ORIGINAL ARTICLE

Evaluation of the Activity of F2-isoprostane in Alzheimer's Disease Rats Given Banana Extract

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ABSTRACT

The objective this study is to analyze f2-isoprostanelevels in the serum of a Wistar rat model of Alzheimer's disease (AD) treated with Musa paradisiaca-Linn (MPL) ethanol extract or banana extract (BE).Twenty wistar rats were randomized into five groups: K0, without AD induction and no BE; K1, AD induction, no BE; K2, AD induction + BE 250 mg/kg body weight (BW); K3, AD induction + BE 500 mg/kg; and K4, AD induction + BE 1000 mg/kg. Alzheimer's disease (AD) induction was performed by $A\beta_{1-42}$ (0.2 ug) injection at the intracerebroventricullary area. F2-isoprostane level measurements were performed by Elisa kit.Paired t-test analysis showed no significant differences of f2-isoprostane serum level before Aβ induction among 5 groups ($p > 0.05$). At 6 weeks post- Aβ induction, there was significant increased f2-isoprostane in all groups except K0 ($p < 0.05$). Notably, after 3 weeks of BE administration, f2-isoprostane serum level was significantly decreased in all BE-treated groups; in the K1 placebo group, f2-isoprostane level increased. The maximum decreased f2-isoprostane level was in group K4 (-BE 1000 mg/kg BW), and minimum was in group K2 (BE 250 mg/kg BW). The results revealed that the ethanol extract of MPL fruit could decrease f2-isoprostane level in AD rat serum.
Keywords: Alzheimer's disease (AD), Oxidative stress, Wistar rat, F2-isoprostane, Musa paradisiaca-L(MPL)

INTRODUCTION

Neurodegenerative disease is a considerable health problem and becoming increasingly prevalent with the aging of general population. Alzheimer's disease (AD) was first described by the German psychiatrist, Alois Alzheimer, in the early 1900[1] and is now considered the most prevalent progressive neurodegenerative disorder, responsible for 75% of all dementia cases and. It affects approximately 35.6 million people worldwide,this will increase with population aging[2]and will probably affect nearly 106.8 million people by 2050[3]. As the aging population increases, the number of patients with AD also increases, with a rate of 13% in older patients over 65 years old and 45% in the group aged over 85 years old[4]. The majority of AD cases occur in women compared with men, the estimated risk for developing AD is about 20% for women and 10% for men for age above 65[5]. Alzheimer's disease (AD) is the most prevalent form of dementia that predominantly affects the elderly populations and is characterized by selective neuronal death and two pathologic hallmarks, i.e., senile plaques formed by extracellular deposits of amyloid-β (Aβ) peptides and neurofibrillary tangles (NFTs) composed of intracellular aggregations of hyperphosphorylated tau protein[6]. Several factors contribute to the progression of the disease including Aβ accumulation, neurofibrillary tangle formation, cholinergic deficit, oxidative stress, neuroinflammation, and apoptosis[7]. Little is known, however, about the molecular mechanisms of oxidative stress by which it mediates neuronal cell death in AD. Oxidative stress is the redox state resulting from an imbalance between the generation and detoxification of reactive oxygen species (ROS). Reactive oxygen species (ROS) are unavoidable physiological byproducts which act as a double-edged sword in the biological system[8]. Oxidative stress is an important contributor to Aβ accumulation and tau hyperphosphorylation, suggesting that it plays an essential role in the pathogenesis of AD[9,10]. It is known that Aβ can cause increased production of ROS and also damage mitochondria, which can lead to further enhanced production. These effects can be seen in the brain of the triple-transgenic mouse model of AD, wherein lipid peroxidation is increased and simultaneously GSH and Vitamin E levels are decreased[11]. On the other hand, experiments have shown that oxidative stress can cause both increased production and accumulation of Aβ[12]. Oxidative stress reduces the activity of α-secretase and promotes the expression and activation of β- and γ-secretases[13,14]. F_2-isoprostane, a lipid peroxidation by-product is a biomarker for oxidative stress[15].F2-isoprostanes (F2-IsoPs) are formed from arachidonic acid via esterification with phospholipids followed by hydrolysis. In AD, increased levels of F2-IsoPs were detected in cerebrospinal fluid (CSF)[16]. Another study also shows higher F2-IsoPs in serum in AD[17]. Many of the current drugs taken to treat the disease, such as, donepezil, have unpleasant side effects and doctors are keen to find alternatives[18]. The drugs designed to slow disease progression are available. Some medicinal herbs from plants may help to improve brain function, but scientific evidence to prove that they can treat Alzheimer's disease, is limited.

