

## Review Article

**Danggui-Shaoyao-San: New Hope for Alzheimer's Disease**Xin Fu<sup>1</sup>, QiuHong Wang<sup>1</sup>, ZhiBin Wang<sup>1</sup>, HaiXue Kuang<sup>1,\*</sup>, Pinghui Jiang<sup>2</sup>

<sup>1</sup>School of Pharmacy, Key Laboratory of Chinese Materia Medica, Heilongjiang University of Chinese Medicine, Ministry of Education, Harbin 150040, China.

<sup>2</sup>College of Electrical and Information Engineering, Heilongjiang Institute of Technology, Harbin 150050, China.

[Received October 11, 2015; Revised December 15, 2015; Accepted December 20, 2015]

**ABSTRACT:** Danggui-Shaoyao-San (DSS), also called Toki-shakuyaku-san (TJ-23) or Dangguijakyak-san (DJS), is a well-known herbal formula (*Angelica sinensis* (Oliv.) Diels., *Ligusticum chuanxiong* Hort., *Paeonia lactiflora* pall., *Poria cocos* (Schw.) Wolf, *Alisma orientalis* (Sam.) Juzep., *Atractylodes macrocephala* Koidz.), which has been widely used in oriental countries for the treatment of various gynecological diseases. Recent studies show that DSS has an effect on free radical-mediated neurological diseases and exhibits anti-inflammatory and antioxidant activities and reduces cell apoptosis in the hippocampus. In addition, DSS mediates the modulation of central monoamine neurotransmitter systems and ameliorates dysfunction of the central cholinergic nervous system and scopolamine-induced decrease in ACh levels. DSS improves the function of the dopaminergic, adrenergic, and serotonergic nervous systems. Interestingly, DSS can alleviate cognitive dysfunction of Alzheimer's disease (AD) patients, suggesting that it is a useful therapeutic agent for AD. This paper reviews the mechanism of DSS for the treatment of AD.

**Key words:** Danggui-Shaoyao-San, Alzheimer's disease, anti-inflammation, antioxidant activity, cell apoptosis

Danggui-Shaoyao-San (DSS), also called Toki-shakuyaku-san (TJ-23) or Dangguijakyak-san (DJS), is a famous herbal formula composed of the following 6 raw herbs: *Angelica sinensis* (Oliv.) Diels (Umbelliferae), *Paeonia lactiflora* Pall. (Paeoniaceae), *Ligusticum chuanxiong* Hort. (Umbelliferae), *Poria cocos* (Schw.) Wolf (Polyporaceae), *Atractylodes macrocephala* Koidz. (Compositae), and *Alisma orientalis* (Sam.) Juzep. (Alismataceae), which has been widely used in the treatment of various gynecological diseases. DSS also has an effect on free radical-mediated neurological diseases, possesses antioxidant capability, attenuates inflammatory reaction, and reduces cell apoptosis in the hippocampus. Analysis by HPLC-DAD-ESI-MS/MS revealed that DSS contains ferulic acid, Z-ligustilide, monoterpene glycosides, phenolic acids, phthalides, sesquiterpenoids and triterpenes, gallic acid, albiflorin, paeoniflorin, benzoic acid, senkyunolide I, coniferyl ferulate,

senkyunolide A, 3-butylphthalide, Z-butylidenphthalide, atractylenolide II, atractylenolide I, levistolide A, and etc [1].

The effects of DSS on neurons are multiple and DSS has been used for the treatment of gynecological symptoms in elderly women. Recently, it was found that DSS is a potential therapeutic agent for Alzheimer's disease (AD) [54]. By ameliorating oxidative stress-induced neuronal apoptosis, DSS has neuroprotective effects in D-gal-induced senescent mice [5]. The function of the dopaminergic nervous system in the hippocampus was stimulated and the function of the adrenergic nervous system was inhibited by DSS [44]. Furthermore, DSS mediates the modulation of central monoamine neurotransmitter systems and ameliorates dysfunction of the central cholinergic nervous system [6]. Several studies have reported that oral administration of DSS immediately increases nerve growth factor (NGF) and

\*Correspondence should be addressed to: Dr. Kuang HaiXue. School of Pharmacy, Key Laboratory of Chinese Materia Medica, Heilongjiang University of Chinese Medicine, Harbin 150040, China. Email: [hxkuang@hotmail.com](mailto:hxkuang@hotmail.com)

prevents the reduction of dopamine (DA) metabolites, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in olfactory-bulb-lesioned mice [46]. DSS has been shown to improve memory impairment and acetylcholine and norepinephrine in the cerebral cortex and hippocampus [47]. However, in recent years, DSS has pharmacological basis on memory dysfunction, modulating metabolism of monoamine neurotransmitters, protecting the ultrastructure of the cortex changed by aging [41], and increasing the level of superoxide dismutase (SOD) [55]. DSS is beneficial for the treatment of cerebrovascular dementia that has indirect neuroprotective activity [64].

It is reported that DSS not only improves microcirculation in patients with asymptomatic cerebral infarction, but also prevents cognitive impairment from AD [53]. The aim of the present review is to focus on mechanisms underlying DSS-mediated neuroprotection for AD patients.

**Table 1.** Mechanisms of protection from neuronal damage and cell apoptosis by DSS

| Mechanism  | Reference |
|--|-----------|
| Increases expressions of nuclear factor- $\kappa$ B and transforming growth factor- $\beta$  | [2]       |
| Suppresses activities of SOD and GSH-PX  | [2]       |
| Attenuates progressive accumulation of type IV collagen  | [2]       |
| Decreases concentrations of the metabolites of monoamines, glutamate, and glutamine  | [3]       |
| Increased the SOD activity of the mitochondrial fraction in the cortex, hippocampus, and striatum  | [3]       |
| Suppresses TBARS formation   | [3]       |
| Reduces the expression of the IL-1 $\beta$ , IL-6, TNF- $\alpha$ mRNA  | [4]       |
| Restores the abnormal activities NOS and levels of CP, MDA, GSH and NO induced by D-gal  | [5]       |
| Attenuates CUS-induced decreases in noradrenaline and dopamine   | [6]       |
| Reverses CUS-induced increase MDA content  | [6]       |
| Suppresses the downregulation of Bcl-2, upregulation of Bax, the release of mitochondrial cytochrome c into cytosol and sequential activation of caspase-9 and caspase-3 | [7]       |
| Reduces 6-OHDA-induced intracellular ROS production and GSH depletion  | [8]       |
| Inhibits mitochondrial membrane instability  | [8]       |
| Protects TH-immunoreactive cells and fibers in the nigrostriatal region from MPTP toxicity   | [9]       |

### Mechanisms underlying DSS-mediated neuroprotection

DSS not only increased the expression of nuclear factor- $\kappa$ B (NF- $\kappa$ B) as well as transforming growth factor- $\beta$  and enhanced the activity of SOD and glutathione peroxidase (GSH-PX), but also attenuated the progressive accumulation of type IV collagen that reflects its antioxidant activity [2]. DSS has an effect on decreasing the concentrations of the metabolites of monoamines, glutamate and glutamine, and increasing SOD activity of the mitochondrial fraction in the cortex, hippocampus and striatum. In addition, DSS significantly suppressed thiobarbituric acid-reactive-substances (TBARS) formation [3]. The impairment of learning acquisition induced by colchicine was attenuated by DSS in the brain [55].

