OPEN ACCESS
Journal of Animal Health and Production

Research Article

Agarwood (*Aquilaria crassna*) Inhibits Beta-amyloid and Tau-protein Formation in a Mouse Obesity Model

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Abstract | Dementia related diseases such as Alzheimer's and Parkinson's became one of the most concerning social issues, almost a prime concern in aging era of todays as crucial as cancers. We have studied Agarwood (*Aquiralia crassna*) extract (AE) and its effects on prevention of dementia related diseases through ICR mice experiment. The mice were fed with 5 mg of AE per day for 16 weeks to see the effect of AE on inhibition of two major biomarkers of beta-amyloid (Aβ) and tau-protein (τ-protein) formed in the brain tissues. Under the hypothesis of obesity inducing potential dementia, mice were fed with high-fat energy diet to gain excessive weight. The experimental groups in this study are 1) high-fat diet fed only (control) and 2) high-fat diet fed with AE added (AE group). After the experiment was terminated, they were slaughtered and brains were obtained. Throughout the experiment, measured were changes of body weight, blood chemical compositions, Aβ and τ-protein expression. Western blot assay was made for Aβ and τ-protein expression. For blood chemical compositions, both LDL and HDL tended to increase in AE group than those in the control. Though body weights increased in the AE group than those in the control, AE significantly reduced both Aβ and τ-protein formation at p=0.05, indicating potentially preventative and medicinal effects of Agarwood against Alzheimer's and other dementia related diseases. Aβ and τ protein formation in AE group were significantly decreased than those in the control group.

Keywords | Agarwood, Beta-amyloid (Aβ), Tau-protein, Dementia, Obesity

Received | February 13, 2022; **Accepted** | March 25, 2022; **Published** | June 01, 2022
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Citation | Jang HM, Kim JH, Park G, Choi YD, Kim SE, Jeon GJ (2022). Agarwood (***aquilaria crassna***) inhibits beta-amyloid and tau-protein formation in a mouse obesity model. J. Anim. Health Prod. 10(2): 221-225.
DOI | http://dx.doi.org/10.17582/journal.jahp/2022/10.2.221.225
ISSN | 2308-2801

INTRODUCTION

Agarwood has long been extensively studied for its medicinal and health problems in many countries, especially south-east nations, middle–east and India. Dementia recently has been a prime issue for modern aging days, its importance is as significant as cancers in health issues, which is utmost important in national medical expenses and aging people's health problems. Among various medicinal plants, Agarwood was shown to exhibit cholinesterase inhibitory activity (Wang et al, 2018, Bahrani et al, 2014). Natural compounds of phytochemicals from various plants were well reviewed for their effects on anti-dementia by (Ahmed et al, 2021). Countless studies have been made for dementia prevention and remedy treatments but once progressed dementia is not reversible and no remedy yet to date. Mental disorders by elderly aging are various and cause the impairment of memory and body movements, which is a typical symptom of dementia. Aging is high-

ly related with various neuronal dysfunction. Dementia is simply and almost universally defined as any impairment or trouble of cognition, speech, behavior, understanding and movement that leads to unfeasibility of our daily living conditions. Dementia could be due to both genetic and environmental causes. Biologically, the formation of beta-amyloid (Aβ) is possibly due to abnormal chopping of the amyloid precursor protein (APP), which produces Aβ, more specifically Aβ42. In early days, Bush et al. (1994) has reported the Alzheimer's disease (AD) is possibly due to the remaining of high concentration of Aβ 1 to 40 which is binding to zinc, killing the neurons.

