● RESEARCH ARTICLE

Kaempferol attenuates cognitive deficit *via* regulating oxidative stress and neuroinflammation in an ovariectomized rat model of sporadic dementia

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Abstract

Alzheimer's disease (AD) is associated with oxidative stress, and ultimately results in cognitive deficit. Despite existing literature on the pathophysiology of AD, there is currently no cure for AD. The present study investigated the effects of kaempferol (Kmp) isolated from the extract of *Mespilus germanica* L. (medlar) leaves on cognitive impairment, hippocampal antioxidants, apoptosis, lipid peroxidation and neuro-inflammation markers in ovariectomized (OVX) rat models of sporadic AD. Kaempferol, as the main flavonoid of medlar extract has been previously known for anti-oxidative, anti-inflammatory and anti-neurotoxic effects. Thirty-two female Wistar rats were ovariectomized, and randomly divided into four groups: sham, OVX + saline, OVX + streptozotocin (STZ) + saline, OVX + STZ + Kmp. Animals received intracerebroventricular injection of STZ (3 mg/kg, twice with one day interval) to establish models of sporadic AD. Intraperitoneal injection of Kmp (10 mg/kg) for 21 days was performed in the OVX + STZ + Kmp group. Spatial learning and memory of rats were evaluated using a Morris water maze. Finally, brain homogenates were used for biochemical analysis by enzyme-linked immunosorbent assay. The results showed a significant improvement in spatial learning and memory as evidenced by shortened escape latency and searching distance in Morris water maze in the OVX + STZ + Kmp group compared with the OVX + STZ group. Kmp also exhibited significant elevations in brain levels of antioxidant enzymes of superoxide dismutase and glutathione, while reduction in tumor necrosis factor-α and malondialdehyde. Our results demonstrate that Kmp is capable of alleviating STZ-induced memory impairment in OVX rats, probably by elevating endogenous hippocampal antioxidants of superoxide dismutase and glutathione, and reducing neuroinflammation. This study suggests that Kmp may be a potential neuroprotective agent against cognitive deficit in AD.

Key Words: Alzheimer's disease; oxidative stress; neuroinflammation; Mespilus germanica L.; Kaempferol; neural regeneration

Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disorder with cognitive and behavioral disturbances that remains the most protracted disease. In 2015, 46.8 million people worldwide lived with AD, and this population will reach 131.8 million by the end of 2050 (Aliev et al., 2016). AD is characterized by the accumulation of senile plaques and neurofibrillary tangles in the brain (Kamat et al., 2014). Senile plaques consist of protein amyloid beta (Aβ), microglia and pro-inflammatory cytokines, particularly tumor necrosis factor-α (TNF-α) (Kar et al., 2004; Batarseh et al., 2016). The most recent studies confirmed the influence of reactive oxygen species (ROS) on the development of different disorders, including AD. Oxidative stress leads to reduction in glutathione (GSH) content, which is one of the important endogenous antioxidants capable to scavenge reactive oxygen species (Wojtunik-Kulesza et al., 2016). Therefore, oxidative stress leads to lipid peroxidation and formation of malondialdehyde (MDA) (Reyazuddin et al., 2016) and TNF-α (Kumar et al., 2017). Finally, production of ROS triggers the release of cyto-chrome c from mitochondria, and the activation of caspase-3 and apoptosis (Redza-Dutordoira and Averill-Batesa, 2016).

There is currently no definite treatment for AD, because the signaling pathways of AD are complex and definite initial causative factors is unknown. The existing therapies improve some behavioral symptoms, but hardly mitigate cognitive deficit. Moreover, at least half of AD patients do not respond to current medications (Farlow et al., 2008). Therefore, it is of great importance to find reagents capable of combating cognitive deficit in AD. Targeting oxidative stress and increasing antioxidant defenses of the brain would be an important strategy for maintaining survival of brain structure.

Recently, there has been an upsurge of interest in the therapeutic potential of flavonoids, a group of natural compounds in plants (Kumar and Pandey, 2013). Flavonoids as free radical scavengers and inhibitors of lipid peroxidation, are capable of passing the blood-brain barrier (Youdim et al., 2004; Banjarnahor and Artanti, 2014), and are involved in synaptic plasticity (Spencer, 2010). Considering the fact

that the brain is rich in peroxidizable fatty acids, elevation in brain free radicals negatively influences physiological functions. Consequently, flavonoids are important in the prevention of neuronal damages by balancing oxidative and anti-oxidative status.