DSS significantly reduced the escape latency and expression of the IL-1 $\beta$ , IL-6, TNF- $\alpha$  mRNA, and neuronal apoptosis by suppressing the level of the nitric oxide (NO) in the hippocampus. Furthermore, DSS shortened the response latency and decreased the error numbers in a step-down passive avoidance test [4]. The abnormal activities of nitric oxide synthase (NOS) and levels of carbonyl protein (CP), malondialdehyde (MDA), glutathione (GSH) and NO induced by D-gal were restored by ethanol extract of DSS (DE). Chronic unpredictable stress (CUS) induced increase in serum MDA content is markedly reversed in mice by DSS [6]. Moreover, by regulating the expression of Bcl-2, Bax and caspase-3, DE improved neuronal survival in the hippocampus of D-gal-treated mice [5]. Recent studies have documented that DSS suppresses the downregulation of Bcl-2, upregulation of Bax, the release of mitochondrial cytochrome c into cytosol and sequential activation of caspase-9 and caspase-3 to protect against H<sub>2</sub>O<sub>2</sub>-induced apoptosis in PC12 cells [7], and DSS also significantly protects dopaminergic neurons from 6-hydroxydopamine (6-OHDA)-induced neurotoxicity and reduces 6-OHDA-induced intracellular reactive oxygen species (ROS) production and GSH depletion and inhibits mitochondrial membrane instability [8]. Furthermore, DSS protects tyrosine hydroxylase (TH)-immunoreactive cells in the nigrostriatal region from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity [9](Table 1).

### Ferulic acid

Ferulic acid (FA), a natural antioxidant and a putative AD neuroprotective compound, is able to reverse morphological defects induced by A $\beta$  oligomers. The antioxidant mechanism includes neutralizing ROS, recovering mitochondrial membrane potential, and

blocking apoptotic pathways [12]. FA can attenuate phosphorylation of ERK1/2 activated by A $\beta$  oligomers and modulate the expression of an antioxidative protein known as Peroxiredoxin [13]. Some studies have indicated that FA greatly attenuates these changes including elevating levels of oxidation as indexed by protein oxidation, lipid peroxidation, and ROS measurement [14]. Moreover, by scavenging free radicals, FA acts as a potent antioxidant compound and has been proposed as a potential treatment for AD [11]. FA not only attenuated impairment induced by mecamylamine (MECA) and scopolamine (SCOP)+MECA as well as central acetylcholinergic neurotoxin ethylcholine mustard aziridinium ion (AF64A), but also activated central muscarinic and nicotinic receptors and antioxidant enzymes, improved cognitive deficits, and could be a key molecule for the development of therapeutics for AD [10].

### Paeoniflorin

DSS have been studied in animals and healthy human volunteers. Paeoniflorin (PF) exerts protection against A $\beta$ -induced neurotoxicity by increases GSH content reduced, suppressing NOS activity and NO level, and decreasing CP and MDA levels [15]. By improving spatial cognitive impairment caused by cholinergic dysfunction, PF may rely on reversal of the muscarinic M1-receptor-mediated inhibition of long-term potentiation (LTP) [16]. PF can modulate Acid-sensing ion channels-(ASIC) activity and protein expression and produce protective effects for PC12 cells against MPP(+) and acidosis-induced cytotoxicity [19]. L-type Ca<sup>2+</sup> channels in NG108-15 cells can be blocked by PF to affect neuronal or neuroendocrine function [17]. In addition, PF is able to produce protective effect on dopaminergic neurodegeneration and significantly attenuate the MPTP-induced toxicity by inhibiting neuroinflammation by activation of an adenosine A1 receptor (A1AR) [20].

A study suggested that PF could ameliorate cerebral hypoperfusion-related learning dysfunction, prevent CA1 neuronal damage, suppress expression of NF- $\kappa$ B [21], and inhibit sodium current in mouse hippocampal CA1 neurons [18]. PF shows an ameliorative effect on the 6-OHDA-induced neurological damage [26] and could activate AIR to produce neuroprotection in cerebral ischemia in the rat [27]. Moreover, PF could attenuate ROS production induced by A $\beta$ 25-35 in SH-SY5Y cells and modulate apoptotic mitochondrial pathway, which includes inhibiting Bax/Bcl-2 ratio, cytochrome c release, decreasing mitochondrial membrane potential and activities of caspase-3 and caspase-9 [22]. PF can reverse neuroinflammatory-induced A $\beta$  clearance and inhibit the

activation of the NALP3 inflammasome, caspase-1, and IL-1 $\beta$  [23]. In the plasma and brain, PF could significantly inhibit upregulation of pro-inflammatory mediators, TNF- $\alpha$ -induced cell apoptosis and neuronal loss are diminished by PF's actions [24]. Furthermore, PF could protect against hypoxia-induced factor-1 $\alpha$  (HIF-1 $\alpha$ ) accumulation and inhibit upregulation of p53 and Bcl-2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3) [25].

### Ligustilide or Z-ligustilide

Studies suggest that ligustilide (LIG) can protect cognitive deficits such as cerebral damage or neurodegenerative disorders. LIG significantly improves behavioral performance of D-gal treated mice. In the brain of D-gal treated mice, LIG is able to reduce cleaved caspase-3 and GFAP levels and the level of MDA as well as increase the activities of Na<sup>+</sup>/K<sup>+</sup>-ATPase and expression of GAP-43 [29]. Lipopolysaccharide-induced upregulation of TLR4 mRNA expression is reduced by LIG dose-dependent in spinal astrocytes [28]. Klotho expression and the AD phenotype was inversely correlated, LIG decreases Akt and Forkhead box class O1 (FOXO1) phosphorylation and upregulates Klotho expression in the cerebral choroid plexus and serum that might contribute to the neuroprotective effect against AD [30].

*In vivo* and *in vitro*, LIG improves NF-E2-related factor 2-(Nrf2) nuclear translocation, and Nrf2 and heme oxygenase-1-(HO-1) protein expression is markedly increased. Furthermore, cell death induced by oxygen-glucose deprivation (OGD) is reduced by LIG treatment [32]. LIG has neuroprotective effect against ischemia-reperfusion injury via upregulation of erythropoietin and inhibiting RTP801 expression [33]. In addition, LIG significantly increases SOD activity and reduces MDA levels, increases choline acetyltransferase activity and inhibits acetylcholinesterase activity in ischemic brain tissues to ameliorate cognitive dysfunction and brain damage induced by permanent forebrain ischemia in rats [34]. LIG significantly increases the Bcl-2 expression and decreases in Bax and caspase-3 immunoreactivities in the ischemic cortex and increases the activities of the antioxidant enzyme GSH-PX and SOD in ischemic brain tissues [35]. BDNF and phosphorylated cAMP-responsive element binding protein (p-CREB) levels and  $\gamma$ -aminobutyric acid (GABA) expression is increased by the component Z-ligustilide of Radix Angelica Sinensis that promotes adult neurogenesis to mediate recovery from cognitive impairment [31].

