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Fimbristylis ovata (Cyperaceae) extract alleviates neuronal degeneration and death through AGEs/RAGE/JNK regulation in human neuroblastoma cell line, SH-SY5Y

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ABSTRACT. Clinical evidence has implicated advanced glycation end products (AGEs) as one of the risk factors for neurological dysfunction and death. However, the neurodegeneration regulation initiated by external AGEs has not yet been fully identified. We investigated the neuroprotective effect of *Fimbristylis ovata* extract, a medicinal plant utilized in Thai traditional medicine, against neuronal cell death in response to AGEs-induced cellular stress stimuli. SH-SY5Y, a human neuroblastoma cell, was used to investigate the relationship between AGEs induction and neuronal cell damage**.** The herbal extract derived from *F. ovata*, a member of the Cyperaceae family, was tested for its properties against AGEs-induced neuronal stress through changes in RAGE, Mn-SOD, p38 MAPK, and JNK protein expression. APP-related genes, including APP, ADAM10, BACE1, PSEN1, and TACE, were also tested. We found that *F. ovata* extract exerted its antioxidant activity by normalized AGEs-mediated RAGE elevation and increased Mn-SOD levels. Total apoptotic cells were also decreased by *F. ovata* extract treatment; this

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perhaps was due to its ability to suppress JNK activation. Additionally, reduction of expression of APP-related genes including APP, PSEN1, and TACE (or ADAM17) was observed in the *F. ovata* extract-treated groups. These results provide evidence that *F. ovata* extract has potential as a neuroprotective agent through its antioxidant and anti-apoptotic properties.

Key words: AGEs; *Fimbristylis ovata*; JNK; Mn-SOD; Neuronal death

INTRODUCTION

Advanced glycation end products (AGEs) are reactive derivatives of the nonenzymatic glycation of proteins, lipids, and nucleic acids known as the Maillard reaction. AGEs formation has been known to progress in the normal aging process. AGEs levels in the brain appear to increase in Alzheimer's disease (AD) (Sasaki et al., 1998). The toxic effects of AGEs trigger several pathological processes involving inflammatory and oxidative stress pathways which are believed to play a pivotal role in various degenerative diseases including AD (Kuzan, 2021). The receptor for advanced glycation end products (RAGE), a common receptor for AGEs, has been reported to trigger various signaling pathways including p38 mitogen-activated protein kinase (MAPK), nuclear factor kappa-B (NF-κB), cell division control protein 42/Ras-related C3 botulinum toxin substrate (CDC42/Rac), stress-activated protein kinase/c-Jun N-terminal kinases (SAPK/JNK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways in different cell types (Soman et al., 2013). Growing evidence supports that RAGE is involved in various pathogenesis of neurological diseases such as multiple sclerosis, amyotrophic lateral sclerosis, AD, and neurological complications of diabetes (Yan et al., 2003; Deane et al., 2004; Maczurek et al., 2008; Qin et al., 2008; Iłżecka, 2009).

AGEs/RAGE activation contribute to the pathogenesis of neuronal dysfunction and death. Consequently, AGEs/RAGE pathways might be interesting novel therapeutics for the early prevention of disease. The role of mitogen-activated protein kinases (MAPK) in the biological process has been extensively reviewed, including cell proliferation, differentiation, and death program responding to extracellular stimuli through intracellular signaling pathways. These regulations have been suggested to function in a cell context-specific and cell type-specific manner (Yue and López, 2020).

Fimbristylis ovata is classified as a member of the Cyperaceae plant family. Its antioxidant and anti-inflammatory properties have been previously described (Sukjamnong and Santiyanont, 2015; Sirirattanakul and Santiyanont, 2021). *Cyperus* plants have been reported to be traditionally used for many diseases. The potential healthcare applications have been variously stated, such as antioxidant activity, anti-inflammatory activity, antimicrobial activity, neuroprotective activity, antidepressant activity, and obesity management. The therapeutic effects of many traditional herbs are due to the presence of natural antioxidants, especially phenolic compounds (Taheri et al., 2021). Herbal plants have been widely used to defend against and minimize cell damage as they are excellent sources of antioxidants and are easily available.

We have previously reported that the extract of *F. ovata* showed possible abilities of a neuroprotective agent by suppressing oxidative stress level, the transcriptional level of

RAGE, and pro-inflammatory cytokines. However, other cellular responses of these plants relevant to neuronal damage and death are rarely known and remained very little data. This study was designed to investigate the *F. ovata* extract to understand its protective role against stress-induced neuronal damage and death through AGEs/RAGE/MAPK regulation.