Vegetables and fruits are two important flavonoids which are rich in diet sources. For example, medlar (*Mespilus germanica L.*) is a small tree from the *Rosaceae* family which has been cultivated for many years in Europe, Turkey, and Iran; and it consists of bioactive antioxidant compounds of flavonoids (Ercisli et al., 2012). Our recent study has demonstrated that alcoholic medlar leaves extract consisted of Kaempferol (Kmp), chrysin, luteolin, myricetin, naringenin, quercetin and rutin, and administration of extract increased passive avoidance learning in rat models of metabolic syndrome (Kouhestani et al., 2017).

Kmp is an anti-inflammatory compound which has the potent iron-chelating capability and ROS scavenging property (Kim et al., 2012). *In vitro* assays showed protective effects of Kmp on oxidative stress-induced cytotoxicity in PC12 cells (Kim et al., 2010). In addition, Kmp administration in parkinsonian mouse models improved motor coordination, raised striatal dopamine level, and increased superoxide dismutase (SOD) activity (Li and Pu, 2011).

Considering above-mentioned results from the previous studies, we assumed that Kmp might have the capability to alleviate cognitive deficit in the animal models of sporadic AD. Therefore, this study was designed to evaluate the effect of Kmp on spatial learning and memory, endogenous antioxidants SOD and glutathione (GSH), inflammatory marker TNF-α, lipid peroxidation marker MDA and apoptosis marker cytochrome c. To approach this, we performed intracerebroventricular (ICV) injection of streptozotocin (STZ) in female ovariectomized (OVX) rats to establish rat models of sporadic AD.

Materials and Methods

Extraction and isolation of Kmp
Mespilus germanica L. (medlar) leaves were collected from Guilan province of Iran in spring and were confirmed (Herbarium code of 6157) by a specialist from the herbarium center of the Guilan University. Extraction and purification of medlar flavonoids were carried out according to our previous work (Kouhestani et al., 2017). Briefly, 5 g of the dried leaves was dissolved in 104 mL of 70% ethanol and kept on a 40°C heater at a speed of 40 m/s. Then 2 M of hydrochloric acid and ethyl acetate were mixed and transferred to a rotary evaporator to achieve pure flavonoids. Two-dimensional paper chromatography was used for detecting all components of extract (at 366 and 254 nm). Then Kmp was isolated by thin layer chromatography and high-performance thin layer chromatography.

Ethics statement
The study protocol was approved by the National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978) modified by the Ethics Committee of Guilan University of Medical Sciences, Rasht, Iran (IR.GUMS.REC.1396.51).

Animals
Thirty-two adult female Wistar rats, aged 3 months, weighing 200 to 250 g, were provided by Guilan University of Medical Sciences, Rasht, Iran. Animals were anesthetized with a mixture of 75 mg/kg ketamine (Rotexmedica GmbH, Trittau, Germany) and 5 mg/kg of xylazine (SciENcelab, Houston, Texas, USA), and ovaries were removed to cease estrus cycle (Babaei et al., 2017). All animals were housed four per cage at standard conditions (22 ± 2°C, 12 hour light/dark cycle, light on 7:00 a.m.) and fed standard-pellet rat chow and tap water *ad libitum*.

Rats were randomly divided into four groups (*n* = 8 per group): sham, OVX + saline, OVX + STZ + saline, OVX + STZ + Kmp. After 3 weeks, animals were bilaterally cannulated under anesthesia, using stereotaxic apparatus (Stoelting, Chicago, IL, USA) according to ventricular coordinates: anterior-posterior = –0.8; medio-lateral = ± 1.5; and dorso-ventral = –3.4 (Paxinos and Watson) (Pourmir et al., 2016; Agrawal et al., 2011).

Drug administration
Animals received ICV injection of STZ (3 mg/kg, 10 μL on each side) (Sigma-Aldrich, St. Louis, MO, USA) on days 1 and 3 after surgery. The OVX + STZ + Kmp group received a daily injection of Kmp (10 mg/kg, intraperitoneal), and the control group received 10 μL of saline (0.9%) for 21 days (Ramezani et al., 2016).

Learning and memory test
Spatial learning and memory of animals were assessed using Morris water maze (MWM) test. The protocol used for MWM included five blocks (four blocks of working memory and one reference memory). Each block consisted of four trials and each trial lasted 90 seconds with an interval of 20 minutes. The apparatus consisted of a circular water tank (148 cm diameter and 60 cm high) with a rectangular platform (10 cm) at a fixed position in the target quadrant, 1.5 cm below the water level. The water temperature was maintained at 26°C. Also, visible test was carried out after the probe test to examine the animal's vision. Total time spent in the target quadrant (TTS), escape latency time to reach the platform and swimming speed were recorded using camera and "Ethovision 11 Noldus" tracking system (Netherland) (Babaei et al., 2017).