**Table 2.** Mechanisms underlying neural protection by individual DSS ingredients

| Main component   | Mechanism  | Reference |
|--|--|-----------|
| <b>Ferulic acid</b>  | Attenuated impairment induced by MECA and SCOP plus MECA and central acetylcholinergic neurotoxin ethylcholine mustard aziridinium ion (AF64A).                      | [10]      |
|  | Scavenging free radicals and enhancing the cell stress.  | [11]      |
|  | Reverse morphological defects induced by A $\beta$ oligomers and neutralizing reactive oxygen species.   | [12]      |
|  | Attenuating phosphorylation of ERK1/2 activated by Abeta oligomers and modulating the expression of an anti-oxidative protein Peroxiredoxin.                         | [13]      |
|  | Protection against hydroxyl and peroxy radical oxidation in synaptosomal and neuronal cell culture systems.  | [14]      |
| <b>Paeoniflorin</b>  | Increasing GSH content, suppressing of NOS activity and NO level   | [15]      |
|  | Decreasing of CP and MDA levels  | [15]      |
|  | Rely on reversal of the muscarinic M1-receptor-mediated inhibition of LTP  | [16]      |
|  | Blocking L-type Ca <sup>2+</sup> channels in NG108-15 cells  | [17]      |
|  | Inhibiting sodium current in mouse hippocampal CA1 neurons   | [18]      |
|  | Modulating ASICs activity and protein expression and producing protective effects for PC12 cells against MPP(+) and acidosis-induced cytotoxicity                    | [19]      |
|  | Protecting effect on dopaminergic neurodegeneration and attenuating the MPTP-induced toxicity  | [20]      |
|  | Preventing CA1 neuron damage and suppressing the expression of NF-kappaB in hippocampus  | [21]      |
|  | Inhibiting Bax/Bcl-2 ratio, cytochrome c release and decreasing mitochondrial membrane potential and activity of caspase-3 and caspase-9                             | [22]      |
|  | Upregulating significantly anti-inflammatory cytokines and downregulating proinflammatory cytokines  | [23]      |
|  | Reversing neuroinflammatory-induced activation of NF- $\kappa$ B signaling pathways and inhibiting the activation of NALP3 inflammasome, caspase-1, and IL-1 $\beta$ | [23]      |
|  | Inhibiting up-regulations of pro-inflammatory mediators (TNF $\alpha$ , IL-1 $\beta$ , iNOS, COX(2) and 5-LOX)   | [24]      |
|  | Protecting against hypoxia-induced factor-1 $\alpha$ (HIF-1 $\alpha$ ) accumulation  | [25]      |
| Inhibiting up-regulation of p53 and Bcl-2/adenovirus E1B 19kDa interacting protein 3 (BNIP3) | [25]   |           |
| <b>Paeoniflorin</b>  | Ameliorating effect on the 6-OHDA-induced neurological damage  | [26]      |
|  | Activating A1R to produce the neuroprotection in cerebral ischemia   | [27]      |
| <b>Ligustilide or Z-ligustilide</b>  | Reducing the expression of upregulation of TLR4 mRNA Lipopolysaccharide-induced  | [28]      |
|  | Reducing the level of MDA as well as increasing the activity of Na(+)-K(+)-ATPase  | [29]      |
|  | Raising the expression of GAP-43 and reducing cleaved caspase-3 and GFAP levels  | [29]      |
|  | Decreasing Akt and Forkhead box class O1 phosphorylation and upregulating Klotho expression  | [30]      |
|  | Increasing BDNF and phosphorylated cAMP-responsive element binding protein (p-CREB) levels and $\gamma$ -aminobutyric acid (GABA) expression                         | [31]      |
|  | Improving Nrf2 nuclear translocation and increasing Nrf2 and HO-1 protein expression   | [32]      |
|  | Up-regulating erythropoietin and inhibiting RTP801 expression  | [33]      |
|  | Increasing SOD activity and reducing malondialdehyde levels  | [34]      |
|  | Increasing the Bcl-2 expression and decreasing in Bax and caspase-3 immunoreactivities   | [35]      |
|  | Increasing the activities of the antioxidant enzyme GSH-PX   | [35]      |
| <b>TMPZ</b>  | Inhibiting the expressions of nitrotyrosine and iNOS to mediate the free radical-scavenging activity   | [36]      |
|  | Suppressing prostaglandin E(2) production and lipopolysaccharide/interferon-gamma-induced inflammation in cultured glial cells                                       | [37]      |
|  | Reducing proinflammatory mediator production and cerebral ischemia/reperfusion-induced inflammatory cell activation  | [37]      |
|  | Reducing cerebral I/R-induced internucleosomal DNA fragmentation, caspase-3, caspase-8 and caspase-9 activation, and cytochrome c release                            | [38]      |
|  | Suppressing the consequent production of monocyte chemoattractant protein 1 (MCP-1)  | [38]      |

### Tetramethylpyrazine

Tetramethylpyrazine (TMPZ) is an active ingredient isolated from a commonly used *Ligusticum chuanxiong* Hort., which markedly inhibits the expressions of nitrotyrosine and iNOS to mediate the free radical-scavenging activity in ischemia-reperfusion brain injury [36]. It was reported that TMPZ markedly suppresses prostaglandin E2 production and lipopolysaccharide/interferon-gamma-induced inflammation in cultured glial cells and reduces pro-inflammatory mediator production and cerebral ischemia/reperfusion-induced inflammatory cell activation that significantly protects the brain against ischemic injury [37]. TMPZ is able to reduce cerebral I/R-induced internucleosomal DNA fragmentation, caspase-3, caspase-8 and caspase-9 activation, cytochrome c release, and suppresses the activation of microglia and/or recruitment of inflammatory cells to the ischemic site as well as the consequent production of monocyte chemoattractant protein 1 (MCP-1) [38].

### Atractylodes macrocephalaon polysaccharides

The levels of Bcl-2 and the ratio of Bcl-2/Bax in hypoxic neurons are significantly increased by Atractylodes macrocephalaon polysaccharides (AMPS), suggesting that AMPS can improve neuronal growth and prevent mitochondrial injury and apoptosis of neurons induced by hypoxia [39].

### Ligusticum chuanxiong Hort. extract

Serum TNF- $\alpha$ , IL-6, IL-8, NO, MIP-1 $\alpha$ , CRP and myocardium MDA levels and serum CK, LDH and AST activities are significantly decreased while myocardium Na<sup>+</sup>/K<sup>+</sup>-ATPase, Ca<sup>2+</sup>/Mg<sup>2+</sup>-ATPase, NOS, SOD, CAT, GSH-Px and TAOC activities are increased by the *Ligusticum chuanxiong* Hort. extract [40].

Together, the mechanism of protecting the neuronal damage and cell apoptosis suggest that DSS could provide more useful ways for developing more comprehensive and potent therapeutic strategies for AD (Table 2).

### The effect on the neurotransmitter and the central nervous system

Alcohol poisoning decreased the activity of choline acetyltransferase (ChAT) and the content of noradrenaline (NA) in forebrain areas such as the cerebral cortex and hippocampus, which contribute to deficiencies in subcortical noradrenergic and cholinergic systems [43]. DSS treatment markedly inhibited CUS-induced decreases in NA and DA concentrations in mouse brain [6]. Moreover, DSS modulates metabolism of monoamine

neurotransmitters including increasing the content of norepinephrine (NE), DA and 5-hydroxytryptamine (5-HT) in brains of aged mice while protecting the cortical ultrastructure [41], which may be the major mechanism of DSS for improving memory dysfunction induced by alcohol. In addition, DSS not only increased ACh and blood flow in intact rats, it also increased ACh in ischemic rats in the dorsal hippocampus (DH), suggesting that DSS can improve the cognition [49].

Single administration of DSS decreased the content of NE in the hippocampus, but increased the contents of DA and HVA in the cerebral cortex and the hippocampus, while repeated administration of DSS, increased the contents of NE, MHPG, DOPAC, 5-HT and 5-HIAA in the cerebral cortex, and NE and DA in the corpus striatum [44]. However, these SCOP-induced decreases in ACh levels were significantly inhibited by a single administration of DSS at 500 mg/kg. In the mouse brain, dysfunction of the central cholinergic nervous system and SCOP-induced decrease in ACh levels were ameliorated by DSS [45]. After oral administration of DSS and a 3-month latency period, the step-through test was significantly prolonged and the content of monoamine neurotransmitters such as NE, DA, and 5-HT were increased in the brains of aged mice and the cortical ultrastructure was protected [48]. DSS administered in a dose-dependent manner significantly suppressed the stress-induced enhancement of hypothalamic NA turnover at a low dose level [56].

