rugosa improves the abnormal glucose metabolism that leads to oxidative stress. Moreover, the rats with STZ-induced diabetes had high serum levels of superoxide and nitrite/nitrate. However, the administration of Rosa rugosa dose-dependently reduced the overproduction of radicals associated with diabetes, suggesting Rosa rugosa is a radical scavenger that would play a crucial role in protecting against diabetic oxidative stress. Furthermore, Rosa rugosa reduced significantly and dose-dependently thiobarbituric acid-reactive substance levels of serum and hepatic and renal mitochondria, implying that Rosa rugosa would alleviate the oxidative stress associated with diabetes by inhibiting lipid peroxidation. This study provides scientific evidence that Rosa rugosa has potential as a treatment for diabetes through attenuating oxidative stress induced by the diabetic condition.

23) **Jung H.A., Chung H.Y., Yokozawa T., Kim Y.C., Hyun S.K., and Choi J.S.: Alaternin and emodin with hydroxyl radical inhibitory and/or scavenging activities and hepatoprotective activity on tacrine-induced cytotoxicity in HepG2 cells.** ***Arch. Pharm. Res.,*** **27: 947-953, 2004.**

Abstract: The antioxidative and hepatoprotective potentials of two anthraquinones, alaternin (2-hydroxyemodin)

and emodin, to scavenge and/or inhibit hydroxyl radicals generated by the Fenton reaction and to protect tacrine-induced cytotoxicity in human liver derived HepG2 cells were evaluated, respectively. The inhibitory activity on hydroxyl radical generated in a cell-free chemical system ($FeSO_4/H_2O_2$) was investigated by a fluorescence spectrophotometer using a highly fluorescent probe, 2',7'-dichlorofluorescein. The hydroxyl radical scavenging activity was determined by electron spin resonance spectroscopy using 5,5-dimethyl-1-pyrroline-N-oxide as hydroxyl radicals trapping agents. Tacrine-induced HepG2 cell toxicity was determined by a 3-[4,5-dimethylthiazole-2yl]-2,5-diphenyltertrazolium bromide assay. Although the scavenging activity of alaternin on hydroxyl radical was similar to that of emodin in dose-dependent patterns, the inhibitory activity exhibited by the former on hydroxyl radical generation was stronger than that of the latter, with IC_{50} values of 3.05 $\pm$ 0.26 μM and 13.29 $\pm$ 3.20 μM, respectively. In addition, the two anthraquinones, alaternin and emodin showed their hepatoprotective activities on tacrine-induced cytotoxicity, and the EC_{50} values were 4.02 μM and 2.37 μM, respectively. Silymarin, an antihepatotoxic agent used as a positive control exhibited the EC_{50} value of 2.00 μM. These results demonstrated that both alaternin and emodin had the simultaneous antioxidant and hepatoprotective activities.

24) **Kim H.Y., Yokozawa T., Nakagawa T., and Sasaki S.: Protective effect of γ-aminobutyric acid against glycerol-induced acute renal failure in rats. *Food Chem. Toxicol.*, 42: 2009-2014, 2004.**

Abstract: To investigate the effect of γ-aminobutyric acid (GABA) on acute renal failure, we used a rat model of acute tubular necrosis induced by glycerol. After deprivation of water for 6 h, the rats received an injection of 50% glycerol into the muscle of the rear limb at 10 ml/kg body weight. GABA was then administered orally to the rats (100 or 500 mg/kg body weight/day) once every 12 h for 3 days. The rats with acute renal failure showed arrested body weight gain and an increase of kidney weight, whereas oral administration of GABA attenuated the physiological changes induced by acute renal failure. However, GABA administration had no significant effect on increased urine volume. Oral administration of GABA at a dose of 100 or 500 mg/kg body weight/day for 3 days significantly improved the markedly elevated levels of blood urea nitrogen and creatinine and the reduced creatinine clearance related to progression of renal failure. Moreover, the rats with acute renal failure exhibited high levels of fractional excretion of sodium (FE_{Na}) due to alteration of tubule function following injection of glycerol. However, administration of GABA lowered the FE_{Na} levels dose-dependently. Furthermore, urine osmolarity was markedly reduced in control rats with acute renal failure as compared with normal rats, whereas it was significantly increased by administration of GABA at a dose of 500 mg/kg body weight/day. These results indicate that GABA has potential as a therapeutic agent against the renal damage involved in acute renal failure.

