# 民族薬物研究センター　Research Center for Ethnomedicine

（平成17年 10 月薬効解析センターより改組）

## 薬効解析部　Division of Biofunctional Evaluation

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### ◇研究目的

民族薬物研究センター薬効解析部は、民族薬物の機能解析に関する研究を推進するために設置された。主な研究内容は以下のとおりであるが、わが国では高齢化や生活習慣病の増加によって疾病構造に大きな変化をきたし、漢方医学の役割や重要性が再認識されてきている。そのためにも基礎的研究を通じて各種疾患、特に慢性疾患に対する漢方薬や個々の生薬、成分の有効性を科学的に実証することが非常に重要な課題であり、これらを中心とした研究を推進している。

### ◇研究概要

1) 腎疾患における病態の解明と腎臓病治療薬の開発
2) 糖尿病性腎症における漢方方剤のアプローチと分子生物学的解明
3) 加齢（老化）及び加齢関連疾患（いわゆる老年病）の発症機序に対するアンチエイジング産業への開拓
4) 生活習慣病分子標的因子の解明と治療薬の創出
5) 神経回路網形成および神経変性疾患（認知症、脊髄損傷、注意欠陥多動性障害）に関する基礎的研究と、それらに有効な伝統薬物の研究

## ◇著書

1) 横澤隆子: 「血管力をつければ病気は治る」(ダイジェスト版), 1-32, リヨン社, 東京, 2005.

2) 小松かつ子, 東田千尋: 民族薬物の謎: 世界の人参を追って.「薬用植物・生薬開発の新展開 New Development of Medical Plant」, 302-324, シーエムシー, 東京, 2005.

## ◇原著論文

1) **Yokozawa T., Cho E.J., Rhyu D.Y., Shibahara N., and Aoyagi K.: Glycyrrhizae Radix Attenuates Peroxynitrite-induced Renal Oxidative Damage through Inhibition of Protein Nitration. *Free Radic. Res.*, 39: 203-211, 2005.**

**Abstract:** We investigated the protective effects of Glycyrrhizae Radix extract against peroxynitrite ($ONOO^-$)-induced oxidative stress under *in vivo* as well as *in vitro*. The extract showed strong $ONOO^-$ and nitric oxide (NO) scavenging effects under *in vitro* system, in particular higher activity against $ONOO^-$. Furthermore, elevations of plasma 3-nitrotyrosine levels, indicative of *in vivo* $ONOO^-$ generation and NO production, were shown using a rat *in vivo* $ONOO^-$-generation model of lipopolysaccharide injection plus ischemia-reperfusion. The administration of Glycyrrhizae Radix extract at doses of 30 and 60 mg/kg body weight/day for 30 days significantly reduced the concentrations of 3-nitrotyrosine and NO and decreased inducible NO synthase activity. In addition, the nitrated tyrosine protein level and myeloperoxidase activity in the kidney were significantly lower in rats given Glycyrrhizae Radix extract than in control rats. However, the administration of Glycyrrhizae Radix extract did not result in either significant elevation of glutathione levels or reduction of lipid peroxidation in renal mitochondria. Moreover, the *in vivo* $ONOO^-$ generation system resulted in renal functional impairment, reflected by increased plasma levels of urea nitrogen and creatinine, whereas the administration of Glycyrrhizae Radix extract reduced these levels significantly, implying that the renal dysfunction induced by $ONOO^-$ was ameliorated. The present study suggests that Glycyrrhizae Radix extract could protect the kidneys against $ONOO^-$ through scavenging $ONOO^-$ and/or its precursor NO, inhibiting protein nitration and improving renal dysfunction caused by $ONOO^-$.