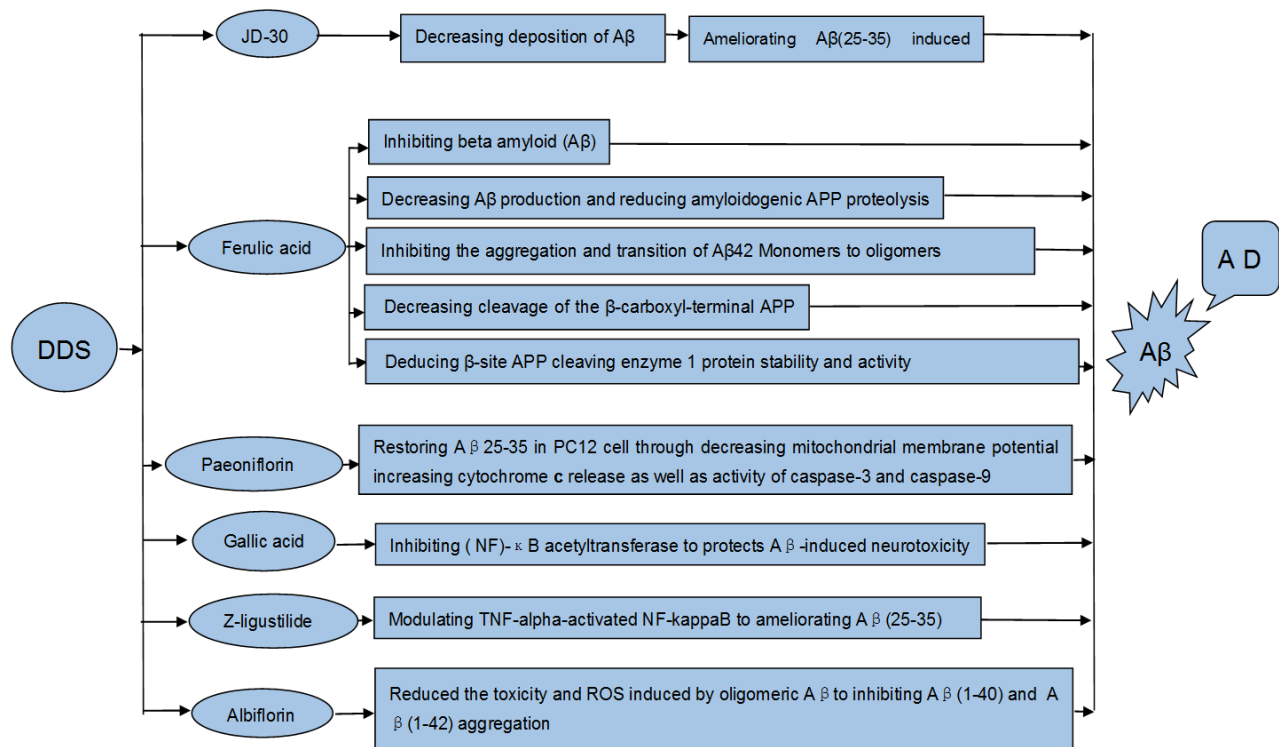
DOPAC and HVA in the olfactory bulb of olfactory-lesioned mice were significantly suppressed by the administration of DSS. DSS can make NGF contents increase at 1 and 2 weeks and within 3 weeks, the expression returns to baseline [46]. Administration of DSS could decrease ChAT activity significantly in the cerebral cortex and the DH of ovariectomized mice along with a decrease of NE contents [47].

Glutamate is the major excitatory neurotransmitter in the brain and has effect on cognition and memory, but alterations of glutamatergic signaling can induce excitotoxicity [57, 58, 59], which is linked to several neurodegenerative disorders and cell death such as AD [64, 66]. Repeated cerebral ischemia markedly decreased GluR2 flop mRNA at 1 and 3 days, but the decrease in GluR2 flop was significantly suppressed by DSS (300 mg/kg) at 3 days [64]. DSS decreases the concentration of glutamate in the cortex, hippocampus, and striatum of female and male SAMP8 and has effect on glutamate, and monoamine metabolites in the aged rat brain [3, 50].

Somatomedin C/insulin-like growth factor 1 (IGF-1) level in medium from the rat corpora lutea incubated *in vitro* was regulated by the effect of herbal components of DSS [51]. DSS has a neuroendocrine effect on ovulation in immature female rats [52]. The Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup>

current components were decreased by DSS in voltage-clamped NG108-15 cells and the peak heights of the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  current components were strongly decreased in the hybrid cells [54]. DSS decreased the content of arginine vasopressin (AVP) in the pituitary and the

expression of AVP mRNA in hypothalamus [42]. In addition, albiflorin can maintain the intracellular  $\text{Ca}^{2+}$  concentration [86] (Fig. 1).



**Figure 1.** DSS-mediated resolution of  $\text{A}\beta$  in Alzheimer's Disease

### To improve the cognitive ability of memory and reduce the damage of amyloid protein

The impairment of learning acquisition induced by colchicine was attenuated by DSS while the level of SOD ( $141 \pm 3$  and  $135.4 \pm 2.0$ ) in the brain was increased by DSS (0.5 and 1.0 g/kg) [60]. DSS treatment tended to improve the score for orientation to place on the Mini Mental State Examination and made regional cerebral blood flow (rCBF) in the posterior cingulate significantly higher [61]. DSS significantly improved erythrocyte differentiation in iron-deficiency anemia, increased the proportion of normal erythrocytes and erythroblasts as well as erythropoietin and transferrin levels in the blood. It also possessed anemia-ameliorating efficacy [62].

$\text{A}\beta$  is a main factor in the pathogenesis of AD [96-100]. DSS inhibited  $\text{A}\beta_{25-35}$ -induced neuronal damage and lactate dehydrogenase (LDH). The  $\text{A}\beta_{25-35}$ -induced neuronal death and lipid peroxidation were significantly reduced by DSS at concentrations of 100 and 300  $\mu\text{g}/\text{mL}$  [63]. Through DSS administration, the SCOP-induced impairment including reference and working memory deficits of a rat's spatial cognition determined in the eight-armed radial maze test was decreased [65]. DSS could also improve the response in the memory-retention test in SAMP8 mice [67]. In addition, JD-30 that is extracted from DSS could improve cognitive dysfunction of mice induced by intracerebroventricular injection of  $\text{A}\beta$ , ameliorate the reduction of LTP, reduce

the neuronal damage in the hippocampus, decrease the prolonged latency in the Morris water-maze test as well as the content and deposition of A $\beta$  in the brains of SAMP8 mice [68-69].

Amyloid Precursor Protein forms ratio (APPr), independent of disease severity, was influenced by cholesterol levels suggesting that cholesterol affects APP processing *in vivo* [70]. The small natural molecule ferulic acid can inhibit A $\beta$  *in vitro* in the early stages of A $\beta$ -fibrillogenesis [76]. FA can improve hyperactivity, object recognition, and spatial, working, and reference memories through decreasing A $\beta$  production and reducing amyloidogenic APP proteolysis [77]. FA reduced IL-1 $\beta$  levels and amyloid deposition in the frontal cortex [78] while pretreatment with FA could suppress increases in immunoreactivities of glial fibrillary acidic protein, the astrocyte marker, and IL-1 $\beta$  in the hippocampus with A $\beta$ 1-42 [79]. FA is likely to inhibit the aggregation of A $\beta$ 42 oligomers; it blocks the hydrogen bond that forms  $\beta$ -sheets thereby interrupting the transition of A $\beta$ 42 monomers to oligomers [80]. FA can decrease cleavage of the  $\beta$ -carboxyl-terminal APP fragment and reduce  $\beta$ -site APP cleaving enzyme 1 protein stability and activity with therapeutic potential against AD [81].

