Artemisia scoparia attenuates amyloid β accumulation and tau hyperphosphorylation in spontaneously hypertensive rats

Kitipong Promyo¹ · Jeong-Yong Cho¹ · Kyung-Hee Park¹ · Lily Jaiswal¹ · Sun-Young Park¹ · Kyung-Sik Ham¹

Received: 8 June 2016 / Revised: 11 November 2016 / Accepted: 17 February 2017 / Published online: 19 June 2017
© The Korean Society of Food Science and Technology and Springer Science+Business Media Dordrecht 2017

Abstract The preventive effects of *Artemisia scoparia* extract (AS) and AS + garlic extract (ASG) on the risk of Alzheimer's disease (AD) were evaluated in spontaneously hypertensive rats. Rats were supplemented with diets containing 2% (w/w) of AS or ASG for 6 weeks. The AS group showed lower levels of amyloid β and beta-site amyloid precursor protein cleaving enzyme 1 expressions and higher expression levels of low-density lipoprotein receptor-related protein 1 than did the control group ($p < 0.05$). In addition, the AS showed remarkably reduced levels of phosphorylated tau proteins and suppressed expression of phosphorylated glycogen synthase kinase 3 β at tyrosine 216 (active form) ($p < 0.05$). The ASG group also suppressed amyloid β accumulation and tau hyperphosphorylation. However, there was no synergistic effect of garlic with AS in lowering the risk of AD. These results indicate that AS could be a potential candidate to ameliorate the risk of AD.

Keywords *Artemisia scoparia* · Alzheimer's disease · Amyloid β · Tau hyperphosphorylation · Oxidative stress

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that weakens neurocognitive skills, including cognition, memory, and behavioral control, in elderly individuals [1].

The worldwide prevalence of AD has been growing rapidly over the last three decades [1]. Extracellular plaques comprising amyloid beta ($A\beta$) and accumulation of hyperphosphorylated tau protein in the form of neurofibrillary tangles are believed to be the causative agents in the progression of AD, as $A\beta$ obstructs neuronal transmission while tangles impede the transport of essential nutrients to neurons, leading to neural death [2]. The severity of AD is aggravated in patients suffering from comorbid metabolic syndromes, such as diabetes mellitus or hypertension [3]. In particular, clinical studies have implicated that hypertension elevates the risk of AD with $A\beta$ accumulation and neurofibrillary tangle formation and impairs memory and cognitive function [4, 5]. Many studies have documented that a high intake of vegetables prevents the risk of AD and delays cognitive decline in the elderly [6, 7]. Therefore, vegetable intake for the clinical prevention of the risks of AD has received attention.

Artemisia scoparia (AS; redstem wormwood, Asteraceae) is an edible halophyte with a unique aroma and bitter taste. Several chemical constituents, such as scopariachromane, coumarins, flavonoids, sterols, and sesquiterpene lactones, have been isolated from this plant [8, 9]. Recently, various biological effects of the extracts, including antioxidant, anti-inflammatory, antipyretic, and liver protective activities, have been demonstrated [9–11]. We previously reported that AS attenuated an increased blood pressure in spontaneously hypertensive rats (SHR) via the suppression of angiotensin II production and inhibition of angiotensin I converting enzyme (ACE) activity [12]. However, the addition of garlic (*Allium sativum*) to AS did not significantly decrease blood pressure, even though garlic is well known to prevent high blood pressure [13]. High blood pressure causes an increase of AD risk and cognitive decline [14]. In particular, angiotensin II

✉ Kyung-Sik Ham
ksham@mokpo.ac.kr

¹ Department of Food Engineering and Solar Salt Research Center, Mokpo National University, Muan, Jeonnam 58554, Republic of Korea

induces A β accumulation and tau hyperphosphorylation and impairs cognitive abilities [15]. Therefore, AS, which inhibited the production of angiotensin II, was expected to ameliorate A β accumulation and tau hyperphosphorylation in SHR. The effects of AS or ASG on AD-related factors in animal models have not yet been assessed.

In this study, the preventive effects of AS or ASG on the risk of AD in SHR that have been used as an animal model system for AD [16] were assessed by analyzing their effects on A β production and tau hyperphosphorylation-related proteins. In addition, the preventative effects of AS or ASG on oxidative stress in the brain tissues of SHR were also assessed.