2) **Yokozawa T., Satoh A., Cho E.J., Kashiwada Y., and Ikeshiro Y.: Protective role of Coptidis Rhizoma alkaloids against peroxynitrite-induced damages to renal tubular epithelial cells. *J. Pharm. Pharmacol.*, 57: 367-374, 2005.**

**Abstract:** A study was conducted to elucidate and compare the protective activities of alkaloids from Coptidis Rhizoma (berberine, coptisine, palmatine, epiberberine, jatrorhizine, groenlandicine and magnoflorine) using a LLC-$PK_1$ cell under peroxynitrite ($ONOO^-$) generation model. Treatment with 3-morpholinosydnonimine (SIN-1) led to an increase of cellular $ONOO^-$ generation in comparison with non-treated cells. However, Coptidis Rhizoma extract and its alkaloids, except for berberine, reduced the cellular $ONOO^-$ level. In addition, DNA fragmentation induced by SIN-1 was significantly decreased by the extract, and also by coptisine, epiberberine, jatrorhizine, groenlandicine and magnoflorine. Moreover, treatment with berberine, coptisine, palmatine and epiberberine exerted a protective effect against $G_0/G_1$ phase arrest of cell cycle induced by SIN-1. The increase in cellular $ONOO^-$ generation, DNA damage and disturbance of the cell cycle by SIN-1 resulted in a decrease of cell viability. However, Coptidis Rhizoma extract, epiberberine, jatrorhizine, groenlandicine and magnoflorine significantly increased cell viability even at a concentration as low as 10 μg/ml. The present findings demonstrate that Coptidis Rhizoma extract and its alkaloids can ameliorate the cell damage associated with $ONOO^-$ generation in renal tubular LLC-$PK_1$ cells, and that the various alkaloids have distinctive mechanisms of action such as $ONOO^-$ scavenging, protection from DNA damage and control of the cell cycle. Furthermore, the data suggest that among the Coptidis Rhizoma alkaloids, coptisine is the most effective for protection against

SIN-1-induced cellular injury in terms of its potency and content

3) **Kim Y.J., Yokozawa T., and Chung H.Y.: Effects of energy restriction and fish oil supplementation on renal guanidino levels and antioxidant defences in aged lupus-prone B/W mice. *Br. J. Nutr.*, 93: 835-844, 2005.**

**Abstract:** Energy restriction (ER) and dietary fish oil (FO) are known to reduce the severity of glomerulonephritis and increase the lifespan of lupus-prone (NZB x NZW) $F_1$ (B/W) mice. In the present study, mice were fed either ad libitum or energy-restricted (a 40 % lower energy intake than the diet ad libitum), semi-purified diets containing 5 % maize oil or 5 % fish oil supplementation. To estimate the renal damage associated with oxidative stress, the total amounts of reactive oxygen species (ROS), cyclooxygenase-derived ROS and levels of guanidino compounds were measured. Additionally, we assessed the putative action of ER and FO on several key antioxidant enzymes measured in the kidney post-mitochondrial fraction. Results showed that the age-related increase in creatinine level was significantly reduced by ER and FO in old mice. In contrast, arginine and guanidino acetic acid levels showed a decrease with age but were increased by ER and FO. The GSH:GSSG ratio showed a significant decrease with age, whereas ER and FO feeding prevented the decrease. The age-related decrease in antioxidant scavenging superoxide dismutase, catalase and glutathione peroxidase activities were all reversed by ER and FO. The moderately decreased glutathione reductase and glutathione-S-transferase activities with age were significantly increased by ER and FO. Furthermore, the increased total ROS and cyclooxygenase-derived ROS levels were effectively reduced by ER and FO. In conclusion, our data strongly indicate that ER and FO maintain antioxidant status and GSH:GSSG ratio, thereby protecting against renal deterioration from oxidative insults during ageing.