Through decreasing mitochondrial membrane potential, increasing cytochrome c release as well as activities of caspase-3 and caspase-9, A $\beta$  25-35 in PC12 cells can be attenuated by PF [82]. Through modulating the TNF- $\alpha$ -activated NF- $\kappa$ B signaling pathway, LIG ameliorated these neurotoxic effects of A $\beta$ 25-35 [83]. Gallic acid (GA), is a NF- $\kappa$ B acetyltransferase inhibitor that protects neuronal cells from A $\beta$ -induced neurotoxicity and restores A $\beta$ -induced cognitive dysfunction [84]. A $\beta$  aggregation and/or the formation of A $\beta$ -derived diffusible neurotoxin ligands were inhibited by GA [85]. Albiflorin reduced the toxicity and ROS induced by both monomeric and oligomeric A $\beta$  species to exhibit a potent inhibitory effect on A $\beta$ 1-40 and A $\beta$ 1-42 aggregation. In addition, albiflorin can maintain the intracellular Ca<sup>2+</sup> concentration [86].

### The effect of DSS on the hypothalamus-pituitary-gonadal axis

Multiple factors lead to AD. Estrogen is a potent factor that may play an important role in the preservation of vascular disease and improve blood flow in regions of the brain affected by AD [73]. DSS increases the latency in step-down test especially in female animals. In the MWM test, SAMP8 mice that were administered DSS orally spent less time finding the platform. DSS could increase the estradiol (E2), NO, and glycine, and ameliorate deterioration of cognition in SAMP8 mice, especially in females [71]. After oral administration of

DSS for 3 weeks, melatonin level was significantly increased at night. Phosphorylation of CREB can be significantly increased by DSS. DSS also had an effect on the beta-adrenergic receptors binding in the pineal glands [72]. *In vivo*, both DSS and Keishibukuryogan can stimulate the production of estradiol-17  $\beta$ , progesterone, and testosterone by preovulatory follicles, but DSS stimulation was more effective than Keishibukuryogan [74]. Further research show that DSS could stimulate the cerebral cortex to produce nicotinic acetylcholine receptors, which directly impacts the brain to accelerate the process of neuroendocrine regulation of ovulation. In addition, Levistilide A is a main component in *Rhizoma Chuanxiong* when paired with DSS, could significantly increase its bioavailability compared with LA alone and *Rhizoma Chuanxiong* alone [75].

### The effect of DSS on metabolic risk factors of AD

Several other studies have also reported that hypercholesterolemia is a potentially modifiable environmental risk factor for AD [92]. Hypercholesterolemia within the membrane is likely to impact APP trafficking, APP metabolism, activities of  $\beta$ ,  $\gamma$  and  $\alpha$  secretases and A $\beta$  synthesis [93, 94]. In addition, other studies have showed an aggregated relative risk of approximately 2.2 that links type 2 diabetes with AD [95]. Type 2 diabetes leads to cognitive decline and increases the risk of late-onset AD. However, hypercholesterolemia and type 2 diabetes are an important risk factor linking AD.

Dietary vitamin E and FA are beneficial for hypercholesterolemia. In particular, FA was added to the vitamin E-rich Western diet that could increase activities of antioxidant enzymes (SOD, catalase, and GSH-PX) and paraoxonase [87]. GA has potent anti-oxidative and anti-obesity activities and can protect against hypercholesterolemia [88]. Moreover, DSS treatment can effectively lower the higher renal levels of Advanced Glycation End Products (AGEs) in streptozotocin (STZ)-diabetic rats [90]. DSS reduces the activities of SOD and GSH-Px in the kidneys of STZ-diabetic rats [91]. FA can also significantly decrease blood glucose and increase serum adiponectin levels for the FA-treated OLETF rats [89]. However, DSS has effects on metabolic risk factors of AD: hypercholesterolemia and type 2 diabetes.

### Conclusion

AD is a complicated brain disease that involves multiple mechanisms. Therefore, DSS might exert beneficial effects on AD. In fact, investigations clearly show that it has recently been a hot target of many research studies related to a strong relationship among the chemical, biological, pharmacological, and medical properties of

these plants. Interestingly, studies indicate that the traditional formula of DSS has potential disease-modifying properties including its well proven effects of anti-inflammation and antioxidant activities, neuroprotective/neurotrophic activities, regulation of the neurotransmitter and the central nervous systems, and modulation of amyloid precursor protein metabolism. Therefore, it might not only contribute to symptomatic improvement, but also play important roles for the treatment of neurodegenerative diseases by interfering with key factors of the disease. These encouraging findings suggest that DSS is a promising candidate for AD.

### Acknowledgment

This work is supported by the Grant (No. 2013BJ10) from the Science Foundation of Heilongjiang Institute of Technology.

### Conflict of Interest?

There are no conflicts of interest.

### Reference

- [1] Chen L, Qi J, Chang YX, Zhu D, Yu B (2009). Identification and determination of the major constituents in Traditional Chinese Medicinal formula Danggui-Shaoyao-San by HPLC-DAD-ESI-MS/MS. *J Pharm Biomed Anal*, 50:127-137.
- [2] Liu IM, Tzeng TF, Liou SS, Chang CJ (2012). Beneficial effect of traditional Chinese medicinal formula danggui-shaoyao-san on advanced glycation end-product-mediated renal injury in streptozotocin-diabetic rats. *Evid Based Complement Alternat Med*, 2012:140103.
- [3] Ueda Y, Kamasu M, Hiramatsu M (1996). Free radical scavenging activity of the Japanese herbal medicine toki-shakuyaku-san (TJ-23) and its effect on superoxide dismutase activity, lipid peroxides, glutamate, and monoaminemetabolites in aged rat brain. *Neurochem Res*, 21:909-914.
- [4] Zhong S, Ma S, Hong Z, Jin X (2011). Anti-inflammation effect of danggui shaoyao san on Alzheimer's diseases. *Zhongguo Zhong Yao Za Zhi*, 36:3155-3160.
- [5] Lan Z, Liu J, Chen L, Fu Q, Luo J, Qu R, et al (2012). Danggui-Shaoyao-San ameliorates cognition deficits and attenuates oxidative stress-related neuronal apoptosis in d-galactose-induced senescent mice. *J Ethnopharmacol*, 141:386-395.
- [6] Huang Z, Mao QQ, Zhong XM, Li ZY, Qiu FM, Ip SP (2012). Mechanistic Study on the Antidepressant-Like Effect of Danggui-Shaoyao-San, a Chinese Herbal Formula. *Evid Based Complement Alternat Med*, 2012: 173565.
- [7] Qian YF, Wang H, Yao WB, Gao XD (2008). Aqueous extract of the Chinese medicine, Danggui-Shaoyao-San, inhibits apoptosis in hydrogen peroxide-induced PC12 cells by preventing cytochrome c release and inactivating of caspase cascade. *Cell Biol Int*, 32:304-311.
- [8] Hwang DS, Kim HG, Kwon HJ, Cho JH, Lee CH, Lee JM, et al (2011). Dangguijakyak-san, a medicinal herbal formula, protects dopaminergic neurons from 6-hydroxydopamine-induced neurotoxicity. *J Ethnopharmacol*, 133: 934-939.
- [9] Lee JM, Hwang DS, Kim HG, Lee CH, Oh MS (2012). Dangguijakyak-san protects dopamine neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity under postmenopausal conditions. *J Ethnopharmacol*, 139:883-888.
- [10] Tsai FS, Wu LY, Yang SE, Cheng HY, Tsai CC, Wu CR, et al (2015). Ferulic acid reverses the cognitive dysfunction caused by amyloid  $\beta$  peptide 1-40 through anti-oxidant activity and cholinergic activation in rats. *Am J Chin Med*, 43:319-335.
- [11] Mancuso C, Santangelo R (2014). Ferulic acid: pharmacological and toxicological aspects. *Food Chem Toxicol*, 65:185-195.
- [12] Picone P, Nuzzo D, Di Carlo M (2013). Ferulic acid: a natural antioxidant against oxidative stress induced by oligomeric A-beta on sea urchin embryo. *Biol Bull*, 224: 18-28.
- [13] Picone P, Bondi ML, Montana G, Bruno A, Pitarresi G, Giammona G, et al (2009). Ferulic acid inhibits oxidative stress and cell death induced by Ab oligomers: improved delivery by solid lipid nanoparticles. *Free Radic Res*, 43:1133-1145.
- [14] Kanski J, Aksenova M, Stoyanova A, Butterfield DA (2002). Ferulic acid antioxidant protection against hydroxyl and peroxy radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure-activity studies. *J Nutr Biochem*, 13: 273-281.
- [15] Zhong SZ, Ge QH, Li Q, Qu R, Ma SP (2009). Peoniflorin attenuates  $A\beta$  ((1-42))-mediated neurotoxicity by regulating calcium homeostasis and ameliorating oxidative stress in hippocampus of rats. *J Neurol Sci*, 280: 71-78.
- [16] Tabata K, Matsumoto K, Watanabe H (2000). Paeoniflorin, a major constituent of peony root, reverses muscarinic M1-receptor antagonist-induced suppression of long-term potentiation in the rat hippocampal slice. *Jpn J Pharmacol*, 83: 25-30.
- [17] Tsai TY, Wu SN, Liu YC, Wu AZ, Tsai YC (2005). Inhibitory action of L-type  $Ca^{2+}$  current by paeoniflorin, a major constituent of peony root, in NG108-15 neuronal cells. *Eur J Pharmacol*, 523: 16-24.
- [18] Zhang GQ, Hao XM, Chen SZ, Zhou PA, Cheng HP, Wu CH (2003). Blockade of paeoniflorin on sodium current in mouse hippocampal CA1 neurons. *Acta Pharmacol Sin*, 24: 1248-1252.
- [19] Sun X, Cao YB, Hu LF, Yang YP, Li J, Wang F, et al (2011). ASICs mediate the modulatory effect by paeoniflorin on  $\alpha$ -synuclein autophagic degradation. *Brain Res*, 1396:77-87.