Materials and methods

Materials

The aerial parts of AS were harvested in Shinan County, South Korea, in May, 2013, and cleaned with tap water. After steaming for 2 min, the aerial parts were dried for 2 days at 45 °C. Garlic was purchased from the local market in Shinan County, in June, 2013. The dried AS aerial parts (4 kg) were added to 40 L of distilled water, extracted for 30 min at 121 °C, and then filtered with filter paper no. 2 (Whatman, Maidenstone, UK). The remaining residues were extracted again with distilled water (20 L) and filtered as described above. The extracted solutions were pooled and freeze-dried to obtain AS extract powder. Similarly, to obtain ASG, the dried AS aerial segments/fresh garlic (9:1, w/w) were mixed, and then extraction, filtration, and freeze-drying were performed using the same procedure as for AS.

Chemicals

Thiobarbituric acid (TBA), malondialdehyde (MDA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), hydroxylamine hydrochloride, 2,4-dinitrophenylhydrazine (DNPH), and acetylthiocholine iodide (ATChI) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Rabbit monoclonal antibodies against beta-site amyloid precursor-cleaving enzyme 1 (BACE1), insulin-degrading enzyme (IDE), low-density lipoprotein receptor-related protein-1 (LRP1), and phosphorylated protein phosphatase 2A at tyrosine 307 (p-PP2A-tyr307) were obtained from Abcam (Burlingame, CA, USA). Antibodies against the receptor for advanced glycation end products (RAGE), glycogen synthase kinase 3 β (GSK3 β), phosphorylated GSK3 β at tyrosine 216 (p-GSK3 β -tyr216), and phosphorylated tau protein at serine 396 (p-tau-ser396) and 404 (p-tau-ser404) were provided by Santa Cruz Biotechnology,

Inc. (Dallas, TX, USA). Antibodies against NF-kappa B (NF- κ B) p65, neprilysin (NEP), PP2A, and horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G were provided from Millipore (Billerica, MA, USA). A monoclonal antibody against tau was provided from Invitrogen (Carlsbad, CA, USA). Rabbit GAPDH and β -actin polyclonal antibodies were provided from Ab Frontier (Seoul, Korea). A human/rat amyloid (1–42) ELISA kit and trichloroacetic acid (TCA) were obtained from Wako Pure Chemical, Inc. (Kyoto, Japan).

Animal study

The Ethics Committee of Mokpo National University approved the animal experimental procedures (no. MNU IACUC-2014-011). Male SHR (4 weeks old) were obtained from Central Lab. Animal Ltd. (Seoul, Korea). The rats were kept under 55 $\pm$ 5% of humidity, 25 $\pm$ 1 °C of temperature, and a 12 h light-dark cycle. Food and water were available ad libitum. After adaption for 3 weeks, the rats were randomly grouped into three groups (each group, n = 8) as follows; control group: rats were fed the AIN-93G diet, AS group: the rats were fed the diet supplemented with 2% (w/w) AS water extract, and ASG group: the rats were fed the diet supplemented with 2% (w/w) ASG water extract. The rats were fed the diets mentioned above for 6 weeks. The rats were anesthetized with diethyl ether and the whole brain was collected, then immediately frozen with liquid nitrogen, and deposited at –80 °C until analysis.

Determination of A β levels

The level of A β (1–42) in the brain was determined by the Wako human/rat amyloid 42 ELISA kit, according to the manufacturer's instructions.

Determination of acetylcholinesterase (AChE) activity and acetylcholine (ACh) level

AChE activity in the brain tissue was evaluated by the spectrophotometric method published by Ellman [17]. The brain tissue (0.15 g) was homogenized in a lysis buffer (0.01 M Tris-HCl, pH 7.4, 1 mL) and centrifuged at 5000 $\times$ g for 10 min at 4 °C. An aliquot (30 μ L) of the supernatant was added to 600 μ L of 0.3 mM DTNB in 0.01 M phosphate buffer (pH 7.0) and 150 μ L of 8.45 mM ATChI. The mixture was monitored at 405 nm at intervals of 30 s for 3 min.

ACh level in the brain tissue was also measured according to the colorimetric method of Hestrin [18]. The supernatant (100 μ L) of the prepared brain tissue homogenate was mixed with 200 μ L of hydroxylamine acid.