4) **Yokozawa T., Nakagawa T., Oya T., Okubo T., Shibahara N., and Juneja L.R.: Green tea polyphenol and dietary fiber protect against kidney damage in rats with diabetic nephropathy. *J. Pharm. Pharmacol.*, 57: 773-780, 2005.**

**Abstract:** In this study, we examined the effect of green tea polyphenols (GTP) and partially hydrolyzed guar gum (PHGG) as dietary fiber on diabetic nephropathy, using rats that had been subjected to subtotal nephrectomy and injection of streptozotocin. The subtotally nephrectomized rats were subjected to resection of three-fourths of the kidney. Rats with diabetic nephropathy were divided into four groups: untreated controls, and animals that had received GTP (100 mg/kg body weight/day), PHGG (100 mg/kg body weight/day) and GTP plus PHGG (50 mg/kg body weight/day plus 50 mg/kg body weight/day). After 50 days of administration, attenuation of urinary protein excretion and the morphological changes peculiar to diabetic nephropathy were observed in all three treated groups. Furthermore, the group treated with GTP plus PHGG showed an improvement of kidney weight and serum levels of urea nitrogen, creatinine and creatinine clearance. Hyperglycemia, as assessed in terms of blood glucose and glycosylated protein levels, was also improved by administration of GTP plus PHGG. On the other hand, GTP administration increased the activity of superoxide dismutase in the kidney to a significant extent. A significant reduction of the total cholesterol concentration was also observed in the PHGG-treated group. These results suggest that GTP and PHGG could be beneficial as additional therapy in the management of diabetic nephropathy.

5) **Piao X.L., Kim H.Y., Yokozawa T., Lee Y.A., Piao X.S., and Cho E.J.: Protective Effects of Broccoli (*Brassica oleracea*) and Its Active Components against Radical-Induced Oxidative Damage. *J. Nutr. Sci. Vitaminol.*, 51: 142-147, 2005.**

**Abstract:** The radical scavenging effect and protective potential from oxidative damage by radical generator, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), in renal epithelial LLC-$PK_1$ cell of broccoli (Brassica oleracea) were investigated and identified the active components under the bioassay-linked fractionation method. The MeOH extract, and fractions of $CH_2Cl_2$, BuOH and $H_2O$ from

broccoli showed the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect in a dose-dependent manner. In addition, they exerted the protective effect against LLC-$PK_1$ cellular damage induced by AAPH dose-dependently. In particular, the BuOH fraction was evaluated as the most active fraction, indicating that the BuOH fraction contains the active components with antioxidative capacity. Employing a bioassay-linked fractionation method, the active principles were isolated and characterized as 1,2-disinapoylgentiobiose and 1-sinapoyl-2-feruloylgentiobiose from the BuOH fraction. These two compounds from broccoli displayed potent antioxidant effects against the DPPH radical, showing the $IC_{50}$ values of 5.18 and 7.52 μg/mL, respectively. Moreover, the compounds significantly and dose-dependently recovered cell viability lowered by AAPH treatment, suggesting the protective roles from cellular oxidative damage. The present study suggests that broccoli has excellent antioxidative potential and the hydroxycinamic acid esters from broccoli, 1,2-disinapoylgentiobiose and 1-sinapoyl-2-feruloylgentiobiose, are considered as the active components with antioxidative effect.

6) **Kim H.Y., Yokozawa T., and Cho E.J.: Mustard Leaf Suppresses Nitric Oxide Synthesis by Mouse Macrophages. *J. Nutr. Sci. Vitaminol.*, 51: 200-203, 2005.**

**Abstract:** The effect of mustard leaf on nitric oxide (NO) production was investigated using lipopolysaccharide (LPS)-stimulated peritoneal macrophages. LPS induced the production of a large amount of nitrite, an indicator of NO. However, the addition of the ethylacetate (EtOAc) or *n*-butanol (BuOH) fraction of mustard leaf to LPS-stimulated peritoneal macrophages inhibited excessive production of nitrite significantly. Moreover, compared with no treatment, LPS impaired cell viability significantly. However, the EtOAc fraction of mustard leaf significantly protected cells that had been exposed to LPS. In addition, the result of nitrite production per cell indicated that the mustard leaf fractions significantly suppressed nitrite synthesis by macrophages. In particular, the EtOAc fraction was a stronger inhibitor of nitrite synthesis than the BuOH fraction. As NO is one of the critical mediators in various disorders, these results may help to explain certain pharmacological activities of mustard leaf.

