

Peer Review File

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Reviewer comments

Reviewer A

The authors describe their studies which aimed to investigate the protective role of traditional Chinese medicines (RSYR, SZRT, LWDH, among others) in Alzheimer's disease treatment. They evaluated the antioxidant activity, cyclooxygenase-2 (COX-2), and acetylcholinesterase (AChE) inhibitory activities. In the present study, the authors demonstrated that 10 Chinese medicines exhibited excellent ORAC activity and the majority of them showed COX-2 inhibition. Besides, RSYR, SZRT, and LWDH showed direct inhibition of AChE activity. Based on these data they proposed some mechanisms through which these herbs could be potential therapeutic agents against Alzheimer's disease.

The manuscript is well written and comprehensive data have been analyzed and presented. It is acceptable for publication in *Biomedicine & Pharmacotherapy* with major revision:

Reply: We thank the reviewer very much for his/her overall positive valuation of this study and appreciate all of the reviewer's comments.

Comment 1. Description of results and discussion should be increased. Discussion should mention more articles about the water solubility for these herbs. Besides, is important to mention what are the common molecules in these herbs and discuss them.

Reply 1: Thank you for this valuable comment which helps us to improve our manuscript. We have added more contents about the solubility and common molecules in the herbs in the discussion parts and cited 20 more references. The changes in the text are as below.

Changes in the text: The contents below were added.

Page 9 line164: All 10 types of Chinese medicinal extracts powders showed good solubilities in both DDW and ethanol the same as the information provided by the manufacturer but left a small amount of sediment.

Page 12, line 243: Chinese medicines are known containing hundreds of bioactive compounds (36). Kobayashi et al used a high performance liquid chromatograph (HPLC) to analyze the constituents in RSYR. Their results showed that RSYR contains ferulic acid, xanthotoxin, dehydropachymic acid, cinnamaldehyde, paeoniflorin, tenuifolin, flavonoids, formononetin, glycyrrhetic acid, liquiritin, gomisins and other organic compounds (17). Among these compounds, paeoniflorin reduces COX-2 expression and inflammatory reactions both in vivo and in vitro (37, 38). Previous studies demonstrated that tenuifolin not only showed inhibitory activity on A β synthesis but also exhibited nootropic activity through inhibiting AChE and promoting

norepinephrine and dopamine production (39-41). The main herb of RSYR, ginseng, has ginsenosides, saponins, flavonoids, polyphenols and other compounds. These bioactive compounds in ginseng showed antioxidant and anti-inflammatory effects in various in vitro and in vivo models (42, 43). Ginsenosides also played a pronounced positive role in the prevention and treatment of AD and other neurological diseases (44). The main compounds of LWDH are 5-hydroxymethyl-2-furoic acid (HMFA), loganin and paeoniflorin. These compounds could be absorbed in to blood stream after oral administration (45) and exhibited antioxidant and anti-inflammatory activities (46, 47). Moreover, LWDH has been demonstrated to against A β -induced paralysis in *Caenorhabditis elegans* through up-regulation of heat shock protein and its antioxidant activity (48). Suanzaoren, the major herb of SZRT, contains saligenin, saponin, flavonoids and sanjoinines. Flavonoids are believed to have various bioactive effects including anti-inflammatory, antioxidant, anti-aging, etc. Dietary flavonoids have been considered as a promising approach to prevent or slow the pathological development of neurodegenerative diseases (49). These evidence confirm our findings in the present study.

Comment 2. More explanation about previous in vitro results with these herbs should be added in the introduction.

Reply 2: Thank you for this valuable comment which helps us to improve our manuscript. We have added more information about previous studies in the introduction and discussion parts.

Changes in the text: The below content was added.

Page 4, line 68: It has been reported that RSYR could promote proliferation of oligodendrocyte precursor cells from aged rat brain (17) and increase nerve growth factor (NGF) secretion in the cultured rat astrocytes (18). LWDH exhibited anti-inflammatory and antioxidant effects in obese rats (19). LWDH and its active fraction combination could improve the cognitive ability and neuronal synaptic function in aging or AD animal models through controlling the neuroendocrine immunomodulation network (20). SZRT showed positive effects on dementia patients with sleep disorders (21, 22),

Comment 3. In the methods from the authors mention that “all experiments were repeated 3 – 5 times independently”, however in the figures is mentioned 3 experiments in each of them. When the experiment was repeated 5 times?

Reply 3: Sorry for our carelessness. The ORAC experiment was repeated 5 times.

Changes in the text: Page 17, line 344, Each bar represents the mean \pm SD of five independent experiments. ***, $p < 0.001$.

Comment 4. Cat number from reagent and antibodies should be mentioned in the methods too (Example: NGF?).

Reply 4: Thank you for your comment. The Cat. Numbers are added in the methods section in the () after each reagent. For example, (SRP3015) is the Cat. Number of NGF purchased from Sigma-aldrich, Tokyo, Japan.

Reviewer B

The manuscript entitled “Ren Shen Yang Rong Tang and other traditional Chinese medicines exhibit antioxidant and anti-inflammatory capacities and suppress acetylcholinesterase activity in PC12 neuronal cells” has been evaluated. The author attempt to assess some Chinese herbal medicine for antioxidant and anti-inflammatory, and anti-acetylcholinesterase activity. Some od plant species showed promising results. The manuscript can be accepted for publication after the minor revision.

Reply: We thank the reviewer very much for his/her overall positive valuation of this study and appreciate all of the reviewer’s comments.

Comment 1. The authors mentioned that Donepezil at 10 μ M shown some activity. However, the herbal concentration was mentioned mg/ml. The authors should mention that Donepezil concentration 3.8 μ g/ml instead of 10 μ M.

Reply 1: Thank you for this comment. We have added (3.8 μ g/ml) after 10 μ M for donepezil in the text (page 10, line 189).

Comment 2. This Ms lacks how the herbal formulation was prepared (Extraction, distillation or powder etc)

Reply 2: Thank you for this comment. The Chinese medicines we used in this study are all provided as extract powders by TSUMURA & CO., Tokyo, Japan. Please kindly see page 5, lines 82 – 86.