After incubating for 2 min at room temperature, pH was adjusted to 1.2 by adding 100 μL of concentrated $\text{HCl}/\text{H}_2\text{O} = 1:2$ (v/v) and 100 μL of 0.1 N HCl containing 0.35 M FeCl_3 . The mixture was centrifuged at $5000\times g$ for 5 min. The supernatant was measured at 530 nm. In the brain tissue, the ACh level was calculated using a calibration curve that used ATChI as a standard.

Measurement of thiobarbituric acid reactive substances (TBARS)

Brain tissue (0.15 g) was homogenized in 1 mL of lysis buffer (0.01 M Tris-HCl, pH 7.4) and centrifuged at $5000\times g$ at 4 °C for 10 min [12]. An aliquot (0.1 mL) of the supernatant was mixed with 0.3 mL of 10 mM K_2HPO_4 solution and 1 mL of 0.15 M Tris-HCl (pH 7.4) and incubated for 20 min at 37 °C in a shaking incubator (100 rpm). Then, protein was precipitated by adding 0.5 mL 20% TCA. The mixture were added to 0.5 mL 0.67% TBA and boiled at 95 °C for 15 min. The absorbance was read at 532 nm and the TBARS level was determined from the standard curve of MDA.

Measurement of protein carbonyls (PCO) content

One milliliter of 2.5 N HCl solution containing 10 mM DNPH was mixed with the protein extract (1.5 mg) of the brain tissue [12]. After incubating at room temperature in the dark and vortexing shortly at intervals of 15 min for 1 h, the protein was precipitated by adding of 10% (w/v) trichloroacetic acid. The protein pellet obtained after centrifugation for 10 min at $3000\times g$ was washed with 2.0 mL of ethanol/ethyl acetate = 1:1 (v/v, three times), dissolved in 6 M guanidine hydrochloride (1.5 mL), and then kept at 37 °C for 10 min. The absorbance was determined at 370 nm, and the PCO content (nmol/mg protein) was calculated using the molar absorption coefficient of $2.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$.

Western blotting

The brain tissue (0.15 g) was homogenized with 1 mL RIPA buffer, which was composed of 0.01 M NaF, 0.01 M EDTA (pH 7.4), 0.01 M sodium pyrophosphate, 2 mM phenylmethylsulfonyl fluoride, 0.1 mg/mL aprotin, 1.0 $\mu\text{g}/\text{mL}$ pepstatin, and 1.0 $\mu\text{g}/\text{mL}$ leupeptin. The homogenate was centrifuged for 40 min at $15,000\times g$ at 4 °C after protein extraction for 2 h at 4 °C. Protein content of the supernatant was measured according to the Bradford method [19]. An aliquot (400 μg protein/40 μL) of the supernatant was mixed with 40 μL tricine buffer containing 2% 2-mercaptoethanol and boiled for 5 min at 95 °C. SDS-PAGE was done, and the proteins were transferred to

a polyvinylidene difluoride (PVDF) membrane. After washing with TBST buffer, the membrane was incubated overnight with primary antibody. The membrane was washed with TBST buffer and incubated for 1 h with secondary horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG antibody. After washing with TBST and TBS buffer, the membrane was further incubated in a solution of the chemiluminescent substrate and photographed. ImageJ software (NIH, Bethesda, Maryland, USA) was used to analyze the intensities of the targeted protein bands.

Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD) using the SPSS (IBM, Armonk, NY, USA) 19.0 software package. Student's *t* test or one-way analysis of variance followed by Tukey's multiple comparison test were done to assess the significance of difference. A value of $p < 0.05$ was considered to indicate significance.

Results and discussion

Preventive effect of AS or ASG on A β accumulation in SHR

The rats supplemented with AS or ASG diets showed lower levels of A β (1–42) in the brain tissue than did the control rats fed a normal diet ($p < 0.05$) [Fig. 1(A)]. In addition, the AS- and ASG-fed rats revealed significantly lower expression of BACE1 compared to the control rats ($p < 0.05$) [Fig. 1(B)]. However, no significant differences in BACE1 expression or A β (1–42) levels between the AS and ASG groups were observed. The BACE1 is an essential enzyme for the production of A β , as it cleaves *N*-terminal of amyloid precursor protein [20]. Excessive A β accumulation induces A β plaques that trigger the development of AD [20], along with a reduction in learning and memory abilities. Our results suggest that AS or ASG diets reduce A β accumulation at least partially through the suppression of BACE1 expression in the brain of SHR.