7) **Nakagawa T., Yokozawa T., Yamabe N., Goto H., Shimada Y., and Shibahara N.: Long-term treatment with Hachimi-jio-gan attenuates kidney damage in spontaneously diabetic WBN/Kob rats. *J. Pharm. Pharmacol.*, 57: 1205-1212, 2005.**

**Abstract:** Diabetes mellitus is now the most common cause of end-stage renal failure. In this study, the effects of Hachimi-jio-gan on diabetic kidney damage in spontaneously diabetic WBN/Kob rats were examined. Oral administration of Hachimi-jio-gan to WBN/Kob rats for 25 weeks significantly suppressed urinary protein excretion. It did not affect body weight loss or blood glucose levels, whereas it reversed the increase in kidney weight of WBN/Kob rats. Hachimi-jio-gan also reduced fibronectin and TGF-$\beta_1$ protein expression in the renal cortex. Furthermore, renal lipid peroxidation levels of WBN/Kob rats given Hachimi-jio-gan were significantly lower than those of untreated controls. Renal superoxide dismutase activity was elevated by Hachimi-jio-gan treatment in a dose-dependent manner. These results suggest that Hachimi-jio-gan prevents diabetic kidney damage by reducing renal oxidative injury and expression of fibronectin and TGF-$\beta_1$ proteins, which are all involved in the pathophysiology of diabetic nephropathy.

8) **Nakagawa T., Yokozawa T., Kim Y.A., Kang K.S., and Tanaka T.: Activity of Wen-Pi- Tang, and Purified Constituents of Rhei Rhizoma and GlycyrrhizaeRadix against Glucose-mediated Protein Damage. *Am. J. Chin. Med.*, 33: 817-829, 2005.**

**Abstract:** Wen-Pi-Tang, an Oriental medical prescription composed of Rhei Rhizoma, Ginseng Radix, Aconiti Tuber, Zingiberis Rhizoma and Glycyrrhizae Radix, is used clinically as a medicine to treat renal failure. This study was conducted to examine the inhibitory activity of the 5 crude drug components of Wen-Pi-Tang and several pure compounds isolated from Rhei Rhizoma and Glycyrrhizae Radix against the protein glycation reaction. Rhei Rhizoma exerted the most potent activity, Zingiberis Rhizoma and

Glycyrrhizae Radix showed relatively moderate activity, whereas Aconiti Tuber and Ginseng Radix showed weak activity. On the other hand, of 20 compounds obtained from Rhei Rhizoma and Glycyrrhizae Radix, tannins, especially rhatannin, RG-tannin and procyanidin B-2 3,3'-di-*O*-gallate, showed significantly strong activities that were more effective than the positive control, aminoguanidine. Some flavones such as licochalcone A and licochalcone B, and anthraquinones such as emodin and aloe-emodin, also showed inhibitory activity. These findings may help to explain, at least in part, certain pharmacological activities of Wen-Pi-Tang, whose clinical efficacy against renal failure is already recognized.

9) **Nakagawa T., Yokozawa T., Kim H.J., and Shibahara N.: Protective Effects of γ-Aminobutyric Acid in Rats with Streptozotocin-Induced Diabetes. *J. Nutr. Sci. Vitaminol.*, 51: 278-282, 2005.**