MATERIAL AND METHODS

Extraction of herbal materials

A single source of *F. ovata* was collected in Bangkok, Thailand. The voucher specimen was botanically identified and given an herbarium number by Professor Kasin Suvatabhandhu Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University, Thailand (voucher No. 013431(BCU)). The fresh herb was cleaned with water, dried in a laboratory oven at 45°C for five days, then ground into fine powder. The fine powder of the herb was then extracted with petroleum ether (Millipore) and methanol (Millipore) 1:10 (w/v) by Soxhlet extraction. The extracts were filtered and evaporated. DMSO (SIGALD) was used as a solvent to dissolve crude extracts. The dissolved extracts were stored as a stock solution of 100 mg/mL at -20°C and protected from light until use.

Cell culture

Human neuroblastoma cell line, SH-SY5Y was a generous gift from Dr. Tewarit Sarachana, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand. SH-SY5Y were cultured in the Minimum Essential Medium: Nutrient Mixture F-12 (MEM/F12) (Hyclone) (1:1) culture medium supplemented with 15% (v/v) FBS (Gibco) and 1% penicillin/streptomycin (Gibco). Cell cultures were maintained at 37°C under 95% humidified and 5% (v/v) CO_2-air environment. Before initiation of experiments, the cells were plated and allowed to grow overnight with fresh medium supplemented with 5% FBS.

Measurement of protein expression levels by western blot analysis

Western blot analysis was applied for a receptor for advanced glycation end products (RAGE) (Millipore), manganese superoxide dismutase (Mn-SOD) (Millipore), p38 mitogen-activated protein kinases (p38 MAPK) (Cell Signaling Technology), and c-Jun N-terminal kinases (JNK) (Cell Signaling Technology). Briefly, SH-SY5Y cells were cultured and seeded in a density of $1x10^5$ cells/cm^2 in 6-well plates. The culture medium was then changed to fresh 5% FBS medium after 24 hours of incubation. Pretreatment was applied for 3 hours with either vehicle control or one of the designated substances: *F. ovata* extracts, N-Acetylcysteine (NAC) (SIGALD). Then, the cells were incubated with 200 µg/mL of Advanced Glycation End Product-bovine serum albumin (AGEs-BSA) (SIGALD) for an hour before protein extraction. Bradford assay was performed to ensure equal loading of protein samples (20 µg). Protein samples were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (70 V, 30 minutes followed by 120 V, 60 minutes), and then transferred to polyvinylidene difluoride (PVDF) blotting membranes (Cytiva) (150 mA, 90 minutes). The membranes were blocked with tris-buffered saline with tween 20 (TBS-T) containing 5% non-fat dry milk for an hour,

washed, and probed with specific primary antibodies overnight at 4ºC. Next, the blots were incubated with peroxidase-conjugated secondary antibodies for an hour. Then, wash with TBS-T. Densitometer analysis using ImageJ software (U.S. National Institutes of Health) (available at https://imagej.nih.gov/ij/) was applied to quantify the intensities of specific bands.

Measurement of messenger ribonucleic acid (mRNA) expression levels by quantitative polymerase chain reaction (qPCR)

SH-SY5Y were grown in a 6-well plate with a density of $1x10^5$ cells/cm^2 and pretreated with either vehicle control or one of the designated substances: *F. ovata* extracts or N-acetylcysteine (NAC) for three hours. After that, we applied 200 µg/mL of AGEs-BSA and incubated for 24 hours. Total RNA was purified using Trizol reagent (Invitrogen) according to the manufacturer's instructions. Absorbance measurement at 260 nm was performed to determine RNA concentration. AccuPower® CycleScript RT PreMix and AccuPower® 2X GreenStar Master Mix Solution (Bioneer) were used for complementary deoxyribonucleic acid (cDNA) synthesis and amplification. Starting with 2 µg of RNA, the cDNA synthesis reaction was performed at 42°C, 60 minutes, followed by 94 °C, 5 minutes. The thermal cycling conditions were composed of an initial denaturation step at 95 °C for 10 minutes, followed by 35 cycles at 95°C for 15 seconds and 62°C for 30 seconds. qPCR was detected under SYBR Green-based Exicycler™ 96 Real-Time Quantitative Thermal Block system. The mRNA expression levels were then normalized with β-actin and calculated as fold-change using the ΔΔCt method (2-ΔΔCt). The specific primer pairs used for all experiments were shown in Table 1.

Table 1. Specific primer pairs of APP, ADAM10, BACE1, PSEN1, TACE, and β –actin for double-stranded (ds) cDNA synthesis and amplification.