Plants contain a large and heterogeneous group of biologically active compounds, including a subgroup of phytochemicals known as phenolic compounds. Phenolics are secondary metabolites synthesized by plants that are ubiquitous throughout the plant kingdom. Phenolics are present in significant amounts in many commonly consumed fruits, vegetables, grains, herbal products, and

beverages. Over 8000 phenolic compounds have been isolated from different natural products, including flavonoids, phenolic acids, coumarins, and tannins. Each group is further divided into subgroups on the basis of chemical structure.Flavonoids are a family of phenolic compounds with strong antioxidant activity present in fruits, vegetables, and other plant foods. More than 5000 distinct flavonoids have been identified in plants, and several hundreds are known to occur in commonly consumed foods[19] such as banana[20], which is rich in polyphenol, flavonoid and tannin substances[21]. Observational studies suggest that an antioxidant-rich diet may reduce the risk of AD[22]**.** Plants provide wealth of bioactive compounds, which exert a substantial strategy for the treatment of neurological disorders such as Alzheimer's disease[23]. Commenges and colleagues found that an intake of flavonoids significantly reduced the risk of dementia and these flavonoids in sufficient amount plays neuroprotective[24]. The Musa paradisiacal-L(MPL)fruit from the musaceaplant family, also known as banana, is commonly consumed and has been used as medication and can decrease oxidative stress caused by neurotoxicity, such as in AD[25]. In our research, we analyzed f2-isoprostane level in an AD rat model induced by injection of Aβ for 6 weeks, which was treated with banana extract (BE)/MPL for 3 weeks. The purpose of this study was to analyze the effect of BE (MPL) administration on the level of the f2-isoprostane in the wistar rats model of Alzheimer's disease, with the hypothesis that BE (MPL) administration for 3 weeks can decrease the level of the f2-isoprostane in wistar rats which is in Aβ induction for 6 weeks. Banana extract (BE) referred to herein is BE or musa paradisiacal-L (MPL) which is extracted using ethanol.

MATERIALS AND METHODS

Experimental procedures were carried out in the Molecular Microbiology and Immunology Laboratory, Faculty of Medicine Hasanuddin University Makassar Indonesia. This research was an experimental study *in vivo* pre- and post-design that was conducted in the period from February to July 2016. The experiment has been approved by the Medical and Health Research Ethics Committee Faculty of Medicine Hasanuddin University Makassar Indonesia (Number: 391/H4.8.4.5.31/PP36-KOMTIK/2016).

Animals

Animal Preparation: We selected twenty Wistar rats ((2.5–3 month, 150–250 grams) from central animal house (Faculty of Medicine Hasanuddin University Makassar Indonesia) for this study. Animals were randomly divided into five groups (= 4 in each group): the control (K0 and K1) and treatment groups (K2, K3 and K4). They were kept in the animal house (The Molecular Microbiology and Immunology Laboratory, Faculty of Medicine Hasanuddin University Makassar Indonesia) for one week for proper acclimatization before starting the experiment under controlled condition of illumination (12 hours light/12 hours darkness) and temperature 23 ± 2°C. They were housed under ideal laboratory conditions, maintained on standard pellet diet and water ad libitum throughout the experimental period. All procedures were in accordance with the internationally accepted guideline for experimental animal use and care of Laboratory animals of the Molecular Microbiology and Immunology Laboratory, Faculty of Medicine Hasanuddin University Makassar

Experimental Design: In this study, total number of twenty Wistar rats were divided into following five groups having four rats in each group: K0=no induction AD model and no BE (MPL) administration; K1=AD induction model without BE (MPL) administration (Just given a placebo); K2=AD induction model with 250 mg/kg BW BE (MPL) administration; K3=AD induction model with 500 mg/kg BW BE (MPL) administration; and K4=AD induction model with 1000 mg/kg BW BE (MPL) administration. F2-isoprostane level was assessed before AD induction, 6 weeks after AD induction and 3 weeks post–BE (MPL) administration.