- [20] Liu HQ, Zhang WY, Luo XT, Ye Y, Zhu XZ (2006). Paeoniflorin attenuates neuroinflammation and dopaminergic neurodegeneration in the MPTP model of the Parkinson's disease by activation of a adenosine A1 receptor. *Br J Pharmacol*, 148: 314-325.
- [21] Liu J, Jin DZ, Xiao L, Zhu XZ (2006). Paeoniflorin attenuates chronic cerebral hypoperfusion-induced learning dysfunction and brain damage in rats. *Brain Res*, 1089: 162-170.
- [22] Wang K, Zhu L, Zhu X, Zhang K, Huang B, Zhang J, et al (2014). Protective effect of paeoniflorin on A $\beta$ 25-35-induced SH-SY5Y cell injury by preventing mitochondrial dysfunction. *Cell Mol Neurobiol*, 34: 227-234.
- [23] Zhang HR, Peng JH, Cheng XB, Shi BZ, Zhang MY, Xu RX (2015). Paeoniflorin Attenuates Amyloidogenesis and the Inflammatory Responses in a Transgenic Mouse Model of Alzheimer's Disease. *Neurochem Res*, 40: 1583-1592.
- [24] Guo RB, Wang GF, Zhao AP, Gu J, Sun XL, Hu G (2012). Paeoniflorin protects against ischemia-induced brain damages in rats via inhibiting MAPKs/NF- $\kappa$ B-mediated inflammatory responses. *PLoS One*, 7: e49701.
- [25] Ji Q, Yang L, Zhou J, Lin R, Zhang J, Lin Q, et al (2012). Protective effects of paeoniflorin against cobalt chloride-induced apoptosis of endothelial cells via HIF-1 $\alpha$  pathway. *Toxicol In Vitro*, 26: 455-461.
- [26] Liu DZ, Zhu J, Jin DZ, Zhang LM, Ji XQ, Ye Y, et al (2007). Behavioral recovery following sub-chronic paeoniflorin administration in the striatal 6-OHDA lesion rodent model of Parkinson's disease. *J Ethnopharmacol*, 112: 327-332.
- [27] Liu DZ, Xie KQ, Ji XQ, Ye Y, Jiang CL, Zhu XZ, et al (2005). Neuroprotective effect of paeoniflorin on cerebral ischemic rat by activating adenosine A1 receptor in a manner different from its classical agonists. *Neurochem Res*, 30: 604-611.
- [28] Qian B, Li F, Zhao LX, Dong YL, Gao YJ, Zhang ZJ (2015). Ligustilide Ameliorates Inflammatory Pain and Inhibits TLR4 Upregulation in Spinal Astrocytes Following Complete Freund's Adjuvant Peripheral Injection. *Cell Mol Neurobiol*, First online: 27 June 2015
- [29] Li JJ, Zhu Q, Lu YP, Zhao P, Feng ZB, Qian ZM, et al (2015). Ligustilide prevents cognitive impairment and attenuates neurotoxicity in D-galactose induced aging mice brain. *Brain Res*, 1595:19-28.
- [30] Kuang X, Chen YS, Wang LF, Li YJ, Liu K, Zhang MX, et al (2014). Klotho upregulation contributes to the neuroprotection of ligustilide in an Alzheimer's disease mouse model. *Neurobiol Aging*, 35: 169-178.
- [31] Xin J, Zhang J, Yang Y, Deng M, Xie X (2013). *Radix Angelica Sinensis* that contains the component Z-ligustilide promotes adult neurogenesis to mediate recovery from cognitive impairment. *Curr Neurovasc Res*, 10: 304-315.
- [32] Peng B, Zhao P, Lu YP, Chen MM, Sun H, Wu XM, et al (2013). Z-ligustilide activates the Nrf2/HO-1 pathway and protects against cerebral ischemia-reperfusion injury in vivo and in vitro. *Brain Res*, 1520: 168-177.
- [33] Wu XM, Qian ZM, Zhu L, Du F, Yung WH, Gong Q, et al (2011). Neuroprotective effect of ligustilide against ischemia-reperfusion injury via up-regulation of erythropoietin and down-regulation of RTP801. *Br J Pharmacol*. 164: 332-343.
- [34] Kuang X, Du JR, Liu YX, Zhang GY, Peng HY (2008). Postischemic administration of Z-Ligustilide ameliorates cognitive dysfunction and brain damage induced by permanent forebrain ischemia in rats. *Pharmacol Biochem Behav*. 88: 213-221.
- [35] Kuang X, Yao Y, Du JR, Liu YX, Wang CY, Qian ZM (2006). Neuroprotective role of Z-ligustilide against forebrain ischemic injury in ICR mice. *Brain Res*. 1102: 145-153.
- [36] Hsiao G, Chen YC, Lin JH, Lin KH, Chou DS, Lin CH, et al (2006). Inhibitory mechanisms of tetramethylpyrazine in middle cerebral artery occlusion (MCAO)-induced focal cerebral ischemia in rats. *Planta Med*, 72: 411-417.
- [37] Liao SL, Kao TK, Chen WY, Lin YS, Chen SY, Raung SL, et al (2004). Tetramethylpyrazine reduces ischemic brain injury in rats. *Neurosci Lett*, 372: 40-45.
- [38] Kao TK, Ou YC, Kuo JS, et al (2006). Neuroprotection by tetramethylpyrazine against ischemic brain injury in rats. *Neurochem Int*. 48: 166-176.
- [39] Hu WX, Xiang Q, Wen Z, He D, Wu XM, Hu GZ, et al (2014). Neuroprotective effect of *Atractylodes macrocephala* polysaccharides in vitro on neuronal apoptosis induced by hypoxia. *Mol Med Rep*, 9: 2573-2581.
- [40] Zengyong Q, Jiangwei M, Huajin L (2011). Effect of *Ligusticum wallichii* aqueous extract on oxidative injury and immunity activity in myocardial ischemic reperfusion rats. *Int J Mol Sci*, 12: 1991-2006.
- [41] Kou J, Zhu D, Yan Y (2005). Neuroprotective effects of the aqueous extract of the Chinese medicine Danggui-Shaoyao-san on aged mice. *J Ethnopharmacol*, 97: 313-318.
- [42] Xu F, Peng D, Tao C, Yin D, Kou J, Zhu D, et al (2011). Anti-depression effects of Danggui-Shaoyao-San, a fixed combination of Traditional Chinese Medicine, on depression model in mice and rats. *Phytomedicine*, 18: 1130-1136.
- [43] Arendt T, Allen Y, Sinden J, Schugens MM, Marchbanks RM, Lantos PL, et al (1988). Cholinergic-rich brain transplants reverse alcohol-induced memory deficits. *Nature*, 332:448.
- [44] Itoh T, Murai S, Saito H, et al (1998). Effects of single and repeated administrations of Toki-shakuyaku-san on the concentrations of brain neurotransmitters in mice. *Methods Find Exp Clin Pharmacol*, 20(1):11-17.
- [45] Itoh T, Michijiri S, Murai S, Saito H, Nakamura K, Itsukaichi O, et al (1996). Regulatory effect of danggui-shaoyao-san on central cholinergic nervous system dysfunction in mice. *Am J Chin Med*, 24: 205-217.
- [46] Song QH, Toriizuka K, Jin GB, Yabe T, Cyong JC (2001). Long term effects of Toki-shakuyaku-san on