Tissue preparation
Following the behavioral test, animals were decapitated under ether anesthesia and their brains were quickly removed, cleaned with ice-cold saline and stored at –80°C. To prepare the homogenized tissue of the brain, lysis buffer (containing Tris-HCl, pH 8.0, NaCl, sodium deoxycholate, SDS, EDTA, Triton X-100, 1 mL diluted protease inhibitor) was used, and supernatants collected after 10-minute centrifugation process (at 4000 r/min) were kept for biochemical analysis (Asadi et al., 2015).
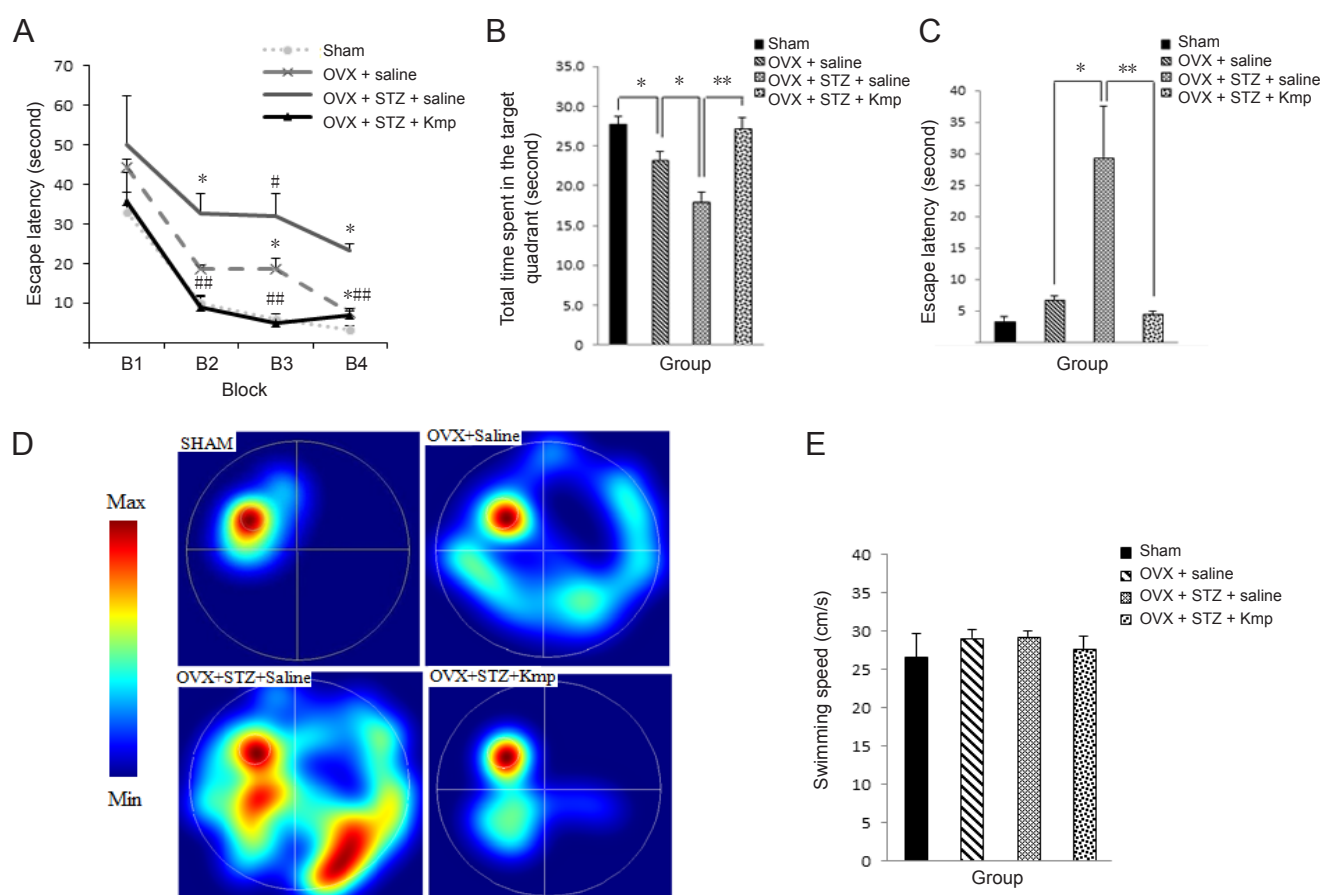
Figure 1 Effects of kaempferol (Kmp) on cognitive function of an ovariectomized (OVX) rat model of sporadic dementia.
(A) Improvement in memory impairment after Kmp treatment. Repeated measures analysis of variance (ANOVA) followed by Tukey's *post-hoc* test showed significant difference between groups (*$P < 0.05$, *vs.* sham group; #$P \leq 0.05$, ##$P < 0.01$, *vs.* OVX + saline group). (B) Total time spent in target quadrant in the Morris water maze probe trial (*$P < 0.05$, **$P < 0.01$). (C) The escape latency in retrieval phase (*$P < 0.05$, **$P < 0.01$). (D) Occupancy plots during the Morris water maze probe test. Increasing color intensity (arbitrary scale) represents increased time spent. (E) Average swimming speed of animals in the Morris water maze. One-way ANOVA followed by Tukey's *post-hoc* test was used. Data are expressed as the mean ± SEM, *n* = 8 rats per group.