RAGE transports A β to the brain across the blood–brain barrier from the systemic circulation, resulting in A β accumulation in the brain, whereas LRP1 removes A β s from the brain through the blood–brain barrier [21]. IDE and NEP are responsible for the degradation of A β , and prevent A β deposition in the brain [22]. In this study, AS- and ASG-fed rats showed significantly lower RAGE and higher LRP1 expression levels than did control rats ($p < 0.05$) [Fig. 1(B)], although no significant differences in their expression levels between the AS and ASG groups were observed. The AS or ASG diets appeared to remove

Fig. 1 Effect of AS or ASG on Aβ accumulation in the brain tissues of experimental rats. **(A)** Aβ(1–42) levels; **(B)** BACE1, LRP1, RAGE, IDE, and NEP expression; **(C)** quantitative analysis normalized against β-actin or GAPDH. All data are represented as mean ± SEM ($n = 8$). * $p < 0.05$; ** $p < 0.01$ versus control

Aβ from the brains of SHR by enhancing LRP1 and suppressing the RAGE expression. However, expression levels of IDE and NEP were not significantly different between groups [Fig. 1(B)], suggesting that AS or ASG intake do not stimulate expression of IDE and NEP, which are involved in degrading Aβ in the brain. The previous experiment reported that Aβ (1–42) level, BACE1, and RAGE expressions increased and LRP1 expression decreased in the brain of SHR [23]. In particular, reduction of hypertension caused the attenuation of Aβ accumulation in SHR [24]. In our previous study, AS or ASG diets were found to reduce oxidative stress and hypertension in SHR [12]. Therefore, these observations suggest that AS or ASG diets attenuate hypertension and lead to decreased Aβ accumulation via suppressing RAGE and BACE1 expressions and enhancing LRP1 expression in the brain of SHR.

Ameliorative effect of AS or ASG on tau hyperphosphorylation in SHR

Tau protein, which is abundantly found in neurons, plays an important role in stabilizing microtubules [25]. Tau balance is regulated by kinases and phosphatases. Excessive hyperphosphorylation of tau causes the formation of

neurofibrillary tangles, which are involved in the pathogenesis of AD [25]. GSK3β is a representative kinase that is involved in the phosphorylation of tau [26]. The expression of PP2A, a representative phosphatase, is greatly decreased in AD patients [27]. In this study, the AS or ASG groups showed lower expression levels of p-tau-ser396 and p-tau-ser404, which are well-known hyperphosphorylated tau proteins, compared to the control group ($p < 0.05$) [Fig. 2(A)]. Moreover, compared with the control group, the AS or ASG groups exhibited lower ratios of p-GSK3β-tyr216 (an active form) to total GSK3β ($p < 0.05$) [Fig. 2(B)]. This result suggests that AS and ASG diets may inhibit GSK-3β activity via suppression of phosphorylation at tyr216 in the brains of SHR. The AS or ASG groups exhibited no change in the expression ratio of p-PP2A-tyr307 (inactive form)/total PP2A (active form), which is involved in dephosphorylation of tau, when compared with the control group [Fig. 2(C)], suggesting that regulation of p-PP2A activity via phosphorylation of p-PP2A at tyr307 may not be affected by AS or ASG diets. It was demonstrated that tau hyperphosphorylation and GSK-3β activity were elevated in SHR [15]. It is also suggested that various antihypertensive drugs could retard a cognitive decline or an incidence of dementia [15, 24]. Therefore, these observations suggest that AS or ASG diets may attenuate tau hyperphosphorylation through the inhibition of GSK3β activity by suppressing phosphorylation of GSK3β at tyr216 in the brains of SHR.

Effect of AS on ACh level and AChE activity in the brain of SHR

ACh is the most common neurotransmitter in the central nervous system and plays an important role in sleeping, attention, and memory [28]. AChE, which is mainly found in neuromuscular junctions and cholinergic brain synapses, hydrolyzes ACh to choline and acetic acid, resulting in the degradation of the signal [28]. In AD patients, reduced ACh content and increased AChE activity have been observed, which have been associated with loss of memory, cognitive, and behavioral abilities [29]. In the present study, significant differences of ACh level and AChE activity between the groups were not observed (Fig. 3). These results suggest that AS or ASG intake do not affect the changes in ACh level and AChE activity in the brain.