Dementia associated with aging was termed senile dementia caused by many reasons and is not simply defined for its causes. One of some factors for causes of dementia in elderly aging over 65 yrs with neurodegenerative dementias such as AD and AD in early ages (in general less than 65 yrs) is possibly due to brain damages by tumor or accidents. Dementia with young ages is termed as early-onset dementia. Recently early-onset-dementia, equally termed as presenile dementia, is also frequently reported (Vieira et al., 2013). Unfortunately, dementia is not yet completely curable and is an irreversible disease. The consequence of dementia is well defined such as the accumulation of Aβ and τ-protein but the causal factors for dementia are not conclusively sound. To date, dementia is classified by the National Institute of Health (NIH) as 1) Alzheimer's disease, 2) Vascular dementia, 3) Lewy body related dementia. 4) Frontotemporal dementia and Mixed dementia (Libro et al., 2016; Raz et al., 2016). AD is mainly and most significantly characterized by neuro-degeneration in the brain releasing acetylcholine particularly in the hippocampus and cortex areas, which causes a gradual loss of cognitive functions such as memory loss, impaired judgement, depression and mental deterioration (Libro et al., 2016). Some studies have published antagonistic and skeptical evidence of association between Aβ and dementia (Walsh and Selkoe, 2020; Selkoe, 2019) and rather, a more noticeable study was earlier given two decades ago to presinlin (PSEN) (Wolfe et al., 1999), which is the transmembrane domain of APP. Aβ exists in a very wide range of forms such as monomers, dimer, oligomer, soluble or insoluble. Worldwide, the symptom of dementia occurs about 7% of above 65 years old and slightly more frequent in developed countries than developing countries (Gale et al., 2018). Common dementia diseases are Alzheimer's, Lewy-body and Parkinson disease. Among these, Alzheimer's is the most common type of dementia and is observed about 5 to 6% in ages of 65 and above and up to 30% in the ages above 85 (Gale et al., 2018). Momtazmanesh et al. (2020) reported that over 40 million people are affected globally, suffering from neurodegenerative diseases. For Alzheimer's, two most common diagnostic evaluations are to examine the detection of Aβ plaques and τ-protein tangles in brain tissues. In brain, microglia and astrocytes are activated by the PRRs to the Aβ plaques and τ-protein tangles in both extracellular and intracellular responses (Heneka et al., 2015). Microglial cells phagocyte the misfolded and aggregated proteins of Aβ and τ-protein as macrophages, in general, do in other than brain tissues. In the study of Gomez et al. (2020), they investigated neuroinflammation in Down syndrome (Gomez et al., 2020). In Down syndrome, chromosome 21 is associated with the production of APP, causing many abnormalities such as Aβ protein aggregation as one gets aging (Momtazmanesh et al., 2020). Rather than, pathological syndrome of dementia, obesity is one of the non-pathological factors to cause dementia. Obesity is highly correlated with late age risk of dementia, which was studied by several (Barrett-Connor, 2007; Prickett et al., 2015; Windham et al., 2017). The evidence of Alzheimer's by obesity is well reviewed by Anjum et al. (2018). And also, adiposity is highly related to dementia as is in obesity. Adiposity is technically defined in a different manner from obesity. Obesity is based on body mass index and adiposity is defined by the state of being fat in body and is in general measured based on the circumference of the hip and waist. Unlike obesity, adiposity is often studied for the risk of dementia by many studies. Kelly et al. (2008) has predicted the obesity and overweight population of adults will increase up to 573 million and 1.35 billion, respectively. Independently of diabetes type II, obesity alone could increase the risk of dementia, which was proven by the study of Whitmer et al. (2008). Pugazhenthi et al. (2017) reviewed the risk of obesity for potent dementia, in which they also pointed out that there is a critical association among Alzheimer's, diabetes and obesity. For natural compounds effective for neuronal disorders, Libro et al. (2016) thoroughly reviewed the natural compounds useful and effective for treatment and prevention of dementia. The objective of this study was to investigate the effect of AE on reduction of Aβ and τ protein formation in dementia related diseases.

MATERIALS AND METHODS

Experimental Units and Period

Six weeks old female ICR mice were purchased from Oriental Bio Co., Ltd, Sungnam city Korea. All mice were acclimated to a condition with 12h light-dark cycle at a temperature of 24±2°C and a relative humidity of 55±5% for 4 weeks. Mice were assigned into 2 groups each with 10 mice; 1) Control group fed with high-fat energy diet (Rodent diet with 60 Kcal% fat (D12492), Research diets, NJ, USA) with no additional additives, 2) High-fat energy diet added with Agarwood extract (EtOH extract, 1 mL/d). Initially mice in both control and treatment groups were fed with high fat energy diet only for 16 weeks. The

experiment was approved by Seoul Hoseo Institutional Animal Care and Use Committee (HS-2019-2) at Seoul Hoseo Occupational Training College, 420 gang-Seo Ro, Seoul, Korea.

Body Weight Changes

The day of administration of the diet was taken as week 0, and the body weights were measured weekly for a whole period of 16 weeks with ad lib supply of high energy fat diet (control group) and high energy fat diet added with AE (AE group). Body weight was measured weekly for 16 weeks.