Biochemical analysis

The hippocampal SOD activity was analyzed using Super Oxide Dismutase Assay Kit (Zellbio GmbH, Ulm, Germany). After adding reagents, samples, and standards into the wells, absorbance was measured at 0 and 2 minutes with a microplate reader (Awareness Technology Inc, Palm city, FL, USA) at 532 nm. The concentration of SOD was expressed as U/mg protein. Then SOD activity was calculated based on the below formula:

$$\text{SOD activity (U/mg protein)} = [(\text{OD}_{\text{sample 2 min}} - \text{OD}_{\text{blank 2 min}}) - (\text{OD}_{\text{sample 0 min}} - \text{OD}_{\text{blank 0 min}})/(\text{OD}_{\text{sample 2 min}} - \text{OD}_{\text{blank 2 min}})] \times 100$$

As an antioxidant enzyme, GSH was determined using Glutathione Assay Kit (Zellbio GmbH, Ulm, Germany), a microplate reader at 412 nm.

TNF-α, an inflammation marker, was assayed using a rat TNF-α ELISA kit (Diaclone SAS, Besancon, France) with a microplate reader at 540 nm.

The MDA as a marker of lipid peroxidation was determined using an MDA assay kit (Zellbio GmbH, Ulm, Germany), and absorbance at 532 nm was measured using a spectrophotometer (UNICCO Inc., Houston, TX, USA).

The level of cytochrome c was analyzed using cytochrome c ELISA Kit (Abcam, Cambridge, UK) with a microplate reader at 450 nm.

Statistical analysis

Normality of variables was estimated by Kolmogorov-Smirnov and Shapiro-Wilk test, and then behavioral data related to working memory were analyzed using repeated measure analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. Molecular variables and reference memory data were analyzed using one-way ANOVA with the Tukey's *post-hoc* test. Results are expressed as the mean ± standard error (SE), and values of $P < 0.05$ were considered statistically significant. SPSS software was used for statistical analysis (version 22, IBM Cooperation, Chicago, IL, USA)

Results

Effects of Kmp on learning and memory function of AD rats

Reduction in escape latency during acquisition trials indicates that all animals successfully learned to find the hidden
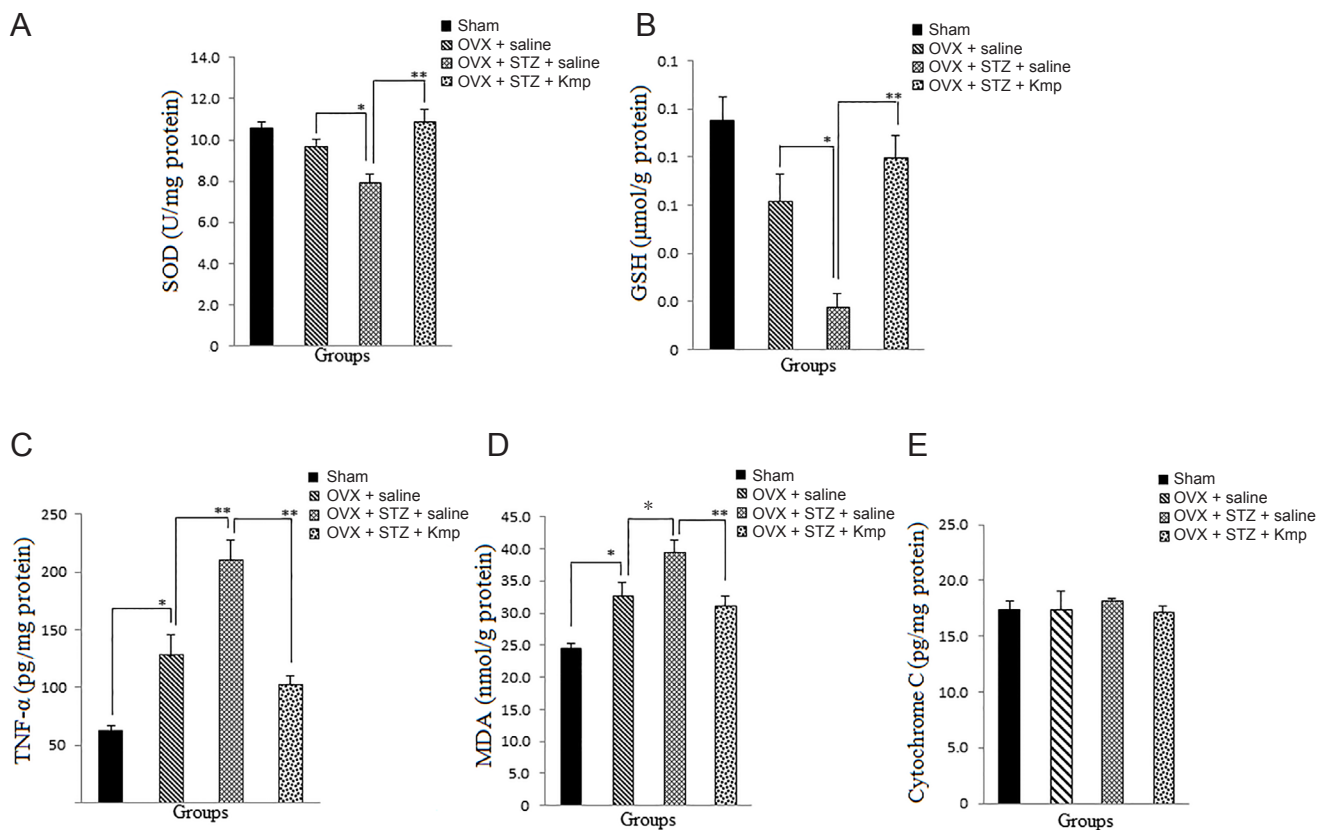
Figure 2 Effect of kaempferol (Kmp) on hippocampal SOD, GSH, TNF-α, MDA, cytochrome c levels in an ovariectomized rat model of sporadic dementia.
(A–E) SOD, GSH, TNF-α, MDA, and cytochrome c levels respectively. *$P < 0.05$, **$P < 0.01$ (one-way analysis of variance followed by Tukey's *post-hoc* test. Data are expressed as the mean ± SEM, $n = 8$ rats per group. SOD: Superoxide dismutase; GSH: glutathione; TNF-α: tumor necrosis factor-α; MDA: malondialdehyde.