Preventive effect of AS or ASG on oxidative stress in the brain of SHR

Oxidative stress and inflammation increase the risk of AD [30]. The increase of blood pressure in SHR induces oxidative stress in the brain [31]. MDA and PCOs are important oxidative stress-related factors in AD [32]. In

Fig. 2 Effect of AS or ASG on tau phosphorylation in rat brain tissue. (A) p-tau-ser396 and p-tau-ser404; (B) tau protein kinase; (C) protein phosphatase. All data are represented as mean $\pm$ SEM ($n = 8$). * $p < 0.05$; ** $p < 0.01$ versus control

addition, an increase in oxidative stress brings about inflammation via activation of NF- κ B and the stimulation of tumor necrosis factor alpha pathway [33]. We previously reported that AS or ASG diets reduced oxidative damage in the liver, skeletal muscles, kidneys, and plasma of SHR [12]. However, whether AS or ASG diets reduce oxidative stress in the brain remained unclear because it was possible that the blood-brain barrier was capable of blocking the passage of active compounds in AS or ASG diets from the periphery to the central nervous system. In this study, the AS- and ASG-fed rats showed lower MDA and PCO levels in the brain tissue than did the control group ($p < 0.05$) [Fig. 4(A), (B)]. Additionally, the AS- or ASG-fed rats exhibited lower NF- κ B p65 expression in the brain tissue

than did the control rats ($p < 0.05$) [Fig. 4(C)]. These results also indicated that AS or ASG diets attenuate oxidative damage and inflammation in the brain of SHR, suggesting that active compounds of AS or ASG are capable of passing through the blood-brain barrier. Therefore, it is possible that a reduction in oxidative damage brought about by the AS or ASG diets attenuates the risk of AD via amelioration of A β accumulation and tau hyperphosphorylation in SHR. However, the significant difference of MDA and PCO levels as well as the expression of NF- κ B p65 between the AS and ASG groups were not observed.

In this study, we found that AS or ASG reduce A β synthesis and tau hyperphosphorylation by suppressing BACE1 and tau protein kinase with the reduction of

Fig. 3 Acetylcholinesterase activity (A) and acetylcholine level (B) in the rats fed AS or ASG diets for 6 weeks. All data are represented as mean $\pm$ SEM ($n = 8$)

Fig. 4 Effect of AS or ASG on oxidative stress in rat brain tissue. (A) Lipid peroxidation; (B) protein carbonyl content; (C) NF-kB p65 expression. All data are represented as mean $\pm$ SEM ($n = 8$). * $p < 0.05$; ** $p < 0.01$ versus control

oxidative stress in the brains of SHR. In addition, we previously reported that AS or ASG reduce blood pressure in SHR by the inhibition of the ACE activity and angiotensin II production and several ACE inhibitors have been identified in the aerial parts of AS [12]. It is known that angiotensin II induces A β synthesis and tau protein hyperphosphorylation [15]. Furthermore, angiotensin II receptor blockers have been proven as a candidate for the treatment of AD [34]. Thus, reduced A β synthesis and tau hyperphosphorylation in AS or ASG group could be due to at least partially ACE inhibitors in AS or ASG. Angiotensin II is also known to induce oxidative stress through angiotensin II type I receptor. Therefore, in addition to antioxidants present in AS or ASG, ACE inhibitors of AS and ASG might play a role in the reduction of oxidative stress in AS or ASG groups.

We could not observe significant differences between AS and ASG in most of the parameters related to AD. Garlic has been found to ameliorate parameters related to AD [35]. Thus, we thought that addition of garlic to AS (ASG) would be more effective than AS only in amelioration of parameters related to AD. However, significant effects by addition of garlic to AS were not observed. One possible explanation for these results could be due to the preparation of the extract of ASG. As described in “Materials and methods”, the dried AS parts were mixed with fresh garlic in the ratio of 9:1 (w/v), then extracted, filtered, and freeze-dried. If water content of fresh garlic is about

90%, dried matter of garlic will be reduced to one tenth. In this case, the garlic content could be too small compared to the AS content to show any effect. This could explain why we could not observe significant differences between AS and ASG groups. Another possible explanation could be that garlic may not be synergistic with AS. These results suggest that AS could be a potential candidate to ameliorate the risk of AD.