**Abstract:** The effects of γ-aminobutyric acid (GABA) in rats with experimental diabetes mellitus were examined. Diabetes mellitus was induced in adult male Wistar rats by streptozotocin (STZ) injection. Oral administration of GABA (100 or 200 mg/kg body weight/day) for 10 days to the diabetic rats resulted in a significant decrease in their serum glucose level. GABA also reduced the level of glycosylated protein in serum, indicating an improvement of hyperglycemic conditions. Rats with STZ-induced diabetes showed arrested body weight gain and an increase in both liver and kidney weight, whereas oral administration of GABA attenuated the organ hypertrophy induced by hyperglycemia. In addition, the degree of serum thiobarbituric acid (TBA)-reactive substance level was significantly lower in the rats treated with 100 mg GABA, and the degree of TBA-reactive substance in the liver and kidney was reduced by GABA in a dose-dependent manner. These results suggest that GABA treatment protects against the development of diabetic complications resulting from impaired glucose metabolism and enhanced oxidative stress.

10) **Nakagawa T., Yokozawa T., Satoh A., and Kim H.Y.: Attenuation of Renal Ischemia-Reperfusion Injury by Proanthocyanidin-Rich Extract from Grape Seeds. *J. Nutr. Sci. Vitaminol.*, 51: 283-286, 2005.**

**Abstract:** The effects of proanthocyanidin-rich extract in rats subjected to renal ischemia-reperfusion were examined. Proanthocyanidin-rich extract, which is prepared from grape seeds (*Vitis vinifera* L.), was given orally at doses of 5 and 10 mg/kg body weight/day for 20 consecutive days prior to ischemia-reperfusion. Administration of proanthocyanidin-rich extract attenuated renal dysfunction, as indicated by serum urea nitrogen and creatinine levels. Additionally, in the ischemic-reperfused kidneys, increased levels of thiobarbituric acid (TBA)-reactive substance and alterations of antioxidant enzyme activities such as superoxide dismutase, catalase and glutathione peroxidase (GSH-Px) were observed. Proanthocyanidin-rich extract-treated groups showed significantly reduced renal TBA-reactive substance levels and enhanced catalase and GSH-Px activities. These results suggest that proanthocyanidin-rich extract has protective effects against ischemia-reperfusion-induced renal damage associated with oxidative stress.

11) **Satoh A., Yokozawa T., Kim Y.A., Cho E.J., Okamoto T., and Sei Y.: The mechanisms underlying the anti-aging activity of the Chinese prescription Kangen-karyu in hydrogen peroxide-induced human fibroblasts. *J. Pharm. Pharmacol.*, 57: 1335-1343, 2005.**

**Abstract:** Our previous study showed that Kangen-karyu extract protected against cellular senescence by reducing oxidative damage through the inhibition of reactive oxygen species generation and regulation of the antioxidative status. Although these findings suggest that Kangen-karyu could delay the aging process, the mechanisms responsible for protection against aging have rarely been elucidated. Therefore, this study was focused on the mechanisms responsible for the anti-aging activity of Kangen-karyu extract using hydrogen peroxide ($H_2O_2$)-induced human diploid fibroblasts, a well-established experimental

model of cellular aging. Kangen-karyu extract exerted a protective effect against the morphological changes induced by $H_2O_2$ treatment and inhibited senescence-associated β-galactosidase activity. In addition, the beneficial effects of Kangen-karyu extract on cell viability and lifespan indicated that Kangen-karyu extract could delay the cellular aging process. The observation that Kangen-karyu extract prevented nuclear factor kappa B (NF-κB) translocation in response to oxidative stress suggested that Kangen-karyu exerted its anti-aging effect through NF-κB modulation and prevention of $H_2O_2$-induced overexpression of heme oxygenase-1 protein. Moreover, pretreatment with Kangen-karyu extract reduced overexpression of bax protein and prevented the mitochondrial membrane potential decline, suggesting that Kangen-karyu extract may protect mitochondria from mitochondrial oxidative stress and dysfunction. These findings indicate that Kangen-karyu is a promising potential for anti-aging agent that may delay the aging process and/or normalize aging process by virtue of its protective activity against oxidative stress-related conditions.