platform in MWM. Results showed a significant difference between groups in escape latency in acquisition phase in Block 1 (B1): [$F(3,12) = 1.06$, $P = 0.042$]; B2: [$F(3,12) = 13.31$, $P = 0.001$]; B3: [$F(3,12) = 16.31$, $P = 0.001$] and B4: [$F(3,12) = 48.22$, $P = 0.001$]. Escape latency was significantly increased in B2, B3 and B4 in the OVX + STZ+ saline group compared with the OVX + saline group ($P = 0.029$, 0.050, 0.001), while the OVX + STZ + Kmp group demonstrated significant reduction in B2, B3 and B4 compared with the OVX + STZ + saline group ($P = 0.001$) (**Figure 1A**).

Moreover, there were significant differences in TTS [$F(3,28) = 15.111$, $P = 0.001$] and escape latency [$F(3,28) = 9.11$, $P = 0.001$]) in probe test between groups. As shown in **Figure 1B, D**, TTS was significantly increased in the OVX + STZ + Kmp group compared with the OVX + STZ + saline group ($P = 0.001$). In addition, escape latency was significantly increased in the OVX + STZ + saline group compared with the OVX + saline group ($P = 0.003$), while that was significantly decreased in the Kmp receiving group compared with the OVX + STZ + saline group ($P = 0.001$) (**Figure 1C**). Results did not show significant difference in swimming speed between experimental groups [$F(3,28) = 0.462$, $P = 0.711$] (**Figure 1E**).

Effects of Kmp on oxidative stress in the brain of AD rats
Significant differences were found in the brain levels of SOD [$F(3,26) = 9.61$, $P = 0.001$] and GSH [$F(3,26) = 11.62$, $P = 0.001$] between groups. SOD and GSH levels were significantly increased in the OVX + STZ + Kmp group compared with the OVX + STZ + saline group ($P = 0.001$; **Figure 2A, B**).

There was significant change in the brain level of TNF-α [$F(3,26)=21.48$, $P = 0.001$] between groups. Brain level of TNF-α was significantly increased in the OVX + STZ + saline group compared with OVX + saline group ($P = 0.001$), while it was significantly decreased in the OVX + STZ + Kmp group ($P = 0.001$) compared with the OVX + STZ + saline group (**Figure 2C**).

A significant difference was found in the brain level of MDA [$F(3,26) = 11.32$, $P = 0.001$] between groups. Brain level of MDA level was significantly increased in the OVX + STZ + saline group compared with the OVX + saline group ($P = 0.049$), while it was significantly decreased in the OVX + STZ + Kmp group compared with the OVX + STZ + saline group ($P = 0.009$) (**Figure 2D**).