Acknowledgements This work was supported by Grant 20130290 to the Solar Salt Research Center of Mokpo National University from the Ministry of Oceans and Fisheries of Korea.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Alzheimer's Association. Alzheimer's disease facts and figures. *Alzheimer's Dement.* 10: e47–92 (2014)
- Kolarova M, García-Sierra F, Bartos A, Ricny J, Ripova D. Structure and pathology of tau protein in Alzheimer disease. *Int. J. Alzheimer's Dis.* 2012: 731526 (2012)
- Duka V, Lee JH, Credle J, Wills J, Oaks A, Smolinsky C, Shah K, Mash DC, Masliah E, Sidhu A. Identification of the sites of tau hyperphosphorylation and activation of tau kinases in synucleinopathies and Alzheimer's diseases. *PLoS One* 8: e75025 (2013)
- Glodzik L, Rusinek H, Pirraglia E, Mchugh P, Tsui W, Williams S, Cummings M, Rich K, Randall C, Mosconi L, Osoria R, Murray J, Zetterberg H, Blennow K, de Leon M. Blood pressure decrease correlates with tau pathology and memory decline in hypertensive elderly. *Neurobiol. Aging* 35: 64–71 (2014)
- Warchol-Celinska E, Styczynska M, Prejbisz A, Przybylowska K, Chodakowska-Zebrowska M, Kurjata P, Piotrowski W, Polakowska M, Kabat M, Zdrojewski T, Drygas W, Januszewicz A, Barcikowska M. Hypertension in patients with Alzheimer's disease—prevalence, characteristics, and impact on clinical outcome. Experience of one neurology center in Poland. *J. Am. Soc. Hypertens.* 9: 711–724 (2015)
- Loef M, Walach H. Fruit, vegetables and prevention of cognitive decline or dementia: a systematic review of cohort studies. *J. Nutr. Health Aging* 16: 626–630 (2012)
- Hu N, Yu JT, Tan L, Wang YL, Sun L, Tan L. Nutrition and the risk of Alzheimer's disease. *Biomed Res. Int.* 2013: 524820 (2013)
- Cho JY, Jeong SJ, Lee HL, Park KH, Hwang DY, Park SY, Lee YG, Moon JH, Ham KS. Sesquiterpene lactones and scopoletins from *Artemisia scoparia* Waldst. & Kit. and their angiotensin I-converting enzyme inhibitory activities. *Food Sci. Biotechnol.* 25: 1701–1708 (2016)
- Yahagi T, Yakura N, Matsuzaki K, Kitanaka S. Inhibitory effect of chemical constituents from *Artemisia scoparia* Waldst. et Kit. on triglyceride accumulation in 3T3-L1 cells and nitric oxide production in Raw 264.7 cells. *J. Nat. Med.* 68: 414–420 (2014)
- Habib M, Waheed L. Evaluation of anti-nociceptive, anti-inflammatory and antipyretic activities of *Artemisia scoparia* hydromethanolic extract. *J. Ethnopharmacol.* 145: 18–24 (2013)
- Wang ZQ, Zhang XH, Yu Y, Tipton RC, Raskin I, Ribnicky D, Johnson W, Cefalu WT. *Artemisia scoparia* extract attenuates non-alcoholic fatty liver disease in diet-induced obesity mice by enhancing hepatic insulin and AMPK signaling independently of FGF21 pathway. *Metabolism* 62: 1239–1249 (2013)
- Cho JY, Park KH, Hwang DY, Lily J, Park YK, Kim SY, Kim HR, Moon JH, Ham KS. Antihypertensive effects of *Artemisia scoparia* Waldst in spontaneously hypertensive rats and identification of angiotensin I converting enzyme inhibitors. *Molecules* 20: 19789–19804 (2015)
- Benavides G, Squadrito G, Mills RW, Patel HD, Isbell TS, Patel RP, Doeller JE, Darley-Usmar VM, Kraus DW. Hydrogen sulfide mediates the vasoactivity of garlic. *Proc. Natl. Acad. Sci. USA.* 204:17977–17982 (2007)
- Duron E, Hanon O. Hypertension, cognitive decline and dementia. *Arch. Cardiovasc. Dis.* 101(3): 181–189 (2008)
- Tian M, Zhu D, Xie W, Shi J. Central angiotensin II-induced Alzheimer-like tau phosphorylation in normal rat brains. *FEBS Lett.* 586: 3737–3745 (2012)
- Benedikz E, Kloskowska E, Winblad B. The rat as an animal model of Alzheimer's disease. *J. Cell. Mol. Med.* 13: 1034–1042 (2009)
- Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of Acetylcholinesterase activity. *Biochem. Pharmacol.* 7: 88–95 (1961)
- Hestrin S. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. *J. Biol. Chem.* 180: 249–261 (1949)
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254 (1976)
- Ohno M, Sametsky EA, Younkin LH, Oakley H, Younkin SG, Citron M, Vassar R, Disterhoft JF. BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease. *Neuron* 41: 27–33 (2004)
- Donahue JE, Flaherty S L, Johanson CE, Duncan JA 3rd, Silverberg GD, Miller MC, Tavares R, Yang W, Wu Q, Sabo E, Hovanesian V, Stopa EG. RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. *Acta Neuropathol.* 112: 405–415 (2006)
- Dorfman VB, Pasquini L, Riudavets M, López-Costa JJ, Villegas A, Troncoso JC, Lopera F, Castaño EM, Morelli L. Differential cerebral deposition of IDE and NEP in sporadic and familial Alzheimer's disease. *Neurobiol. Aging* 31: 1743–1757 (2010)
- Qiao J, Wang X, Lu W, Wang X, Ma A. Abnormal expression level of BACE1 and RAGE of hippocampus related to cognitive impairment in SHR. *Ann. Clin. Exp. Hypertension* 2: 1010 (2014)
- Kurata T, Lukic V, Kozuki M, Wada D, Miyazaki K, Morimoto N, Ohta Y, Deguchi K, Yamashita T, Hishikawa N, Matsuzono K, Ikeda Y, Kamiya T, Abe K. Long-term effect of telmisartan on Alzheimer's amyloidogenesis in SHR-SR after tMCAO. *Transl. Stroke Res.* 6: 107–115 (2015)
- Obloh G, Akinyemi AJ, Ademiluyi AO, Bello FO. Inhibitory effect of some tropical green leafy vegetables on key enzymes linked to Alzheimer's disease and some pro-oxidant induced lipid peroxidation in rats' brain. *J. Food Sci. Tech.* 51: 884–891 (2014)
- Hampel H, Blennow K, Shaw LM, Hoessler YC, Zetterberg H, Trojanowski JQ. Total and phosphorylated tau protein as biological markers of Alzheimer's disease. *Exp. Gerontol.* 45: 30–40 (2010)
- Sontag E, Luangpirom A, Hladik C, Mudrak I, Ogris E, Speciale S, White CL. Altered expression levels of the protein phosphatase 2A AB α C enzyme are associated with Alzheimer disease pathology. *J. Neuropathol. Exp. Neurol.* 63: 287–301 (2004)
- Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: Cholinergic signaling shapes nervous system function and behavior. *Neuron* 76: 116–129 (2012)