Blood Composition Analysis

Blood was taken from each of the mice separately after the termination of experiment. The samples were sent to KPC co. ltd., Korea for the various blood components analysis.

Aβ and τ-protein Expression

After slaughtering the mice from each group, the brains were taken out and stored in EtOH tubes. The brains were ground with liquid nitrogen and they were put into the tube for centrifuge. Into the tube, RIPA lysis buffer and Protease inhibitor (Sigma, USA) were added and, then, they were centrifuged for 20 minutes at 4° C. After collecting the supernatant, the method followed the standard and conventional Western blot assay. The biopsy of the mice brains followed a conventional protein analysis with an aid of the method described in the published paper of Koob (2012). The antibodies used were Aβ and τ-protein (Abcam, USA).

Statistical Analysis

For the comparison between the control and treatment groups, only two means were compared and thus, a student t-test was made at p=0.05 in all analyses.

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RESULTS

Body Weight Changes

In comparison of control group and AE group, started with similar body weights in both groups, AE group tends to significantly increase body weight over the control group. However, the difference was not significant at the end of the experiment. The difference of body weights between the two groups at the termination was less than the mid-experimental period. As shown in Figure. 1, after about 9 weeks, the weight changes were almost to asymptotically plateau.

Biochemical Analysis of Blood

As shown in Figure 2 in analysis of blood compositions, 6 characteristics were measured for liver functions (GOT, GPT) and lipid related characteristics (TG, Cholesterol, LDL and HDL). The differences between control and AE group were not much different except for cholesterol and HDL. Unexpectedly, the result for cholesterol, LDL and HDL were antagonistic, which expected less cholesterol with higher HDL and lower LDL. However, AE group showed slightly higher HDL, LDL and cholesterol values than control group.

Western blot assay for Aβ and τ-protein

Identification of Aβ and τ-protein formation was made by Western blot assay from which the results were shown in Figure. 3, indicating that both Aβ and τ-protein were expressed less in AE group than the control. By looking at the band patterns, control group showed very distinct and thicker band types while AE group showed significantly lower expression of both Aβ and τ-protein at p=0.05. The relative band size of AE group over control was less than 25% in both Aβ and τ-protein, suggesting that Agarwood has a potential effect of reducing the Aβ and τ-protein formation more than half.

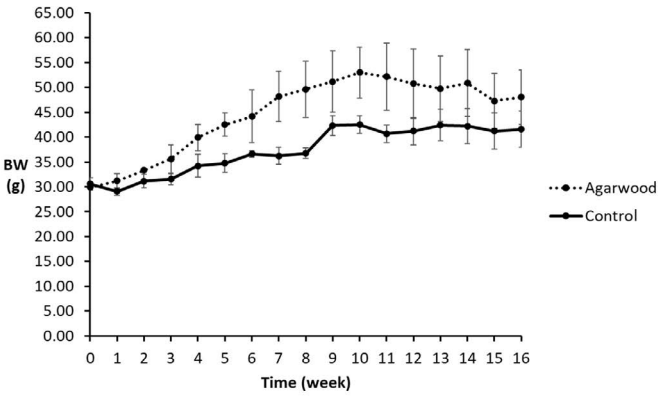

Figure 1: Body weight (BW) changes over time for control and AE groups.

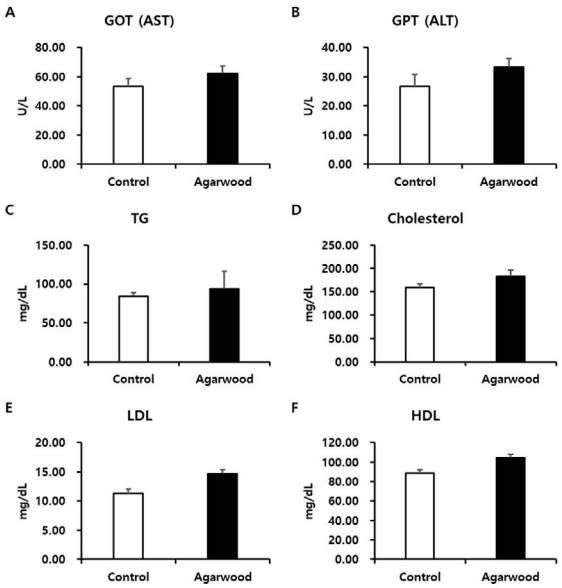

Figure 2: Blood biochemical analysis for control and AE groups.