Cytochrome c level did not change significantly in either of the groups [$F(3,19) = 1.501$, $P = 0.264$]; however, it was decreased slightly, but insignificantly, in the OVX + STZ + Kmp

compared with the OVX + STZ + saline group (**Figure 2E**).

Discussion

In the present study, we investigated the effect of Kmp on STZ-induced memory impairment and hippocampal endogenous antioxidants SOD and GSH. In addition, TNF-α, MDA, and cytochrome c levels were measured because of their roles in the inflammation, lipid peroxidation, and apoptosis respectively.

The obtained data clearly demonstrated that: (1) OVX and ICV injection of STZ produced learning and memory deficits, decreased SOD and GSH levels in the hippocampus, while elevated MDA, TNF-α, and cytochrome c levels. (2) Kmp (10 mg/kg per day) reversed the STZ-induced cognitive dysfunction significantly and enhanced hippocampal SOD and GSH levels. (3) Kmp also reduced the levels of inflammatory markers MDA and TNF-α, but not the apoptotic factor of cytochrome c.

Consistent with previous studies (Su et al., 2012; Parker et al., 2014; Solmaz et al., 2015), results of the current study showed that OVX increases the risk of cognitive decline, parallel with enhancement in lipid peroxidation and neuroinflammation. Moreover, ICV injection of STZ doubled memory impairment, induced oxidative stress, lipid peroxidation, and neuroinflammation. STZ has been known to induce neuronal damage by producing free radicals, Aβ deposits (Veerendra Kumar and Gupta, 2003; Huang et al., 2016), and lipid peroxidation (Rai et al., 2014). Santos et al. (2012) previously reported disruption of working memory 3 hours after STZ injections, which was followed by degenerative processes in the hippocampus (Santos et al., 2012). Elevation in MDA (a marker of lipid peroxidation), and TNF-α in the present study reflects oxidative stress and inflammatory status (Gustaw-Rothenberg et al., 2010). TNF-α, as an inflammatory marker, plays an essential role in Aβ plaque accumulation, cell death, and cognitive deficits (Chang et al., 2017).

On the other hand, treatment with Kmp for 21 days significantly reversed STZ-induced cognitive dysfunction, increased hippocampal SOD and GSH levels, and reduced MDA and TNF-α levels. Many studies including ours, suggest that flavonoids have the potential to enhance memory and learning function (Veerendra Kumar and Gupta, 2003; Kouhestani et al., 2017). These components have been known to activate intracellular signaling pathways of memory storage such as mitogen-activated protein kinases, phosphoinositide 3-kinase, protein kinase B (Krishnaveni, 2012), and cAMP response element-binding protein (CREB) (Spencer, 2010).

Elevation in SOD and GSH levels in the present study reflects improvement in brain endogenous antioxidants. SOD and GSH are the most important first-line antioxidant defense systems against toxicity of ROS (Wojtunik-Kulesza et al., 2016). GSH works *via* scavenging ROS and removing hydrogen and lipid peroxidase (Halliwell and Gutteridge, 1989), and improves neurological functions.

Kmp, the essential flavonoid of medlar leaves extract, has been shown to augment cellular antioxidant defense capacity (Kampkotter et al., 2007; Hong et al., 2009). Kmp also scavenges hydroxyl radical and peroxynitrite increases the activity of antioxidant enzymes and prevents lipid peroxidation (Ozgova et al., 2003; Kampkotter et al., 2007).

Furthermore, the present study showed that Kmp caused 21% reduction in hippocampal MDA level, 52% reduction in hippocampal TNF-α level, and only 6% reduction in hippocampal cytochrome c level. These findings reflect the ability of Kmp to alleviate neurotoxicity induced by STZ.

In line with our findings, Sharoar et al. (2012) and Li and Pu (2011) reported that chronic administration of Kmp leads to attenuating neurotoxicity induced by Aβ and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine respectively. Also Kmp exhibited anti-parkinsonian activity which was related to increases in SOD and glutathione peroxidase activities and reduction in MDA level. In addition, Chen et al. (2012) reported that Kmp attenuated lung injury in mice. Considering no significant change in cytochrome c in our study explains that Kmp might not be effective on the mitochondrial intrinsic pathway of apoptosis. However, the effect of Kmp on other pro-apoptotic factors remains to be elucidated in future studies.

Taken together, findings from this study suggest potential important clinical relevance of considering anti-inflammatory and antioxidant properties of Kmp, as it was reported in metabolic syndrome (Hoang et al., 2015), Parkinson (Li and Pu, 2011) and cardiovascular diseases (Perez-Vizcaino and Duarte, 2010).