Administration of MPL Ethanol Extract (BE): Banana fruits were obtained from a market in Bogor, West Java Province, Indonesia. The fruits were cut longitudinally into chips of about 5 mm thickness and air-dried for 4 days after which they were grinded and made into flour. Three doses of the plantain flour were prepared: : 250 mg/kg/day, 500 mg/kg/day and 1000 mg/kg/day. The flour was dissolved in 2 ml of double distilled water, for easy administration. Banana (MPL) ethanol extract or BE was obtained by maceration methods at Balitro Bogor Agricultural Department Laboratory, West Java Province, Indonesia and plant determination process from Central Biology Research, Bogor LIPI, West Java Province, Indonesia (Number: 147/IPH.1.02/lf.07/I/2016). Previous research by Ittiyavirah and Anurenj (2014) showed that a 200 mg/kg BW dose of MPLethanol extract (BE) given over 21 days produced a significant effect on anti-stress activity[26]. In our research, we increased the dosage with the consideration that the current study is for AD and the BE used on K2 is 250 mg/kgBW, K3 500 mg/kg BW and K4 1000 mg/kg BW for experimental analyses; treatments were given orally for 21 days (3 weeks) each day. While in K1 only given a placebo with the same time of administration is 3 weeks. Overall both BE (MPL) and placebo were administered by mouth-feeding

Animal model: In this research we do advance preliminary research to develop an animal model of AD. To develop an AD model, we used a dose of $A\beta_{1-42}$ of 0.2 µg injected in ICV and observed for 6 weeks[27]. The AD animal model was generated using $A\beta_{1-42}$ peptide from Abcam, code number.Ab120959 (Cambridge, MA, USA). We injected mice with 0.2 µg $A\beta_{1-42}$ at ICV and observed the mice for 6 weeks. Aβ levels were measured using the SAA Mouse Elisa Kit.

Blood Collection: Blood samples were collected at three time points: day 0 before Aβ injection (baseline); day 42 (week: 6) ie on the last day of post AB induction observation or the day before the MPL intervention; And last is the day 63, the last day of BE intervention which done in 21 days from day 42 (weeks:9, changes). Blood was taken from the tail vein using a 0.1-ml hematocrit needle . The samples were centrifuged and serum was kept in a sterile tube at -20°C until analyses.

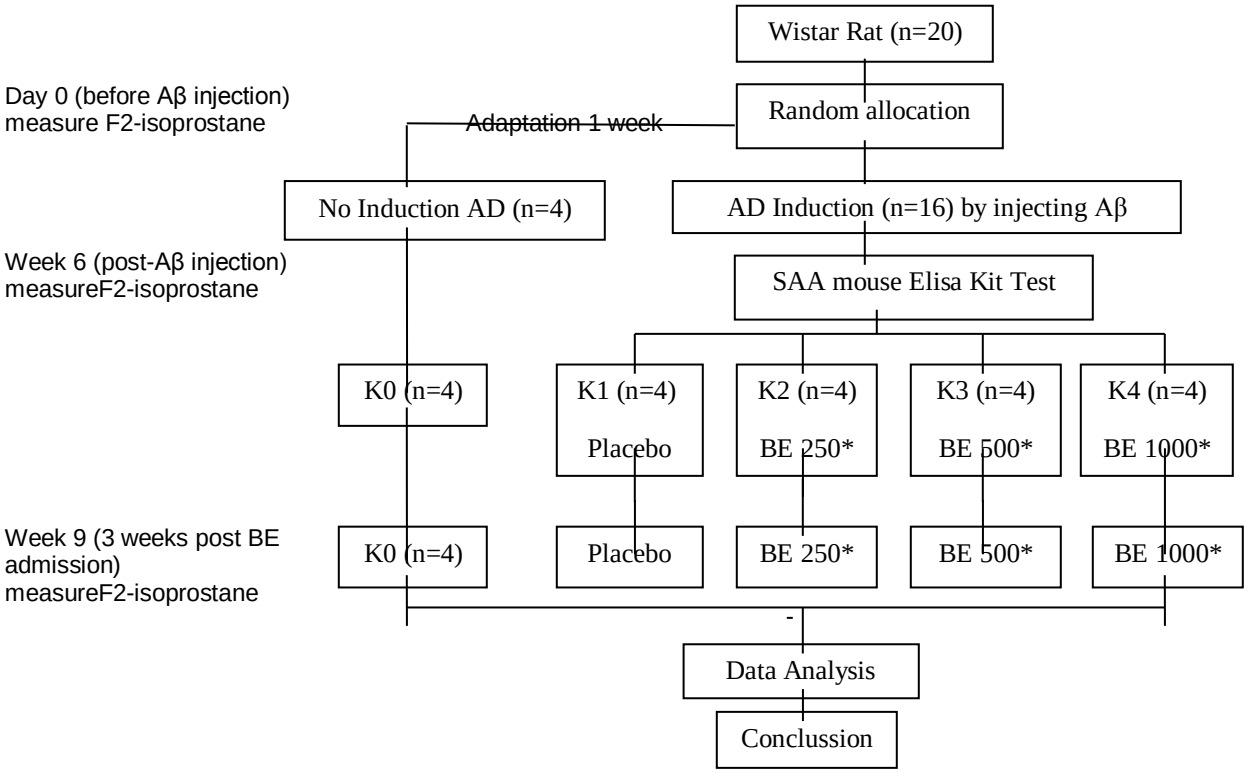
Enzyme-Linked Immunosorbent Assay (ELISA) Kit: Serum levels of f2-isoprostane (8 Isoprostane) were determined by an Enzyme linked ImmunoSorbent Assay (ELISA) kit (Abcam Ab175819, USA); a competitive