- brain dopamine and nerve growth factor in olfactory-bulb-lesioned mice. *Jpn J Pharmacol*, 86: 183-188.
- [47] Toriizuka K, Hou P, Yabe T, Iijima K, Hanawa T, Cyong JC (2000). Effects of Kampo medicine, Tokishakuyaku-san (Tang-Kuei-Shao-Yao-San), on choline acetyltransferase activity and norepinephrine contents in brain regions, and mitogenic activity of splenic lymphocytes in ovariectomized mice. *J Ethnopharmacol*, 71: 133-143.
- [48] Kou J, Zhu D, Yan Y (2005). Neuroprotective effects of the aqueous extract of the Chinese medicine Danggui-Shaoyao-san on aged mice. *J Ethnopharmacol*, 97:313-318.
- [49] Hatip-Al-Khatib I, Hatip FB, Yoshimitsu Y, Iwasaki K, Egashira N, Liu AX, et al (2007). Effect of Tokishakuyaku-san on acetylcholine level and blood flow in dorsal hippocampus of intact and twice-repeated ischemic rats. *Phytother Res*, 21:291-294.
- [50] Komatsu M, Ueda Y, Hiramatsu M (1999). Different changes in concentrations of monoamines and their metabolites and amino acids in various brain regions by the herbal medicine/Toki-Shakuyaku-San between female and male senescence-accelerated mice (SAMP8). *Neurochem Res*, 24:825-831.
- [51] Usuki S (1991). Blended effects of herbal components of tokishakuyakusan on somatomedin C/insulin-like growth factor 1 level in rat corpus luteum. *Am J Chin Med*, 19: 61-64.
- [52] Koyama T, Hagino N, Cothron AW, Saito M (1989). Neuroendocrine effect of toki-shakuyaku-san on ovulation in rats. *Am J Chin Med*, 17: 29-33.
- [53] Kitabayashi Y, Shibata K, Nakamae T, Narumoto J, Fukui K (2007). Effect of traditional Japanese herbal medicine tokishakuyakusan for mild cognitive impairment: SPECT study, *Psychiatry and Clinical Neurosciences*, 61: 447-448.
- [54] Enomoto K, Higashida H, Maeno T (1992). Effects of toki-shakuyaku-san (Tsumura TJ-23) on electrical activity in neuroblastoma cells and frog neuromuscular junctions. *Neurosci Res*, 15: 81-89.
- [55] Lu MC (2001). Danggui shaoyao san improve colchicine-induced learning acquisition impairment in rats. *Acta Pharmacol Sin*, 22:1149-1153.
- [56] Iizuka S, Ishige A, Komatsu Y, Matsumiya T, Inazu M, Takeda H (1998). Effects of Toki-shakuyaku-san on electric footshock stress in ovariectomized mice. *Methods Find Exp Clin Pharmacol*, 20: 39-46.
- [57] Rudy CC, Hunsberger HC, Weitzner DS, Reed MN2 (2015). The Role of the Tripartite Glutamatergic Synapse in the Pathophysiology of Alzheimer's Disease. *Aging and Disease*, 6: 131-148.
- [58] Curtis DR, Phillis JW, Watkins JC (1960). The chemical excitation of spinal neurones by certain acidic amino acids. *The Journal of physiology*, 150: 656-682.
- [59] Sheldon AL, Robinson MB (2007). The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. *Neurochemistry international*, 51:333-355.
- [60] Lu MC (2001). Danggui shaoyao san improve colchicine-induced learning acquisition impairment in rats. *Acta Pharmacol Sin*, 22: 1149-1153.
- [61] Matsuoka T, Narumoto J, Shibata K, Okamura A, Taniguchi S, Kitabayashi Y, et al (2012). Effect of tokishakuyaku-san on regional cerebral blood flow in patients with mild cognitive impairment and Alzheimer's disease. *Evid Based Complement Alternat Med*, 2012:245091.
- [62] Akase T, Hihara E, Shimada T, Kojima K, Akase T, Tashiro S, Aburada M (2007). Efficacy of Tokishakuyakusan on the anemia in the iron-deficient pregnant rats. *Biol Pharm Bull*, 30: 1523-1528.
- [63] Egashira N, Iwasaki K, Akiyoshi Y, Takagaki Y, Hatip-Al-Khatib I, Mishima K, et al (2005). Protective effect of Toki-shakuyaku-san on amyloid beta25-35-induced neuronal damage in cultured rat cortical neurons. *Phytother Res*, 19:450-453.
- [64] Pu F, Mishima K, Egashira N, et al (2005). Post-ischemic treatment with toki-shakuyaku-san (tang-gui-shao-yao-san) prevents the impairment of spatial memory induced by repeated cerebral ischemia in rats. *Am J Chin Med*, 33:475-489.
- [65] Hatip-Al-Khatib I, Egashira N, Mishima K, Iwasaki K, Iwasaki K, Kurauchi K, et al (2004). Determination of the effectiveness of components of the herbal medicine Toki-Shakuyaku-San and fractions of *Angelica acutiloba* in improving the scopolamine-induced impairment of rat's spatial cognition in eight-armed radial maze test. *J Pharmacol Sci*, 96: 33-41.
- [66] Hardingham GE, Bading H (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat Rev Neurosci*, 11: 682-696.
- [67] Mizushima Y, Kan S, Yoshida S, Irie Y, Urata Y (2003). Effect of Choto-san, a Kampo medicine, on impairment of passive avoidance performance in senescence accelerated mouse (SAM). *Phytother Res*, 17:542-545.
- [68] Hu ZY, Liu G, Yuan H, Yang S, Zhou WX, Zhang YX, et al (2010). Danggui-Shaoyao-San and its active fraction JD-30 improve A $\beta$ -induced spatial recognition deficits in mice. *J Ethnopharmacol*, 128: 365-372.
- [69] Hu ZY, Liu G, Cheng XR, Huang Y, Yang S, Qiao SY, et al (2012). JD-30, an active fraction extracted from Danggui-Shaoyao-San, decreases  $\beta$ -amyloid content and deposition, improves LTP reduction and prevents spatial cognition impairment in SAMP8 mice. *Exp Gerontol*, 47: 14-22.
- [70] Borroni B, Colciaghi F, Lenzi GL, Caimi L, Cattabeni F, Di Luca M, et al (2003). High cholesterol affects platelet APP processing in controls and in AD patients. *Neurobiol Aging*, 24: 631-636.
- [71] Huang Y, Hu ZY, Yuan H, Shu L, Liu G, Qiao SY, et al (2014). Danggui-Shaoyao-San Improves Learning and Memory in Female SAMP8 via Modulation of Estradiol. *Evid Based Complement Alternat Med*, 2014:327294.
- [72] Qu HG, Cheng SW, Tian RB, Li ZL, Lei WL, Wang HQ, et al (2008). Effects of the aqueous extract of the