In conclusion, this study demonstrated for the first time that Kmp ameliorates STZ-induced cognitive dysfunction possibly through regulating antioxidants and neuroinflammation in ovariectomized rat hippocampus, however, the underlying mechanism remains unclear.

References

Agrawal R, Tyagi E, Shukla R, Nath C (2011) Insulin receptor signaling in rat hippocampus: a study in STZ (ICV) induced memory deficit model. Eur Neuropsychopharmacol 21: 261-273.

Aliev G, Obrenovich ME, Reddy VP, Shenk JC, Moreira PI, Nunomura A, Zhu X, Smith MA, Perry G (2008) Antioxidant therapy in Alzheimer's disease: theory and practice. Mini Rev Med Chem 8:1395-1406.

Asadi F, Jamshidi AH, Khodagholi F, Yans A, Azimi L, Faizi M, Vali L, Abdollahi M, Ghahremani MH, Sharifzadeh M (2015) Reversal effects of Crocin on amyloid β-induced memory deficit: modification of autophagy or apoptosis markers. Pharmacol Biochem Behav 139:47-58.

Babaei P, Ghorbani Shirkouhi S, Hosseini R, Soltani Tehrani B (2017) Vitamin D is associated with metabotropic but not neurotrophic effects of exercise in ovariectomized rats. Diabetol Metab Syndr 9:91.

Banjarnahor SD, Artanti N (2014) Antioxidant properties of flavonoids. Med J Indones 23:239-244.

Batarseh SY, Duong QV, Mousa MY, Rihani SB.AL, Elfakhri KH, Kaddoumi A (2016) Amyloid-β and astrocytes interplay in amyloid-β related disorders. Int J Mol Sci 17:338.

Chang R, Yee KL, Sumbria RK (2017) Tumor necrosis factor α Inhibition for Alzheimer's disease. J Cent Nerv Sys Dis 9:1-5.

Chen X, Yang X, Liu T, Guan M, Feng X, Dong W, Chu X, Liu J, Tian X, Ci X, Li H, Wei J, Deng Y, Deng X, Chi G, Sun Z (2012) Kaempferol regulates MAPKs and NF-κB signaling pathways to attenuate LPS-induced acute lung injury in mice. Int Immunopharmacol 14:209-216.

Ercisli S, Sengul M, Yildiz H, Sener D, Duralija B, Voca S, Purgar DD(2012) Phytochemical and antioxidant characteristics of medlar fruits (Mespilusgermanica L.). J Appl Bot Food Qual 85:86-90.

Farlow MR, Miller ML, Pejovic V (2008) Treatment options in Alzheimer's disease: maximizing benefit, managing expectations. Dement Geriatr Cogn Disord 25:408-422.

Gustaw-Rothenberg K, Kowalczuk K, Stryjecka-Zimmer M (2010) Lipids' peroxidation markers in Alzheimer's disease and vascular dementia. Geriatr Gerontol Int 10:161-166.

Halliwell B, Gutteridge JM (1989) Free radicals in biology and medicine. Oxford, UK: Clarendon Press.

Hoang MH, Jia Y, Mok B, Jun HJ, Hwang KY, Lee SJ (2015) Kaempferol ameliorates symptoms of metabolic syndrome by regulating activities of liver X receptor-β. J Nutr Biochem 26:868-875.

Hong JT, Yen JH, Wang L, Lo YH, Chen ZT, Wu MJ (2009) Regulation of heme oxygenase-1 expression and MAPK pathways in response to kaempferol and rhamnocitrin in PC12 cells. Toxicol Appl Pharmacol 237:59-68.

Huang WJ, Zhang X, Chen WW (2016) Role of oxidative stress in Alzheimer's disease. Biomed Rep 4:519-522.

Kamat PK, Rai S, Swarnkar S, Shukla R, Nath C (2014) Mechanism of synapse redox stress in okadaic acid (ICV) induced memory impairment: role of NMDA receptor. Neurochem Int 76:32-41.

Kampkötter A, Gombitang Nkwonkam C, Zurawski RF, Timpel C, Chovolou Y, Wätjen W, Kahl R (2007) Effects of the flavonoids kaempferol and fisetin on thermotolerance, oxidative stress and FoxO transcription factor DAF-16 in the model organism Caenorhabditis elegans. Arch Toxicol 81:849-858.

Kar S, Slowikowski SP, Westaway D, Mount HT (2004) Interactions between beta-amyloid and central cholinergic neurons: implications for Alzheimer's disease. J Psychiatry Neurosci 29:427-441.

Kim GN, Kim ES, Kwon YI, Jang HD (2012) Potential mechanism of kaempferol against Cu^{2+}-induced oxidative stress through chelating activity and regulation of nuclear factorerythroid-2-related factor 2 signaling. Food Sci Biotechnol 21:1469-1475.