Gene	Primer pairs
APP	Forward: 5'-TGGTTCGAGAGGTGTGCTCTG-3'
	Reverse: 5'-CGCCACATCCGCCGTAAAAG-3'
ADAM10	Forward: 5'-CTGCCCAGCATCTGACCCTA-3'
	Reverse: 5'-GAACCTGCACATTGCCCATT-3'
BACE1	Forward: 5'-CCACGGGCACTGTTATGGGA-3'
	Reverse: 5'-ATCGTGCACATGGCAAGCG-3'
PSEN1	Forward: 5'-TGGCGGGTTCAGTGAGGAAT-3'
	Reverse: 5'-ACTCCCCTTTCCTCTGGGTCTT-3'
ADAM17	Forward: 5'-CCGGGAACATGAGGCAGTCT-3'
	Reverse: 5'-TCAAGCTTCTCGAGTCTCTGGTG-3'
β -actin	Forward: 5'-CTTCCTGGGCATGGAGTCCTGT-3'
	Reverse: 5'-CTTTGCGGATGTCCACGTCAC-3'

Measurement of apoptotic event

Muse™ Annexin V & Dead Cell Kit (Millipore) was used to determine the percentage of live, early apoptotic, late apoptotic, total apoptotic, and dead cells of

cellular samples. SH-SY5Y were seeded in a density of $1x10^5$ cells/cm^2 in 24-well plates. The cells were pretreated for 3 hours with either vehicle control or one of the following substances including *F. ovata* extracts, and NAC. After that, 200 µg/mL AGEs-BSA was applied for 24 hours. Analyzing step was performed on Muse™ Cell Analyzer according to the manufacturer's instructions. Harvested cells were resuspended in suspension with at least 1% BSA, and 1% FBS ($1x10^5$ cells/mL). Resuspended cells were stained with a staining solution of Annexin V and 7-AAD. Stained cells were analyzed by Muse™ Cell Analyzer with Muse™ software.

Statistical analysis

All experiments were performed at least in triplicate. Results are presented as mean ± standard error of the mean (mean ± SEM). Statistical significances were analyzed by GraphPad Prism 9 for Windows (available at https://www.graphpad.com/) using Student's *t*-test. P values less than 0.05 were defined as statistically significant.

RESULTS

RAGE protein expression levels upon initiation of AGEs in SH-SY5Y

As a major receptor for advanced glycation end products (AGEs), the receptor for advanced glycation end products (RAGE) was observed by western blot analysis. As shown in Figure 1A, 200 µg/mL AGEs-BSA incubation for 1 hour significantly increased RAGE protein levels (P = 0.0217). Pretreatment with 100 mg/mL *Fimbristylis ovata* (*F. ovata)* methanol extract (met.) for 3 hours exhibited a possibility to normalize RAGE protein expression compared to AGEs treated (P = 0.0451). This result suggests that the extract of *F. ovata* might exert its role as a neuroprotective agent against AGE-induced cytotoxicity as demonstrated by RAGE reduction.

Change of Mn-SOD protein expression levels in SH-SY5Y

To determine whether initiation of oxidative stress by AGEs affects antioxidant components of cells. Superoxide dismutase (SOD), one of the main enzymatic scavengers responsible for preventing the formation or neutralization of free radicals, was observed by western blotting. As shown in Figure 1B, an hour of incubation of AGEs lower manganese superoxide dismutase (Mn-SOD) levels (P= 0.0402). 3 hours of pretreatment of 100 mg/mL *F. ovata* petroleum ether extracts (pet.) significantly raised Mn-SOD levels (P = 0.0280) compared to AGEs treated, which is compared to the pretreatment of 10 mM N-Acetylcysteine (NAC) (P = 0.0309). While pretreatment with 100 mg/mL *F. ovata* (met.) slightly increased Mn-SOD levels but without statistical significance (P > 0.05).