immunoassay for the quantification of total (both free and esterified) f2-isoprostane. This kit is specific for the measurement of f2-isoprostane in serum or plasma. The concentration of f2-isoprostane herein is determined by an enzymatic test, absorbance was measured by colometric at a wavelength of 450 nm. Using such ingredients: 8 isoprostane HRP konyugate, 8 standard isoprostane, horse radish peroxidase (HRP) buffer, procoated microplate 96 well, dilution buffer, TMB substrate, wash buffer (WB). Serum samples were thawed and prepared according to the manufacturer's instructions. All samples were run in a single batch.Preparation of measurements: preparing the reagents by balancing all the reagents, samples and controls at room temperature (18°C-25°C), next prepare the standard by way of dilution series. Measurements on standards, controls and samples are duplicated. Add 200 µl sample dilution buffer (SDB) into the blank well and 100 µl into the control well.Add 100 µl from each standard into the sample and put it into the well, add 100 µlkonyugate IV HRP into all wells. Incubated at room temperature for 2 hours,wash the plate with 400 µl WB then dry. Add 200 µl of TMB substrate into well, incubated at room temperature for 15-30 minutes.Add 50 µl 2N sulfuric acid into the well.For examination of Elisa Kit f2-isoprostane: SDB from the concentration kit 10 times, diluted to 1 time (by taking 5 ml SDB + 45 ml aquades), then distributed into 36 tubes and each contents 200 µl+4 µl serum and vortex, idle for 2 hours,then put into plate wells each contents 50 µl, then plastic cover, incubation at 37°C for 2 hours, remove sample and wash 3 times using WB. Add konyugate 50 µl, plastic cover and incubation for 0.5 hours at 37°C, discard and wash 3 times. Add TMB 50 µl then cover the plastic and incubate at 37°C for 30 minutes. Turn on the Elisa machine: click start measurement and select the method-isoprostane-enter. At this time the reaction was stopped by adding 50ml reaction stop at each well (stop reaction is H2SO4 0,5 molar)and absorbance was measured at 450 nm on a microplate reader. Standard curves were calculated and plotted for each plate with the percent bound on the y-axis and (log) standard concentration on the x-axis. R2 for each standard ranged from 0.94 to 0.97. Sample concentrations of 8-iso-PGF2a (ng/ml) were determined by interpolation from the standard curves for each plate.

Statistical Analysis: The data f2-isoprostane levelwere statistically analyzed and the significance calculated using one way 'ANOVA'(Bonferroni) followed by Tukey's test. All numerical values were expressed as Mean ± SD and the value of $P<0.05$ was considered as statistically significant.

Figure 1.Schematic of the research process. Twenty Wistar rats were divided into 5 groups: K0=no induction AD model and no BE administration; K1=AD induction model without BE administration; K2=AD induction model with 250 mg/kg BW BE administration; K3=AD induction model with 500 mg/kg BW BE administration; and K4=AD induction model with 1000 mg/kg BW BE administration. F2-isoprostane level was assessed before AD induction, 6 weeks after AD induction and 3 weeks post–BE administration.