Kim JK, Choi SJ, Cho HY, Hwang HJ, Kim YJ, Lim ST, Kim CJ, Kim HK, Peterson S, Shin DH (2010) Protective effects of kaempferol (3,4',5,7-tetrahydroxyflavone) against amyloid beta peptide (Abeta)-induced neurotoxicity in ICR mice. Biosci Biotechnol Biochem 74:397-401.

Kouhestani S, Zare S, Babaei P (2017) Effects of pure flavonoid of medlar leaves on passive avoidance learning and memory in Alzheimer model of ovariectomized rats. J Guilan Univ Med Sci 26:62-71.

Krishnaveni M (2012) Flavonoid in enhancing memory function. J Pharmacy Res 5:3870-3874.

Kumar M, Kaur D, Bansal N (2017) Caffeic acid phenethyl ester (CAPE) prevents development of STZ-ICV induced dementia in rats. Pharmacogn Mag13:10-15.

Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Sci World J 2013:1-16.

Li S, Pu XP (2011) Neuroprotective effect of kaempferol against a 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson's disease. Biol Pharm Bull 34:1291-1296.

Ozgova S, Hermanek J, Gut I (2003) Different antioxidant effects of polyphenols on lipid peroxidation and hydroxyl radicals in the NADPH-, Fe-ascorbate- and Fe-microsomal systems. Biochem Pharmacol 66:1127-1137.

Parker WH, Broder MS, Chang E, Feskanich D, Farquhar C, Liu Z, Shoupe D, Berek JS, Hankinson S, Manson JE (2009) Ovarian conservation at the time of hysterectomy and long-term health outcomes in the nurses' health study. Obstet Gynecol 113:1027-1037.

Perez-Vizcaino F, Duarte J (2010) Flavonols and cardiovascular disease. Mol Aspects Med 31:478-494.

Pourmir M, Babaei P, Soltani Tehrani B (2016) Kisspeptin-13 ameliorates memory impairment induced by streptozotocin in male rats via cholinergic system. Physiol Pharmacol 20:38-57.

Rai S, Kamat PK, Nath C, Shukla R (2014) Glial activation and post-synaptic neurotoxicity: the key events in Streptozotocin (ICV) induced memory impairment in rats. Pharmacol Biochem Behav 117:104-117.

Ramezani M, Darbandi N, Khodagholi F, Hashemi A (2016) Myricetin protects hippocampal CA3 pyramidal neurons and improves learning and memory impairments in rats with Alzheimer's disease. Neural Regen Res 11:1976-1980.

Redza-Dutordoira M, Averill-Batesa DA (2016) Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta 1863:2977-2992.

Reyazuddin M, Azmi SA, Islam N, Rizvi A (2014) Oxidative stress and level of antioxidant enzymes in drug-naïve schizophrenics. Indian J Psychiatry 56:344-349.

Santos TO, Mazucanti CH, Xavier GF, Torrão AS (2012) Early and late neurodegeneration and memory disruption after intracerebroventricular streptozotocin. Physiol Behav 107:401-413.

Sharoar MG, Thapa A, Shahnawaz M, Ramasamy VS, Woo ER, Shin SY, Park IS (2012) Keampferol-3-O-rhamnoside abrogates amyloid beta toxicity by modulating monomers and remodeling oligomers and fibrils to non-toxic aggregates. J Biomed Sci 19:104.

Solmaz V, Çınar BP, Yiğittürk G, Çavuşoğlu T, Taşkıran D, Erbaş O (2015) Exenatide reduces TNF-α expression and improves hippocampal neuron numbers and memory in streptozotocin treated rats. Eur J Pharmacol 765:482-487.

Spencer JP (2010) The impact of fruit flavonoids on memory and cognition. Br J Nutr 3:40-47.

Su J, Sripanidkulchai K, Hu Y, Wyss JM, Sripanidkulchai B (2012) The effect of ovariectomy on learning and memory and relationship to changes in brain volume and neuronal density. Int J Neurosci 122:549-559.

Veerendra Kumar MH, Gupta YK (2003) Effect of Centellaasiatica on cognition and oxidative stress in an intracerebroventricular streptozotocin model of Alzheimer's disease in rats. Clin Exp Pharmacol Physiol 30:336-342.

Wojtunik-Kulesza KA, Oniszczuk A, Oniszczuk T, Waksmundzka-Hajnos M (2016) The influence of common free radicals and antioxidants on development of Alzheimer's disease. Biomed Pharmacother 78:39-49.

Youdim KA, Qaiser MZ, Begley DJ (2004) Flavonoid permeability across on in situ model of the blood-brain barrier. Free RadicBiol Med 36:592-604.