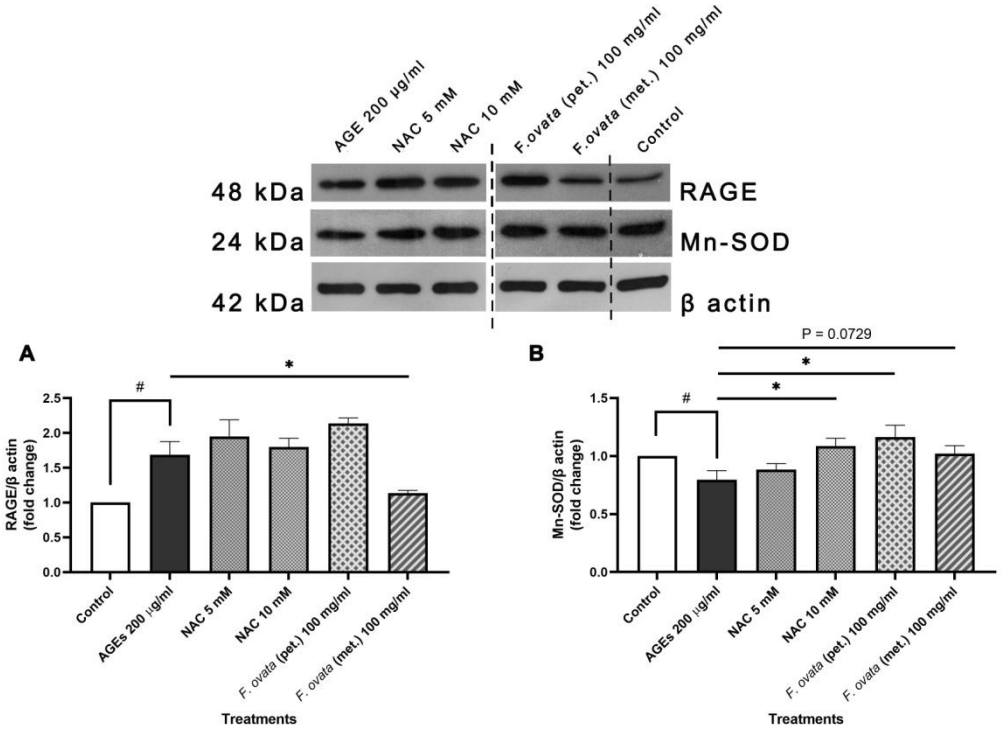

Figure 1. The effect of *Fimbristylis ovata* extract on RAGE and Mn-SOD protein levels in SH-SY5Y. Results were calculated as fold change to untreated control and expressed as mean ± SEM. Outcomes with $P < 0.05$ were considered as statistically significant (#, $P < 0.05$; *, $P < 0.05$). (A) The difference in RAGE protein level fold change between control and 200 µg/mL AGEs (1.688 ± 0.1882) was statistically significant ($P = 0.0217$). Pretreatment with 100 mg/mL *F. ovata* (met.) (1.134 ± 0.0389) significantly reduced RAGE protein level compared to AGEs treated ($P = 0.0451$). (B) Statistical differences in fold change of Mn-SOD level between control and 200 µg/mL AGEs (0.7961 ± 0.0782) have been observed ($P = 0.0402$). Pretreatment with 10 mM NAC (1.086 ± 0.0674) and 100 mg/mL *F. ovata* (pet.) (1.164 ± 0.1011) significantly increased Mn-SOD levels compared to AGEs treated ($P = 0.0309$ and 0.0280, respectively). Pretreatment with 100 mg/mL *F. ovata* (met.) slightly increased Mn-SOD level but without statistically significant ($P = 0.0729$). pet.: petroleum ether, met.: methanol.

Effects of *F. ovata* extract encountering AGEs treated on protein expression levels of canonical MAP kinases classes

As one of the signaling cascades contributing to neuronal degeneration through its involvement in cellular processes such as cell proliferation, cell survival, and apoptosis, the change of mitogen-activated protein kinases (MAPK) upon AGEs-treated and pretreated with either vehicle control containing 0.1% DMSO or one of the selected substances: *F. ovata* (pet.), *F. ovata* (met.), or NAC were evaluated. p38 MAPK and c-Jun N-terminal kinases (JNK) were measured using western blot analysis. Results demonstrated that there was no significant change in total p38 MAPK and JNK levels after treatment of 200 µg/mL AGEs-BSA for 1 hour (Figure 2). As shown in Figure 2A, an increase in the ratio of phosphorylated p38 MAPK to total p38 MAPK protein level was observed with 10 mM NAC ($P = 0.0274$) and 100 mg/mL *F. ovata* (pet.) ($P =$

0.0039) pretreatment groups compared to AGEs treated. Figure 2B revealed that the ratio of phosphorylated JNK to total JNK levels can be reduced upon pretreatment with 100 mg/mL *F. ovata* (pet.) or 100 mg/mL *F. ovata* (met.) (P = 0.0024 and 0.0193, respectively).