12) **Rao T.P., Sakaguchi N., Juneja L.R., Wada E., and Yokozawa T.: Amla (*Emblica officinalis* Gaertn.) Extracts Reduce Oxidative Stress in Streptozotocin-Induced Diabetic Rats. *J. Med. Food*, 8: 362-368, 2005.**

**Abstract:** The antioxidant properties of amla extracts and their effects on the oxidative stress in streptozotocin-induced diabetes were examined in rats. Amla in the form of either the commercial enzymatic extract SunAmla (Taiyo Kagaku Co. Ltd., Yokkaichi, Japan) (20 or 40 mg/kg of body weight/day) or a polyphenol-rich fraction of ethyl acetate extract (10 or 20 mg/kg of body weight/day) was given orally for 20 days to the streptozotocin-induced diabetic rats. Amla extracts showed strong free radical scavenging activity. Amla also showed strong inhibition of the production of advanced glycosylated end products. The oral administration of amla extracts to the diabetic rats slightly improved body weight gain and also significantly alleviated various oxidative stress indices of the serum of the diabetic rats. The elevated serum levels of 5-hydroxymethylfurfural, which is a glycosylated protein that is an indicator of oxidative stress, were significantly reduced dose-dependently in the diabetic rats fed amla. Similarly, the serum level of creatinine, yet another oxidative stress parameter, was also reduced. Furthermore, thiobarbituric acid-reactive substances levels were significantly reduced with amla, indicating a reduction in lipid peroxidation. In addition, the decreased albumin levels in the diabetic rats were significantly improved with amla. Amla also significantly improved the serum adiponectin levels. These results form the scientific basis supporting the efficacy of amla for relieving the oxidative stress and improving glucose metabolism in diabetes.

13) **Kim Y.J., Yokozawa T., and Chung H.Y.: Suppression of oxidative stress in aging NZB/NZW mice: Effect of fish oil feeding on hepatic antioxidant status and guanidino compounds. *Free Radic. Res.*, 39: 1101-1110, 2005.**

**Abstract:** Oxidative stress caused by excessive reactive species (RS) and lipid peroxidation is known to be casually linked to age-related inflammation. To test the hypothesis that fish oil (FO) intake has a beneficial effect on nephritis due to its suppressive action of oxidative stress and the enhancement of antioxidant defenses, we examined the effect of dietary FO on various oxidative stress-related parameters and guanidino compound (GC) levels using (NZB x NZW) $F_1$ (B/W) mice. These mice were fed diets supplemented with either 5% corn oil (control) or 5% FO. At 4 and 9 months of age, the hepatic oxidative status was estimated by assessing RS generation produced from xanthine oxidase, the prostaglandin pathway and lipid peroxidation. To evaluate the effect of FO on redox status, including antioxidant defenses, GSH and GSSG levels and antioxidant enzyme activities were measured. To correlate the extent of oxidative status with the nephritic condition, creatinine, guanidino acetic acid and arginine levels were measured. Results indicated that increased levels of lipid peroxidation, RS generation and xanthine oxidase activity with age were all significantly suppressed by FO feeding. Furthermore, reduced GSH

levels, GSH/GSSG ratio and antioxidant enzyme activities in the FO-fed mice were effectively enhanced compared to the corn oil-fed mice. Among several GCs, the age-related increase of creatinine level was blunted by FO. Based on these results, we propose that dietary FO exerts beneficial effects in aged, nephritic mice by suppressing RS, superoxide and lipid peroxidation, and by maintaining a higher GSH/GSSG ratio and antioxidant enzyme activities.