25) **Kim H.Y., Yokozawa T., Cho E.J., and Yamabe N.: Protective effects of the Chinese prescription Hachimi-jio-gan against diabetic oxidative stress. *J. Pharm. Pharmacol.*, 56: 1299-1305, 2004.**

Abstract: We used rats with streptozotocin (STZ)-induced diabetes to investigate the effects of Hachimi-jio-gan on diabetic oxidative stress. Oral administration of Hachimi-jio-gan, at a dose of 50, 100 or 200 mg/kg body weight/day, for 10 days to rats with STZ-induced diabetes resulted in significant dose-dependent decreases in serum levels of glucose and glycosylated protein, implying that Hachimi-jio-gan improves the abnormal glucose metabolism that leads to oxidative stress. Hachimi-jio-gan also showed a tendency to reduce the urine volume and significantly reduced the elevated urinary protein level. Moreover, rats with STZ-induced diabetes had high serum levels of superoxide and nitrite/nitrate. However, the administration of Hachimi-jio-gan dose-dependently reduced the overproduction of radicals associated with diabetes, suggesting the role of Hachimi-jio-gan as a radical scavenger that could protect against diabetic oxidative stress. Furthermore, thiobarbituric acid-reactive substance levels of serum, and hepatic and renal mitochondria were dose-dependently lower in the Hachimi-jio-gan-treated groups than in the

control diabetic group, which implies that Hachimi-jio-gan would alleviate the oxidative stress associated with diabetes through the inhibition of lipid peroxidation. These results indicate that Hachimi-jio-gan is a potential therapeutic agent that will reduce the damage caused by oxidative stress involved in diabetes.

26) **Yokoyama K., Shimada Y., Hori E., Nakagawa T., Takagi S., Sekiya N., Kouta K., Nishijo H., Yokozawa T., and Terasawa K.: Effects of Choto-san and hooks and stems of *Uncaria sinensis* on antioxidant enzyme activities in the gerbil brain after transient forebrain ischemia. *J. Ethnopharmacol.*, 95: 335-343, 2004.**

Abstract: Previously, we revealed that oral administrations of Choto-san, a Kampo formula, and the hooks and stems of *Uncaria sinensis* Haviland (Rubiaceae), a medicinal plant comprising Choto-san, enhanced superoxide anion and hydroxyl radical scavenging activities in the hippocampus, and prevented delayed neuronal death of pyramidal cells in the hippocampal CA1 region in a transient forebrain ischemia gerbil model. In the present study, for the purpose of clarifying whether the endogenous antioxidant enzymes contribute to these mechanisms, we investigated the effects of Choto-san extract (CSE) and *Uncaria sinensis* extract (USE) on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities in the brain by using the same experimental model. 1.0% CSE or 3.0% USE were dissolved in water and provided to gerbils ad libitum from 7 days prior to ischemia/reperfusion (i/rp). Seven days of continuous administrations of CSE or USE without i/rp procedure enhanced CAT activity but not SOD and GSH-Px activities in both the hippocampus and cortex. CSE elevated CAT activity in the hippocampus at 7 days and in the cortex at 3 h after i/rp. USE raised CAT activity in both the hippocampus and cortex at 3 h and 7 days after i/rp. These results suggest that one of the mechanisms of the protective effects of CSE and USE against transient brain ischemia-induced neuronal damage may be their enhancing effect on CAT activity in the brain.

27) **Cho E.J., Yokozawa T., Rhee S.H., and Park K.Y.: The role of Coptidis Rhizoma extract in a renal ischemia-reperfusion model. *Phytomedicine,* 11: 576-584, 2004.**

Abstract: The role of Coptidis Rhizoma extract in ischemia-reperfusion rats was examined. The blood levels of urea nitrogen and creatinine increased significantly more in rats subjected to 24-h reperfusion than those subjected to 6-h reperfusion following 1-h ischemia, indicating functional kidney damage was more severe after the longer reperfusion time. These parameters were reduced by oral administration of Coptidis Rhizoma extract. Greater activity was exerted in rats given the extract for 30 days than for 10 days prior to ischemia-reperfusion. In addition, the serum malondialdehyde level was lower, while the glutathione/glutathione disulfide ratio and the activities of the antioxidation enzymes, superoxide dismutase and catalase, were higher in rats given Coptidis Rhizoma extract orally for 30 consecutive days prior to 1-h ischemia and 24-h reperfusion in comparison with control rats given water, indicating that Coptidis Rhizoma has a protective action against the renal dysfunction caused by the ischemia and reperfusion process. Furthermore, renal DNA of rats given Coptidis Rhizoma extract orally showed a significantly lower DNA fragmentation rate, which was dose-dependent, implying that the extract afforded the kidneys protection against oxidative stress-mediated apoptosis during the process. Our results suggest that Coptidis Rhizoma has a protective effect against renal ischemia-reperfusion injury, in that tissue damage due to oxidative stress is reduced, thus ameliorating renal function impairment.