29. Čolović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase inhibitors: Pharmacology and toxicology. *Curr. Neuropharm.* 11: 315–335 (2013)
30. Agostinho P, Cunha RA, Oliveira C. Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. *Curr. Pharm. Des.* 16: 2766–2778 (2010)
31. Lee MC, Shoji H, Miyazaki H, Yoshino F, Hori N, Toyoda M, Ikeda Y, Anzai K, Ikota N, Ozawa T. Assessment of oxidative stress in the spontaneously hypertensive rat brain using electron spin resonance (ESR) imaging and in vivo L-Band ESR. *Hypertens. Res.* 270: 485–492 (2004)
32. Greilberger J, Koidl C, Greilberger M, Lamprecht M, Schroecksnadel K, Leblhuber F, Fuchs D, Oettl K. Malondialdehyde, carbonyl proteins and albumin-disulphide as useful oxidative markers in mild cognitive impairment and Alzheimer's disease. *Free Radic. Res.* 42: 633–638 (2008)
33. Schütze S, Wiegmann K, Machleidt T, Krönke M. TNF-induced activation of NF-kappa B. *Immunobiology* 193: 193–203 (1995)
34. Saavedra JM. Evidence to consider angiotensin II receptor blockers for the treatment of early Alzheimer's disease. *Cell. Mol. Neurobiol.* 36: 259–279 (2016)
35. Gupta VB, Indi SS, Rao KS. Garlic extract exhibits anti-amyloidogenic activity on amyloid-beta fibrillogenesis: relevance to Alzheimer's disease. *Phytother Res.* 23: 111–115 (2009)