**14) Yamabe N., Yokozawa T., Kim H.Y., and Cho E.J.: Protective effect of Hachimi-jio-gan against renal failure in a subtotal nephrectomy rat model. *J. Pharm. Pharmacol.*, 57: 1637-1644, 2005.**

**Abstract:** The protective effect of Hachimi-jio-gan extract against chronic renal failure in a subtotal nephrectomy rat model was investigated. The level of serum urea nitrogen by nephrectomy was increased over 15 weeks from 18.4 mg/dl to 60.6 mg/dl, but the administration of Hachimi-jio-gan at 50 and 200 mg led to the decrease to 45.2 and 35.1 mg/dl, respectively. In addition, the levels of creatinine (Cr), urinary methylguanidine (MG) and MG/Cr were increased, whereas Cr clearance dramatically decreased in nephrectomized rats. However, the oral administration of Hachimi-jio-gan extract at 100 and 200 mg prevented the elevation of these uremic toxins in serum and urine, and the production of hydroxyl radical. Moreover, nephrectomy led to a significant decline in superoxide dismutase (SOD) and catalase activities from 34.89 U/mg protein to 22.81 U/mg protein and from 107.64 U/mg protein to 63.82 U/mg protein, but increased glutathione peroxidase activity compared with normal levels, indicating an abnormal antioxidative system. The increased activity of both SOD and catalase by the oral administration of Hachimi-jio-gan at 200 mg to 30.28 and 82.28 U/mg protein, respectively suggested that SOD and catalase are associated with the protective role of Hachimi-jio-gan extract against oxidative stress by nephrectomy. Moreover, the decrease of serum albumin in nephrectomized control rats was increased and proteinuria was ameliorated by the administration of Hachimi-jio-gan with improved glomerular hyalinosis, interstitial fibrosis and inflammation, suggesting the beneficial effect of Hachimi-jio-gan to prevent glomerular sclerosis and progressive renal fibrosis. This study suggests that Hachimi-jio-gan plays the protective role from the progression of chronic renal failure through the decline in uremic toxins, elevation of antioxidative enzyme activity such as SOD and catalase and amelioration of histopathological lesions in kidney.

**15) Kim H.J., Yokozawa T., Kim H.Y., Tohda C., Rao T.P., and Juneja L.R.: Influence of Amla (*Emblica officinalis* Gaertn.) on Hypercholesterolemia and Lipid Peroxidation in Cholesterol-Fed Rats. *J. Nutr. Sci. Vitaminol.*, 51: 413-418, 2005.**

**Abstract:** The effects of amla on low-density lipoprotein (LDL) oxidation and cholesterol levels were investigated *in vitro* and *in vivo* using $Cu^{2+}$-induced LDL oxidation and cholesterol-fed rats. SunAmla and ethyl acetate (EtOAc) extract of amla significantly inhibited thiobarbituric acid (TBA)-reactive substance level in the $Cu^{2+}$-induced LDL oxidation and the effects were stronger than probucol. In addition, the administration of SunAmla (at a dose of 20 or 40 mg/kg body weight/day) or EtOAc extract of amla (at a dose of 10 or 20 mg/kg body weight/day) for 20 days to rats fed 1% cholesterol diet significantly reduced total, free and LDL-cholesterol levels in a dose-dependent manner, respectively and EtOAc extract of amla exhibited more potent serum cholesterol-lowering effect than SunAmla in the same amount. Furthermore, the oxidized LDL level in serum was markedly elevated in cholesterol-fed control rats as compared with normal rats, while it was significantly decreased by the administration of SunAmla or EtOAc extract of amla. Moreover, the serum TBA-reactive substance level was also significantly decreased after oral administration of SunAmla or EtOAc extract of amla. These results suggest that amla may be effective for hypercholesterolemia and prevention of atherosclerosis.