28) **Yokozawa T., Sekiya M., Cho E.J., Kurokawa M., and Shiraki K.: Effect of Wen-Pi-Tang extract on lung damage by influenza virus infection. *Phytomedicine,* 11: 625-632, 2004.**

Abstract: The effect of Wen-Pi-Tang extract on influenza virus infection in mice was investigated. The administration of Wen-Pi-Tang extract at a dose of 100 mg/kg body weight for 8 consecutive days to influenza virus-infected mice reversed the lack of body weight gain and prevented the increase in lung weight caused by the infection in

comparison with uninfected mice, while allopurinol, a xanthine oxidase (XOD) inhibitor, did not show these effects. The serum levels of uric acid and allantoin in influenza virus-infected mice were reduced by Wen-Pi-Tang extract administration. Moreover, Wen-Pi-Tang extract reduced the uric acid level more as the dose administered, 100, 200 and 400 mg/kg body weight, increased although it exerted lower activity than allopurinol. The XOD activity of the lungs was elevated by influenza virus infection, but Wen-Pi-Tang extract administration inhibited this activity, indicating prevention of lung damage by oxygen free radicals generated by XOD. After the administration of Wen-Pi-Tang extract to influenza virus-infected mice, the lung superoxide dismutase activity was not significantly different from that of uninfected mice, whereas lung catalase activity was lower in the former than the latter, but slightly higher than that of influenza virus-infected mice, suggesting that Wen-Pi-Tang extract may prevent the generation of highly toxic hydroxyl radicals in the lung. In addition, the administration of both Wen-Pi-Tang extract and allopurinol reduced the degree of lung consolidation caused by influenza virus infection. In particular, Wen-Pi-Tang extract reduced the consolidation score in a dose-dependent manner and more markedly than allopurinol did. This study suggests that Wen-Pi-Tang extract could improve pathological conditions of the lungs induced by influenza virus infection.

29) **Yokozawa T., Kim H.Y., and Yamabe N.: Amelioration of diabetic nephropathy by dried Rehmanniae Radix (Di Huang) extract. *Am. J. Chin. Med.*, 32: 829-839, 2004.**

Abstract: The effects of dried Rehmanniae Radix (Di Huang) extract were investigated using a diabetic nephropathy model: rats given streptozotocin after nephrectomy. The results showed that this crude drug reduced the magnitudes of the increases in glucose, urea nitrogen, 5-hydroxymethylfurfural and thiobarbituric acid-reactive substance levels, with the effects being most marked in the high blood glucose group. The renal histopathological lesions, which were conspicuous in rats not given dried Rehmanniae Radix extract, were ameliorated considerably in the high blood glucose group given this extract. It appears that dried Rehmanniae Radix extract may be useful as a therapeutic agent for inhibiting the progression of diabetic nephropathy. On the basis of these results, the possible mechanisms of action of this crude drug are discussed.

◇総　説　Review papers

1）Ma C. M., Nakamura N., and Hattori M.: Natural products and their derivatives, having anti-HIV-1 protease activity. *Current Topics in Medicinal Chemistry,* **3**: 77-99, 2003.

2）Rao T.P., Yokozawa T., and Juneja L.R.: Preventive effects of green tea polyphenols against oxidative stress of renal disease. *International Journal of Tea Science,* **3**: 239-250, 2004.

3）横澤隆子：効能最前線「茶と腎臓」. 茶, 57(4)：2-3, 2004.

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2）横澤隆子, 源　伸介, 金　武祚：糖尿病性腎症におけるエピガロカテキンガレートの有用性. 日本農芸化学会2004年会, 2004, 3, 28-31, 広島.

3）安　恩美, 中村憲夫, 赤尾光昭, 服部征雄, 西原　力：マメ科生薬千斤抜に含まれる1,1-(dimethyl)-genistein のエストロゲン作用について. 日本薬学会第124年会, 2004, 3, 29-31, 大阪.

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18) 中川孝子, 横澤隆子, 後藤博三, 柴原直利, 嶋田 豊 : 自然発症糖尿病ラット (WBN/Kob) における八味地黄丸の腎保護作用. 第21回和漢医薬学会大会, 2004, 8, 21-22, 富山.
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27) 金　英愛, 横澤隆子, 佐藤亜希子：高濃度グルコース誘導酸化ストレスに及ぼす冠元顆粒の効果－腎上皮細胞での検討－. 第16回腎とフリーラジカル研究会, 2004, 9, 11, 京都.

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34) 服部征雄, 東田千尋, 小松かつ子, 土屋真澄, 中村憲夫：コーヒー豆のトリゴネリンと脳神経細胞. 第7回くすりと食物シンポジウム「シーズとニーズ」, 2004, 11, 19, 東京.