Figure 1. Outline of research process



RESULTS

A total of twenty Wistar rats mean age 2.5–3 month, with 150-250 mg / kg body weight were included in the analysis. The mean, standard deviation and range of study variables presented in Table 2. The animals are grouped into groups induced by Aβ (K1, K2, K3 and K4) and those not induced by Aβ (K0), then continuous variables between the groups are compared.In Table 1 it shown that the mean level of f2-isoprostane was significantly different.F2-isoprostane levels were assessed before induction of Aβ and 6 weeks after induction of Aβ, and 3 weeks after BE treatment. In this study, we examined the effect of f2-isoprostane level evaluation in a single dose (0.2 µg) injected Aβ in the ICV region. Amyloid beta (Aβ) used in this study: peptide from Abcam, code number.Ab120959 (Cambridge, MA, USA). The levels of f2-isoprostane level at various time points throughout measurements were performed by ELISA kit and the analysis was evaluated. The f2-isoprostane levels in all observation groups are shown in Table 1.

Table 2 show that f2-isoprostane serum levels before Aβ induction had no significant differences (p >0.05) between the 5 groups. After 6 weeks post Aβ induction there were significant increased (p <0.05) in all groups except K0 group. The mean increased level of f2-isoprostane serum levels in each group were (464,48[b]) in K1 from 36,49 (2,96) to 500,96 (74,92); (381,59[b]) in K2 from 35,84 (3,10) to 417,43 (10,75); (409,34[b]) in K3 from 36,84 (2,14) to 446,18 (20,63); (408,99[b]) in K4 from 26,90 (2,32) to 445,89 (15,61). There were no significant differences in f2-isoprostane serum increased (p >0.05) between induction groups. After 3 weeks post BE treatment, there were significant decreased(p < 0.05) in all group except K0 and K1 group. The mean decreased level of f2-isoprostane serum in each group were(-79,19[c]) in K2 from 417,43 (10,75) to 338,24 (15,14); (-176.34[d]) in K3 from 446.18 (20.63) to 269.84 (40.32); (-179.03[d]) in K4 from 445.89 (15.61) to 266.86 (25.67).While in the group that was not given BE (ie placebo) increased (p <0,05) amount (0,89[a]) in K0 from 35,44 (3,86) to 36,33 (4,16); (156,53[b]) in K1 from 500,96 (74,92) to 657,49 (34,89).Paired t-test and one-way ANOVA (Bonferroni) were used to examine changes in f2-isoprostane serum expression after 3 weeks post-BE admission, after 3 weeks of BE administration, there was a significant increase in f2-isoprostane in the group without BE (placebo) compared with the levels at week 6 before BE treatment (p< 0.05). Notably, in all groups receiving BE, all showed a significant decrease in f2-isoprostane serum expression compared with the levels at week 6 before BE treatment (p< 0.05). After 3 weeks of BE admission during the post-induction period, f2-isoprostane serum expression decreased in all groups given BE. In comparison, in the group given only placebo, the f2-isoprostane levels continued to increase. The maximum decrease in f2-isoprostane serum expression was observed in the K4 group, which received a BE dosage of 1000 mg/kg BW. The smallest decrease was observed in group K2, which received a BE dosage of 250 mg/kg BW.

Table 1: F2-isoprostane levels in each group.

VARIABLE	GROUPS	OBSERVATION TIME				
		BEFORE INDUCTION (BASELINE WEEK 0)	6 WEEKS AFTER INDUCTION (WEEKS 6)	3 WEEKS POST BE TREATMENT (WEEK 9)	0-6 P*	6-9 P
F2-ISOPROSTANE LEVEL MEAN (SD)	K0 (N=4)	35,07(3,56)	35,44(3,86)	36,33(4,16)	0,656	0,064
	K1 (N=4)	36,49(2,96)	500,96(74,92)	657,49(34,89)	0,001	0,040
	K2 (N=4)	35,84(3,10)	417,43(10,75)	338,24(15,14)	<0,001	0,006
	K3 (N=4)	36,84(2,14)	446,18(20,63)	269,84(40,32)	<0,001	0,002
	K4 (N=4)	26,90(2,32)	445,89(15,61)	266,86(25,67)	<0,001	0,001

K0=without AD induction and no BE; K1=AD induction+Placebo; K2= AD induction+BE 250*; K3= AD induction + BE 500*; K4= AD induction+BE 1000*