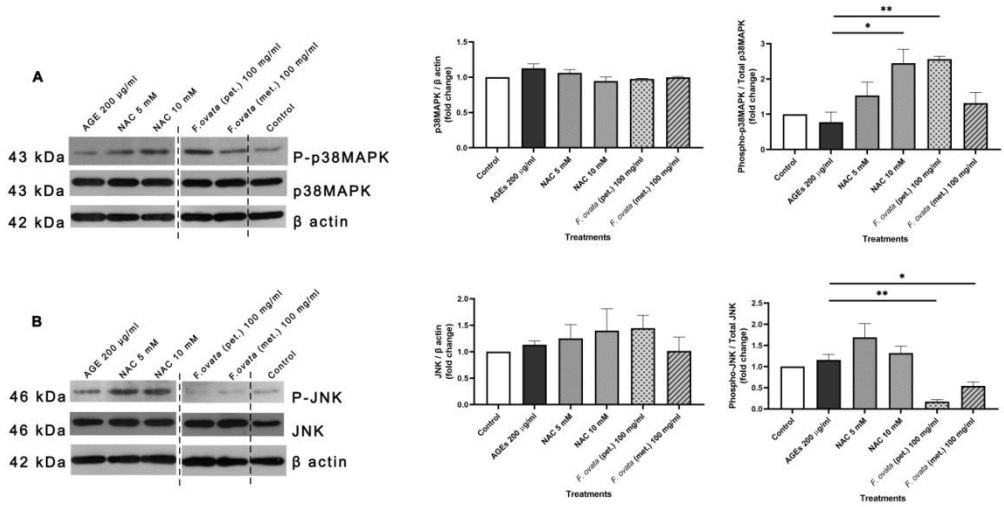

Figure 2. Protein expression levels of canonical mitogen-activated protein kinases (MAPK) classes resulting from *Fimbristylis ovata* extract treatment. Results were calculated as fold change to untreated control and expressed as mean ± SEM. Outcomes with P < 0.05 were considered as statistically significant (*, P < 0.05; **, P < 0.01). (A) Pretreatment with 10 mM NAC (2.445 ± 0.3984) and 100 mg/mL *F. ovata* (pet.) (2.565 ± 0.0787) significantly increased the ratio of phosphorylated p38MAPK to total p38MAPK compared to AGEs treated (0.7747 ± 0.2888) (P = 0.0274 and 0.0039, respectively). (B) Pretreatment with 100 mg/mL *F. ovata* (pet.) (0.1708 ± 0.0483) significantly decreased the ratio of phosphorylated JNK to total JNK compared to AGEs treated (1.155 ± 0.1354) (P = 0.0024). Pretreatment with 100 mg/mL *F. ovata* (met.) (0.5438 ± 0.0879) slightly decreased the ratio of phosphorylated JNK to total JNK compared to AGEs treated (0.5438 ± 0.0879) (P = 0.0193). pet.: petroleum ether, met.: methanol.

Variable of cellular death events upon *F. ovata* pretreatment to encounter AGEs induction

As one of the key pathological features in all neurodegenerative diseases, total levels of neuronal cell death have been studied. To induce apoptosis, cells were exposed to 200 µg/mL AGEs-BSA for 24 hours. The treated cells were harvested and analyzed using flow cytometry. Our result demonstrated that the total apoptotic cell level was increased upon AGEs initiation (P = 0.0023) (Figure 3). Either *F. ovata* extracts or NAC were applied to observe the effect of *F. ovata* extract encountering AGEs. Data showed that 100 mg/mL *F. ovata* (pet.) significantly decreased total apoptotic cells when compared to the AGEs treated group (P = 0.0091) (Figure 3). The influence of 100 mg/mL *F. ovata* (pet.) was comparable to 10 mM NAC.