**16) Kuboyama T., Tohda C., and Komatsu K.: Neuritic regeneration and synaptic reconstruction induced by withanolide A. *Br. J. Pharmacol.*, 144: 961–971, 2005.**

**Abstract:** We investigated whether withanolide A (WL-A), isolated from the Indian herbal drug Ashwagandha (root of *Withania somnifera*), could regenerate neurites and reconstruct synapses in severely damaged neurons. We also investigated the effect of WL-A on memory-deficient mice showing neuronal atrophy and synaptic loss in the brain. Axons, dendrites, presynapses, and postsynapses were visualized by immunostaining for phosphorylated neurofilament-H (NF-H), microtubule-associated protein 2 (MAP2), synaptophysin, and postsynaptic density-95 (PSD-95), respectively. Treatment with Aβ(25-35) (10 μM) induced axonal and dendritic atrophy, and pre- and postsynaptic loss in cultured rat cortical neurons. Subsequent treatment with WL-A (1 μM) induced significant regeneration of both axons and dendrites, in addition to the reconstruction of pre- and postsynapses in the neurons. WL-A (10 μmol/kg/day, for 13 days, p.o.) recovered Aβ(25-35)-induced memory deficit in mice. At that time, the decline of axons, dendrites, and synapses in the cerebral cortex and hippocampus was almost recovered. WL-A is therefore an important candidate for the therapeutic treatment of neurodegenerative diseases, as it is able to reconstruct neuronal networks.

## ◇総　説

1) Yokozawa T., Yamabe N., Cho E.J.: A novel therapeutic approach of Hachimi-jio-gan to diabetes and its complications. *Oriental Pharmacy and Experimental Medicine*, **5**: 75-91, 2005.
2) 横澤隆子: ラジカル惹起モデルにおける薬用人参サポニンRdの役割. *JIM*, **15:** 74-77, 2005.
3) 杉野豪俊, Rao T.P., Hlahla H., 坂口　騰, 大久保　勉, 朱　政治, Juneja L.R., 横澤隆子: アムラ抽出物の抗酸化活性. *機能性食品と薬理栄養*, **3:** 91-95, 2005.
4) Tohda C., Komatsu K., Kuboyama T.: Scientific basis for the anti-dementia drugs of constituents from Ashwagandha (*Withania somnifera*). 和漢医薬学雑誌, **22(Suppl.1):** 176-182, 2005.
5) Tohda C., Kuboyama T., Komatsu K.: Search for natural products related to regeneration of the neuronal network. *Neurosignals,* **14:** 34-45, 2005.
6) Komatsu K., Tohda C., Zhu S.: Ginsengs Drugs - Molecular and chemical characteristics and possibility as antidementia drugs. *Current topics in Nutraceutical Research,* **3(1):** 47-65, 2005.

## ◇学会報告　(*: 特別講演、シンポジウム、ワークショップ等)

1) 安田直美, 金　賢柱, 杉野豪俊, Rao T.P., 大久保　勉, 横澤隆子, ジュネジャ L.R.: アムラの動脈硬化抑制作用. 日本農芸化学会 2005 年度大会, 2005, 3, 28-30, 札幌.
2) 山辺典子, 中川孝子, 柴原直利, 横澤隆子: 八味地黄丸の糖尿病性腎症治療薬への展望. 日本薬学会第 125 年会, 2005, 3, 29-31, 東京.
3) Kang K.S., Kim H.Y., Park J.H., Yokozawa T.: Peroxynitrite Scavenging Activity of Phenolic Compounds from Sun Ginseng. 日本薬学会第 125 年会, 2005, 3, 29-31, 東京.
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## ◇共同研究

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## ◇研究費取得状況

1) 科学技術振興機構（分担：横澤隆子）「血管障害性生活習慣病に対する予防食品の開発研究」
2) 学内特別経費「戦略的経費」（代表：横澤隆子）「健康食品素材としての柿ポリフェノールオリゴマー」
3) つくし奨学・研究基金（代表：横澤隆子）「糖尿病性腎症に対する漢方方剤治療の基礎的研究」
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5) 文部科学省研究費補助金、基盤研究 C　（代表：東田千尋）　「新規の抗痴呆薬となる withanolide 類を用いたシナプス形成機序の解明」
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井口江美子：糖尿病における冠元顆粒の検討