- Chinese medicine Danggui-Shaoyao-San on rat pineal melatonin synthesis. *Neuro Endocrinol Lett*, 29: 366-372.
- [73] Birge-SJ (1997). The role of estrogen in the treatment of Alzheimer's disease. *Neurology*, 48:S36.
- [74] Usuki S (1990). Effects of tokishakuyakusan and keishibukuryogan on steroidogenesis by rat preovulatory follicles in vivo. *Am J Chin Med*, 18: 149-156.
- [75] He WQ, Lv WS, Zhang Y, Qu Z, Wei RR, Zhang L, et al (2015). Study on Pharmacokinetics of Three Preparations from Levistolide A by LC-MS-MS. *J Chromatogr Sci*, 53: 1265-1273.
- [76] Bramanti E, Fulgentini L, Bizzarri R, Lenci F, Sgarbossa A (2013).  $\beta$ -Amyloid amorphous aggregates induced by the small natural molecule ferulic acid. *J Phys Chem B*, 117: 13816-13821.
- [77] Mori T, Koyama N, Guillot-Sestier MV, Tan J, Town T (2013). Ferulic acid is a nutraceutical  $\beta$ -secretase modulator that improves behavioral impairment and alzheimer-like pathology in transgenic mice. *PLoS One*, 8: e55774.
- [78] Yan JJ, Jung JS, Kim TK, Hasan A, Hong CW, Nam JS, et al (2013). Protective effects of ferulic acid in amyloid precursor protein plus presenilin-1 transgenic mouse model of Alzheimer disease. *Biol Pharm Bull*, 36: 140-143.
- [79] Cho JY, Kim HS, Kim DH, Yan JJ, Suh HW, Song DK (2005). Inhibitory effects of long-term administration of ferulic acid on astrocyte activation induced by intracerebroventricular injection of beta-amyloid peptide (1-42) in mice. *Prog Neuropsychopharmacol Biol Psychiatry*, 29: 901-907.
- [80] Cui L, Zhang Y, Cao H, Wang Y, Teng T, Ma G, et al (2013). Ferulic Acid Inhibits the Transition of Amyloid- $\beta$ 2 Monomers to Oligomers but Accelerates the Transition from Oligomers to Fibrils. *J Alzheimers Dis*, 37: 19-28.
- [81] Mori T, Koyama N, Guillot-Sestier MV, Tan J, Town T (2013). Ferulic acid is a nutraceutical  $\beta$ -secretase modulator that improves behavioral impairment and Alzheimer-like pathology in transgenic mice. *PLoS One*, 8:e55774.
- [82] Li J, Ji X, Zhang J, Shi G, Zhu X, Wang K (2014). Paeoniflorin attenuates A $\beta$ 25-35-induced neurotoxicity in PC12 cells by preventing mitochondrial dysfunction. *Folia Neuropathol*, 52: 285-290.
- [83] Kuang X, Du JR, Chen YS, Wang J, Wang YN (2009). Protective effect of Z-ligustilide against amyloid beta-induced neurotoxicity is associated with decreased pro-inflammatory markers in rat brains. *Pharmacol Biochem Behav*, 92: 635-641.
- [84] Kim MJ, Seong AR, Yoo JY, Jin CH, Lee YH, Kim YJ, et al (2011). Gallic acid, a histone acetyltransferase inhibitor, suppresses  $\beta$ -amyloid neurotoxicity by inhibiting microglial-mediated neuroinflammation. *Mol Nutr Food Res*, 55: 1798-1808.
- [85] Bastianetto S, Yao ZX, Papadopoulos V, Quirion R (2006). Neuroprotective effects of green and black teas and their catechin gallate esters against beta-amyloid-induced toxicity. *Eur J Neurosci*, 23: 55-64.
- [86] Ho SL, Poon CY, Lin C, Yan T, Kwong DW, Yung KK, et al (2015). Inhibition of  $\beta$ -amyloid aggregation by albiflorin, aloemodin and neohesperidin and their neuroprotective effect on primary hippocampal cells against  $\beta$ -amyloid induced toxicity. *Curr Alzheimer Res*, 12: 424-433.
- [87] Kwon EY, Cho YY, Do GM, Kim HJ, Jeon SM, Park YB, et al (2009). Actions of ferulic acid and vitamin E on prevention of hypercholesterolemia and atherogenic lesion formation in apolipoprotein E-deficient mice. *J Med Food*, 12: 996-1003.
- [88] Chao J, Huo TI, Cheng HY, Tsai JC, Liao JW, Lee MS, et al (2014). Gallic acid ameliorated impaired glucose and lipid homeostasis in high fat diet-induced NAFLD mice. *PLoS One*, 9: e96969.
- [89] Choi R, Kim BH, Naowaboot J, Lee MY, Hyun MR, Cho EJ, et al (2011). Effects of ferulic acid on diabetic nephropathy in a rat model of type 2 diabetes. *Exp Mol Med*, 43: 676-683.
- [90] Chakrabarti S, Sinha M, Thakurta IG, Banerjee P, Chattopadhyay M (2013). Oxidative stress and amyloid beta toxicity in Alzheimer's disease: intervention in a complex relationship by antioxidants. *Curr Med Chem*, 20: 4648-4664.
- [91] Santos RX, Correia SC, Wang X, Perry G, Smith MA, Moreira PI, et al (2010). A synergistic dysfunction of mitochondrial fission/fusion dynamics and mitophagy in Alzheimer's disease. *J Alzheimers Dis*, 20: S401-S412.
- [92] Evans RM, Emsley CL, Gao S, Sahota A, Hall KS, Farlow MR, et al (2000). A synergistic dysfunction of mitochondrial fission/fusion dynamics and: a population-based study of African Americans. *Neurology*, 54: 240-242.
- [93] Wolozin B (2001). A fluid connection: cholesterol and A $\beta$ . *Proc Natl Acad Sci USA*, 98: 5371-5373.
- [94] Puglielli L, Tanzi RE, Kovacs DM (2003). Alzheimer's disease: the cholesterol connection. *Nat Neurosci*, 6: 345-351.
- [95] Luchsinger JA, Tang MX, Shea S, Mayeux R (2004). Hyperinsulinemia and risk of Alzheimer disease. *Neurology*, 63:1187-1192.
- [96] Masters CL, Beyreuther K (1987). Neuronal origin of cerebral amyloidogenic proteins: their role in Alzheimer's disease and unconventional virus diseases of the nervous system. *Ciba Foundation symposium*, 126: 49-64.
- [97] Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, et al. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature*, 325: 733-736.
- [98] Glenner GG, Murphy MA (1989). Amyloidosis of the nervous system. *Journal of the neurological sciences*, 94: 1-28.
- [99] Palmert MR, Podlisny MB, Witker DS, Oltersdorf T, Younkin LH, Selkoe DJ, et al. (1989). The betaamyloid protein precursor of Alzheimer disease has soluble derivatives found in human brain and cerebrospinal

fluid. Proceedings of the National Academy of Sciences of the United States of America, 86:6338-6342.

[100] Joachim CL, Selkoe DJ (1992). The seminal role of beta-amyloid in the pathogenesis of Alzheimer disease. Alzheimer disease and associated disorders, 6:7-34