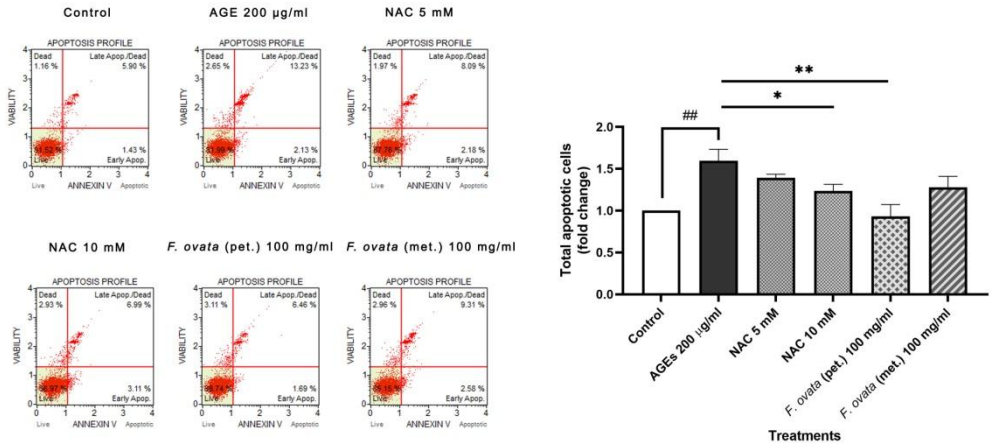

Figure 3. Apoptosis profile variable in SH-SY5Y cells. Results were calculated as fold change to untreated control and expressed as mean ± SEM. Outcomes with $P < 0.05$ were considered as statistically significant ($^{\#\#}$, $P < 0.01$; *, $P < 0.05$; **, $P < 0.01$). The difference in fold change of total apoptotic cell levels between the control and 200 µg/mL AGEs (1.5980 ± 0.1355) was significant ($P = 0.0023$). Pretreatment with 10 mM of NAC (1.236 ± 0.0782), and 100 mg/mL of *Fimbristylis ovata* (pet.) (0.9331 ± 0.1390) significantly decreased total apoptotic cell levels compared to AGEs treated ($P = 0.0496$, and 0.0091, respectively). pet.: petroleum ether, met.: methanol.

Change of APP-related mRNA expression following *F. ovata* extract pretreatment on AGEs-treated cell

The effect of AGEs on Amyloid beta precursor protein (APP) and its related mRNA levels had been assessed by 200 µg/mL AGEs-BSA incubation in SH-SY5Y overnight. Expression levels of APP, Beta-secretase 1 (BACE1), Presenilin 1 (PSEN1), ADAM metallopeptidase domain 10 (ADAM10), and Tumor necrosis factor-alpha converting enzyme (TACE) were then measured using qPCR. Results showed that exposure to AGEs significantly increase APP ($P < 0.0001$), BACE1 ($P < 0.0001$), PSEN1 ($P = 0.0049$), and TACE ($P = 0.0275$) (Figure 4A-C, E). These results indicated that AGEs-mediated neuronal injury may occur through the change of these transcriptional levels.

To find out the effect of *F. ovata* extract encountering AGEs on APP-related mRNA level, SH-SY5Y cells were pretreated with either vehicle control containing 0.1% DMSO or one of the selected substances: *F. ovata* (pet.), or *F. ovata* (met.) before AGEs treatment.

Our results demonstrated that the extract of *F. ovata* could normalize APP, BACE1, PSEN1, and TACE upon AGEs-induced neuronal change (Figure 4A-C, E). Additionally, *F. ovata* (pet.) was found to be able to enhance ADAM10 expression compared to AGEs-treated ($P = 0.0235$) (Figure 4D). Hence, the extract of *F. ovata* may exert its role as a neuroprotective agent against stress stimuli through these cascades.