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◇その他　Others

1) 服部征雄：植物資源の新機能の探索. *Fods & Food Ingredients Journal of Japan,* **209**, 1-2, 2004.

2) 服部征雄：クシャラスートラ結紮系について. アーユルヴェーダ通信シャーンティ・マールガ, **15**, 36-39, 2004.

3) 服部征雄：難波恒雄先生の御逝去を悼む. *Natural Medicine,* **58**, i-ii, 2004.

4) 服部征雄, 井出範男：霊芝清談. れいし倶楽部 **1**, 1-8, 2004.

5) 服部征雄：ヒト腸内細菌による和漢薬成分の活性化. 漢方薬・生薬研修会, 東京, 2004. 1. 25.

6) 服部征雄：Metabolic activation of natural medicines by human intestinal bacteria. Pocala Nepal, 2004. 3. 17.

7) 服部征雄：Development of anti-viral agents from natural resources. 瀋陽薬科大学, 瀋陽, 中国, 2004. 3. 24.

8) 服部征雄：Development of anti-viral agents from natural resources. 遼寧中医学院, 瀋陽, 中国, 2004. 3. 25.

9) 服部征雄：Recent progress in the researches on metabolic activation by human intestinal bacteria. 大連大学, 大連, 中国, 2004. 3. 26.

10) 服部征雄：Anti-HIV agents from natural resources. 嘉南薬理科技大学薬学院, 台南市, 台湾, 2004. 4. 30.

11) 服部征雄：ヒト腸内細菌による和漢薬成分の活性化. 漢方薬・生薬研修会, 東京, 2004. 5. 16.

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13) 横澤隆子 : 糖尿病性腎症における漢方方剤の有用性. 和漢薬 No. 616, pp.2-3, 2004.
14) 中村憲夫 : 海洋性菌類をターゲットとした防御物質で海草は身を守る？ ファルマシア（トピックス欄）Vol. 40 No. 5（トピックス欄）2004.

◇共同研究　Co-oparative researches

国内

1）下遠野邦忠（京都大学ウイルス研究所), 下遠野久美子（共立薬科大学), 垣内信子（金沢大学薬学部) :「C 型肝炎 RNA ポリメラーゼ阻害活性を指標とした抗HCV剤の開発研究」

海外

1）Ida S. Owens (National Institute of Health, USA) :「エストロゲンおよび非ステロイド抗炎症薬の代謝研究」
2）鄭　海泳（釜山大学薬学部), 青柳一正（筑波技術短期大学), 柏田良樹（新潟薬科大学), 金　賢栄（ソウル大学薬学部) :「抗酸化研究」
3）Byung Pal Yu (The University of Texas), 趙　恩珠（釜山大学生活科学部) :「老化研究」

◇研究費取得状況　Acquisition of research funds

1）日本科学協会 平成16年度笹川科学研究助成（条美智子 代表）55万円
2）「富山産ヤマブシタケに含まれる PEP 阻害物質の分離同定」富山県受託研究（服部征雄 代表）20万円.
3）「漢方薬の効果を遺伝子発現レベルで評価する系の開発」経済産業省 地域新生コンソーシアム研究開発事業（服部征雄 代表）3800万円.
4）「糖尿病性腎症に対する漢方方剤治療の基礎的検討」つくし奨学・研究基金（横澤隆子 代表）120万円.
5）「C型肝炎ウイルスレプリカーゼ及びプロテアーゼをターゲットとした抗 HCV 剤開発の試み」ウイルス肝炎研究財団（中村憲夫 代表）100万円.
6）「ヒト腸内細菌により代謝活性化される植物エストロゲン様作用物質に関する研究」富山第一銀行奨学財団（中村憲夫 代表）40万円.

◇受賞　Award s

1）服部征雄 : 5th International Congress on Natural Medicine, 大会賞, 中国瀋陽.
2）高　江静 : 5th International Congress on Natural Medicine, 青年賞, 中国瀋陽.

◇学位（修士、博士）取得者　Academic degrees and theses

薬学士：
近藤 直子：乳酸菌によるリグナン類の変換反応の検討
和田 江美子：冠元顆粒は糖尿病に有効か？

修士（薬学）：
平川 暁子：台湾産樟芝菌糸体の成分およびその生理活性
山辺 典子：八味地黄丸の糖尿病性腎症治療薬への可能性

博士（薬学）：
高　江 静：霊芝苦味成分に関する研究
安　恩 美：千斤抜のエストロゲン及び抗エストロゲン活性に関する研究

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大学院後期1年：山辺 典子, 鄭　美和, 佐々木 澄代, 藤井　創, 姜　奇成 (10月入学), Ali Mahmoud (10月入学)

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機関研究員：左　風 (博士)

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事務補佐員：黒岩 純子