DISCUSSION

The F_2-isoprostane (8-iso-$PGF_{2\alpha}$,) was specifically chosen as the representative marker of oxidative damage. Our results showed that detected an increase of f2-isoprostane serum level at 6 weeks after Aβ induction,which is similar to the result presented by Montine et al, studies have shown f2-isoprostanes to be increased in postmortem ventricular CSF obtained from autopsy-verified AD cases[28], as well as in antemortem CSF from individuals diagnosed with AD dementia[29]. The research found high f2-isoprostane activity levels in hypocampus, temporal and parietal cortexs in aged mice injected with $A\beta_{1-42}$[30]. Amyloid β (Aβ) has been proposed as the main factor in the AD pathophysiology mechanism and the major component of senile plaques, is considered to play a central role in neuronal cell death[31]. Currently, many blood markers of oxidative stress have been identified in AD patients or related animal models, including f2-isoprostanes (F2-IsoPs)[32]. In this research we also found that the placebo group continued to show significantly increased f2-isoprostane levels for the next 3 weeks. However, in groups treated with MPL (BE), we observed the opposite trend, with a significant decrease of f2-isoprostane serum level (p< 0.05). The maximum decreased level of f2-isoprostane serum level was observed in K4, the group that received the highest dose of MPL (BE) in this study (1000 mg/kg BW BE), and the minimum decrease was observed in group K2, which recieved 250 mg/kg BW BE. This result is consistent with the study by Vijayakumar et al., who reported findings of flavonoid antioxidant (MPL/BE) activity in mice[33].The administration of BE suppressed the serum f2-isoprostane levels in animal model AD.The mechanism of this effect are not clear, but the following may explain the results:i)The effects of banana extracts on neuron cells and found that the phenolic phytochemicals of the fruits prevented neurotoxicity on the cells against oxidative stress-induced

and may play an important role in reducing the risk of neurodegenerative disorders such as Alzheimer's disease[34]. Bioactive compound such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids are present in banana peel. This bioactive compound is reported to exert pharmacological effect, especially as an antioxidant[35]. Flavonoids were also reported to function in neurons to initiate neurogenesis at the hippocampus, as well as to slow down the initiation of pathological development in AD[36]. The antioxidant activity of the extracted flavonoids from M. paradisiaca in rats stimulated the activities of superoxide dismutase (SOD) and catalase which might be responsible for the reduced level of peroxidation products such as malondialdehyde, hydroperoxides and conjugated dienes[33].Heo et al. used BE (MPL) to protect neurons from oxidative stress by neurotoxicity. The study also showed that BE (MPL) could reduce oxidative stress risk caused by neurodegenerative disease, such as AD[25]. Flavonoids and their metabolites can modulate several neurological processes, as shown by interactions with neuronal-glial signaling pathways involved in neuronal survival and function[36,37].ii) The oxidative stress-induced β-site APP-cleaving enzyme 1, PS1 expression, and γ-secretase activation are mediated by activation of the c-Jun N-terminal kinase pathway[38],the show that H_2O_2 via c-Jun N-terminal kinase (JNK) enhances the activity of γ-secretase, leading to accelerated APP processing and Aβ accumulation.Oxidative stress induces expression of β-secretase through JNK-dependent regulation of γ-secretase, thereby providing a forward feedback between γ- and β-secretase for the cleavage of APP[38]. Consequently, the relation between oxidative stress and AD suggest that oxidative stress is anessential part of the pathological process, and antioxidants may be useful for AD treatment[39].

CONCLUSION

Clearly, oxidative stress is a significant element in AD pathogenesis and it is an essential pathological marker of AD,and induces expression of β-secretase through JNK-dependent regulation of γ-secretase, thereby providing a forward feedback between γ- and β-secretase for the cleavage of APP[38], characterized by a f2-isoprostane biomarker.In sum, oxidative stress plays an important role in Aβ pathologyand is one of the first consequences of Aβ overproduction in the brain,that can cause increased production of ROS and also damage mitochondria[11].The antioxidants are potential therapeutics by eliminating ROS and exerting neuroprotective effects on neurons in AD, the reduced level of peroxidation products such as malondialdehyde, hydroperoxides and conjugated dienes[33]. These findings will help contribute to finding an effective AD therapy and this study provide further knowledge to mechanisms involved in Aβ -induced apoptosis in neuronal cell. Herbs may play a promising role in the early treatment of Alzheimer's and other conditions involving poor memory and dementia. One of the chief benefits is that they have a low side effect compared to pharmaceutical agents.

Conflict of interest: The authors declare no conflict of interest.

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