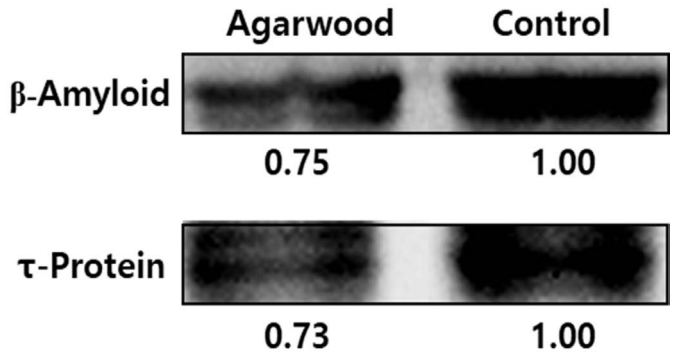

Figure 3: Western blot analysis of β-Amyloid and τ-protein for control and AE groups. The degrees of Aβ (0.75) and τ-protein (0.73) expression were denoted as the proportion to the control group (1.00).

DISCUSSION

The excessive increase of body weight changes over a whole experimental period is due to the high fat energy diet fed ad lib. Anjum et al. (2018) well reported the review of the relationship between obesity and dementia. As pointed earlier by Anstey et al. (2011), obesity in mid-life is highly likely to have dementia problems compared to normal body mass index. The increased body weights over time were verified for their consequences on formation of Aβ and τ-protein in this study.

Deposit of Aβ and τ-protein in brain leads a cascade of events including oxidative stress and inflammation (Barage and Sonawane, 2015), Aβ activates microglial cells which release pro-inflammatory cytokines, reactive oxygen species (ROS, a form of very harmful and dangerous molecule) and reactive nitrogen species (RNS), which leads to mitochondrial dysfunction, causing glutamate release and excitotoxic neuronal cell death. Additionally, τ-protein tangles, the Neurofibrillary Tangles (NFTs) form insoluble filaments that disrupt the transportation of neurotransmitters such as acetylcholine (ACh) and interfere with communication between neurons contributing with Aβ oligomers to affect synaptic transmission, leading to cognitive impairment (Libro et al., 2016). Since many studies have been published on dementia, as usual in scientific studies, integration of all those causal factors in dementia is quite complicated to clearly identify. Yet, more have been studied for partial factors for dementia but will hopefully be well defined by upcoming technologies of cross-linking information of biological and environmental factors.

The importance of Aβ hypothesis has long been questioned with pro-and con- discussion. Walsh and Selkoe (2020) argued that Aβ hypothesis in AD is questionable and argued that treatment against Aβ has failed in Phase 3 clinical trials. Other than Aβ and τ-protein in regards to AD, many other biochemical factors are involved and one of the major factors at genetic levels is ApoE gene with 3 different alleles of E2, E3 and E4 for which E2 is inhibitor functioning, E3 being neutral and E4 being the highly related to AD patients. Any combination of these 3 ApoE alleles, E4/E4 genotypes is significantly increasing the potential AD. To date, the challenge of reducing Aβ has been focused on β-secretase cleavage enzyme 1 (BACE1). In conventional dementia, more to Alzheimer's, the secretases of α-, β-, γ cleavage enzymes have been of utmost concern. However, other than these secretases cutting the APP, other enzymes are also of interest to scientists about an ***eta***-secretase pathway (Willem et al., 2015). Aβ also triggers to stimulate the formation of τ-protein.

CONCLUSION

AE was highly attractive for potential therapeutic and preventative effects on dementia related diseases. Conventional biomarkers in dementia patients have been Aβ and τ-protein which were significantly reduced by AE in mice brain. Thus, it is highly recommended to be further studied in the natural plant therapies for dementia prevention.

ACKNOWLEDGEMENTS

We appreciate Chimhyang-In Co., Ltd. for providing Agarwood chips and Seoul Hoseo Occupational Institute, Seoul, Korea for the use of the animal care facility.

AUTHORS CONTRIBUTION

Jang, Hye-myoung and Gwang Joo Jeon equally contributed to this work as the 1st author.

CONFLICT OF INTEREST

There is no conflict of interest.

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