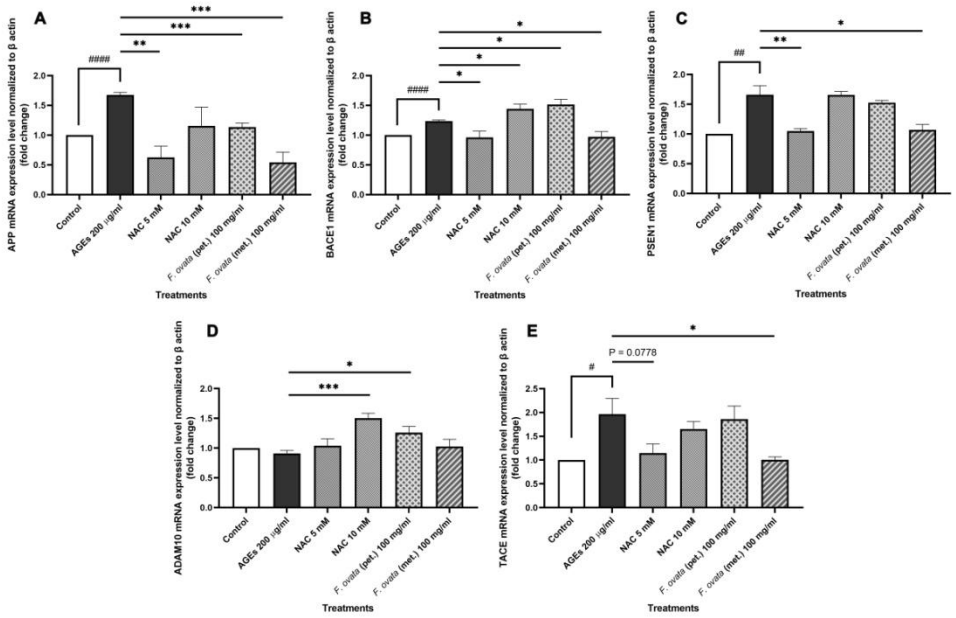

Figure 4. Change of APP-related mRNA expression following *Fimbristylis ovata* extract pretreatment on AGEs-treated cell. Results were calculated as fold change to untreated control and expressed as mean ± SEM. Outcomes with $P < 0.05$ were considered as statistically significant ($^{\#}$, $P < 0.05$; $^{\#\#}$, $P < 0.01$; $^{\#\#\#}$, $P < 0.001$; $^{\#\#\#\#}$, $P < 0.0001$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$). (A) 200 µg/mL AGEs significantly increased APP mRNA level (1.6780 ± 0.0419) ($P < 0.0001$). Pretreatment with 5 mM NAC (0.6238 ± 0.1912), 100 mg/mL *F. ovata* (pet.) (1.134 ± 0.0698), and 100 mg/mL *F. ovata* (met.) (0.5401 ± 0.1740) significantly decreased APP levels compared to AGEs treated ($P = 0.0017$, 0.0005, and 0.0007, respectively). (B) AGEs significantly enhanced BACE1 levels (1.235 ± 0.0207) compared to untreated control ($P < 0.0001$). Pretreatment with 5 mM NAC (0.9631 ± 0.1053), and 100 mg/mL *F. ovata* (met.) (0.9725 ± 0.0888) significantly decreased BACE1 levels compared to AGEs treated ($P = 0.0447$, and 0.0282, respectively). (C) The difference in fold change of PSEN1 mRNA expression level between control and 200 µg/mL AGEs (1.660 ± 0.1521) was significant ($P = 0.0049$). Pretreatment with 5 mM NAC (1.048 ± 0.0419), and 100 mg/mL *F. ovata* (met.) (1.067 ± 0.0929) significantly reduced PSEN1 levels compared to AGEs treated ($P = 0.0082$, and 0.0159, respectively). (D) There was no difference in ADAM10 mRNA expression level fold change between the control and 200 µg/mL AGEs (0.9111 ± 0.0498). Pretreatment with 10 mM NAC (1.504 ± 0.0812) significantly increased ADAM10 level compared to AGEs treated ($P = 0.0008$). 100 mg/mL *F. ovata* (pet.) (1.259 ± 0.1041) also demonstrated the potential to raise ADAM10 level compared to AGEs treated ($P = 0.0235$). (E) The difference in TACE mRNA expression level fold change between control and 200 µg/mL AGEs (1.963 ± 0.3327) was significant ($P = 0.0275$). Pretreatment with 100 mg/mL *F. ovata* (met.) (1.006 ± 0.0612) significantly lessened TACE levels compared to AGEs treated ($P = 0.0299$). pet.: petroleum ether, met.: methanol.

DISCUSSION

It has been previously suggested that AGEs occur not only through normal aging processes, but are also triggered by acute stress, ROS generation, inflammation, and physical injury. RAGE upregulation is a key consequence of AGEs accumulation. The central roles of this axis have been highlighted in diabetes, inflammation, dementia, and Alzheimer's disease complications (Ramasamy et al., 2005). Our study investigated the association between AGEs and neuronal injury through neuronal cell death regulation. In this study, we also examined the ability of *F. ovata* extracts to neutralize these changes. In a

previous study, we demonstrated the neurotoxicity of AGEs in which the increase of ROS production and proinflammatory cytokines regulation have been involved with the exposure of AGEs. These changes are alleviated by *F. ovata* extract (Sirirattanakul and Santiyanont, 2021). We then speculated that *F. ovata* extract may have the ability to induce a change in neuronal cell death resulting from AGEs induction. The use of oxidative stress biomarkers may help provide evidence of a causal relationship between cellular damage caused by AGEs and the protective effect of *F. ovata* extract. This study confirmed that RAGE enhancement was affected by AGEs. A slight reduction in Mn-SOD levels was also observed in the AGEs-treated group. The *F. ovata* extract revealed its potential by suppressing the RAGE expression level. Moreover, the Mn-SOD level was also improved upon pretreatment with *F. ovata* extract, which was comparable to the NAC-treated group.

The stress-activated MAPK activity and their downstream signaling play a key role in balancing cell survival and death responding to cellular stress. MAPK family member including p38 MAPK, JNK, and ERKs cascades not only promote pro-apoptotic processes but are also involved in anti-apoptotic processes (Yue and López, 2020). Hence, we observed cellular changes centering around MAPK regulation. *F. ovata* extract proved its potential by increased phosphorylated p38 MAPK and decreased phosphorylated JNK. These are perhaps due to its different activation cascades, concentration, and timing (Shigiyama et al., 2019). It has been previously reported that under physical-chemical stresses and proinflammatory cytokines stimulation, p38 MAPK promotes neuronal survival through myocyte enhancer factor 2 (MEF2) regulation (Okamoto et al., 2000; Yin et al., 2012). JNK has been reported to play a role in apoptotic signaling through various targets. The induction of pro-apoptotic genes such as TNFα, Fas-L, and Bak via the JNK-AP1 pathway was discovered. Several other transcription factors were also phosphorylated by JNK. The potentiate apoptotic role of JNK was previously demonstrated by the inhibition of anti-apoptotic proteins such as Bcl2 (Liu and Lin, 2005; Dhanasekaran and Reddy, 2008). The neuroprotective effect of JNK inhibitors against cell death has also been reported (Guan et al., 2006). In concordance with the above-mentioned cascades, the regulation of AGEs stimuli might occur through these signaling molecules. The possibility of the neuroprotective effect of *F. ovata* extract mediating these processes is worth mentioning.

Previous researchers have suggested a potential link between AGEs toxicity and Alzheimer's disease (AD) that AGEs trigger ROS production, APP processing, and promote neuronal cell death pathways. The aggregated proteins such as Tau and amyloid β protein in Alzheimer's disease were also found to be involved in AGEs formation, hence the positive feedback loop of oxidative stress formation and neuronal dysfunction and death has been created (S. D. Yan et al., 1994). AD-related proteins including phosphorylated tau and APP, and APP processing proteins including BACE and PS1 (Pugazhenthi et al., 2017). Additionally, activation of JNK has been reported as its pathophysiologic feature in AD through APP processing (Colombo et al., 2009). We then wondered whether the AGEs/RAGE/JNK-mediated mechanism triggered other APP-related Aβ formation. Aβ deposition is a biomarker related to the early pathophysiological process of neurodegenerative diseases including AD and Parkinson's disease (PD) (Selkoe, 2001; Araki et al., 2019). APP proteolytic process involving proteolytic enzyme β and γ secretase is the source of Aβ. The production of Aβ is a normal metabolic event influenced by the developmental and physiological state of the cells. There are two potential pathways of the APP proteolytic process: nonamyloidogenic and amyloidogenic pathways. Both pathways

begin with an extracellular cleavage, followed by an intramembranous cleavage. The proteolytic event at the extracellular domain will determine the APP pathway. If the cleavage occurs at the α-secretase site within the Aβ domain, then Aβ is not generated (the nonamyloidogenic pathway). On the other hand, APP can undergo cleavage by β-secretase (beta-site amyloid precursor protein–cleaving enzyme 1; BACE1), which cuts at the N-terminal region of the Aβ sequence to produce Aβ (amyloidogenic pathway). Following the APP cleavage at either of these sites, an intramembranous cleavage at the C-terminus of Aβ by the γ-secretase complex (containing presenilin, nicastrin, Pen-2, and Aph-1) will be carried out. In the nonamyloidogenic pathway, a secreted sAPPα and an 83-residue carboxy-terminal APP fragment (C83) will be created upon the first cut. C83 is then cleaved by γ-secretase to produce extracellular peptide P3 and the APP intracellular domain (AICD) which further translocate to the nucleus to trigger transcription activation. In contrast, during a first cut of the amyloidogenic pathway, a secreted sAPPβ and a 99-residue carboxy-terminal APP fragment (C99) will be formed. After that, Aβ and AICD will be generated (Mattson, 2004; Tanzi and Bertram, 2005; Querfurth and Laferla, 2010). Our finding revealed that AGEs-mediated change in APP-related transcriptional levels by increasing APP, BACE1, PSEN1, and TACE levels. The expression level of ADAM10 did not affect by AGEs initiation. These findings support those previous publications that link AGEs toxicity to AD progression perhaps through amyloidogenic pathway enrichment. The potential of *F. ovata* extracts to alleviate these changes has been demonstated. Our study indicated APP, BACE1, PSEN1, and TACE reduction following *F. ovata* extract treatment, especially with the methanolic extract. These results suggested the neuroprotective effect of *F. ovata* extract-mediated amyloidogenic pathway suppression. ADAM10, a major α-secretase in neurons, is a key factor in the nonamyloidogenic pathway (Marcello et al., 2017). We found an increased trend toward ADAM10 expression upon *F. ovata* extract treatment. Thus, the *F. ovata* extract might exert its role as a neuroprotective substance by preventing Aβ generation mediated ADAM10-related nonamyloidogenic pathway.

Altogether, this study delineates the potential efficacy of *F. ovata* extract toward neuronal damage and death in response to cell stress stimuli. This work has supported the biological activity of *F. ovata* extract as antioxidant, anti-apoptotic, and anti-APP production (suppressed amyloidogenic pathway and enhanced nonamyloidogenic pathway regulation).

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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