Songklanakarin J. Sci. Technol.
43 (5), 1367-1373, Sep. - Oct. 2021

http://www.sjst.psu.ac.th

Original Article

The effects of ethanolic extract of Okra fruit, *Abelmoschus Esculentus* (L.) Moench on cellular senescence in aging neuron

Pichaya Jumnongprakhon[1], Krittalak Pasakawee[2], Panida Banjongsinsiri[2], Nowwapan Donrung[2], Supawadee Daodee[3], and Pennapa Chonpathompikunlert[2*]

[1] *Department of Anatomy, Faculty of Medical Science, Naresuan University, Mueang, Phitsanulok, 65000 Thailand*

[2] *The Expert Centre of Innovative Health Food* (*InnoFood*), *Thailand Institute of Scientific and Technological Research* (*TISTR*), *Khlong Luang, Pathum Thani, 12120 Thailand*

[3] *Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Mueang, Khon Kaen, 40000 Thailand*

Received: 3 September 2020; Revised: 8 October 2020; Accepted: 9 November 2020

Abstract

Cellular senescence is characterized by morphological and metabolic changes with emerging neurodegeneration. Recently, senescence has been described as having excessive amount of reactive oxygen species (ROS) and over-activation of senescence associated β-galactosidase (SA-β-gal) prior mediated loss of synaptic plasticity. The purpose of this study was to evaluate the therapeutic effects of ethanolic extract of Okra fruit (OKR) on neuronal aging. SK-N-SH cells were pre-incubated with hydrogen peroxide (H_2O_2) for 4 h and then treated with 10, 25, 50, or 75 µg/mL OKR for 24 h. The results demonstrated that OKR significantly promoted cell viability with reduction of ROS content and SA-β-gal positive cells. Additionally, the synaptic plasticity was also improved by inhibiting the AChE activity in OKR administration compared to aging neurons. Interestingly, the OKR was comparable to N-acetylcysteine (NAC). Hence, we propose that OKR could alleviate the negative responses to aging in neurons.

Keywords: antioxidant, cellular senescence, cellular dysfunction, neurodegeneration, okra fruit

1. Introduction

In response to persistent cellular stress and DNA damage, induction into cell senescence promotes age-related diseases, which facilitates cellular dysfunction and death (Hernandez-Segura, Nehme, & Demaria, 2018). Interestingly, emerging findings have revealed that program induction of cellular senescence plays a pivotal role in regulating brain diseases such as Alzheimer's disease (AD). In addition, the limitations in eliminating senescent cells can contribute to their persistence in brain tissue, leading to progression of the disease (Baker & Petersen, 2018). The pathological hallmark of cellular senescence, and potentially a cause of it, is an excessive amount of free radicals causing disequilibrium in oxidants and antioxidants, with a simultaneously increase in reactive oxygen species (ROS) production that encourages several negative responses such as lipid peroxidation, inflammation, and apoptosis (Feng, Yingxia, Nai-Kei, Jia, & Kwok-Fai, 2017). Moreover, an intracellular antioxidant defense system imbalance is also affected by prolonged oxidative stress both *in vitro* and *in vivo*, by dis-regulation of phase II enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Wang & Geng, 2019). In accordance with the oxidant/antioxidant imbalance, the cells are certainly stressed and can initiate cell cycle arrest and aging by promoting over-activation of senescence associated β-galactosidase enzyme

*Corresponding author
Email address: pennapa@tistr.or.th

(SA-β-gal) and telomere shortening (Hernandez-Segura, Brandenburg, & Demaria, 2018). The SA-β-gal is currently of high interest because of its high potency in the induction of synaptic plasticity deficit in brain disease by decreasing neurotransmitters such as acetylcholine, serotonin, and dopamine (Geng, Guan, Xu, & Fu, 2010). The down-regulation of synaptic proteins and up-regulation of enzyme degraded-synaptic protein activity such as monoamine oxidase (MAO) and acetylcholine esterase (AChE) is characteristic in synaptic loss from aging (Fernández-Gómez *et al*., 2010). In particular, it appears that AChE may interact with free radicals in a manner that increases the deposition of SA-β-gal and progressively turns into senescence and apoptosis (Martínez-Cué & Rueda, 2020). Recent studies have demonstrated that antioxidant compounds may contribute to reduction of AChE activity in brain tissue by suppressing the upstream mechanisms of AChE, such as inflammatory response in NF-κB. Moreover, several studies of antioxidant agents such as flavonoids and N-acetyl cysteine (NAC) have shown different outcomes in AChE activity of brain tissue (Haroon, Marya, Mohammad & Seema, 2018; Giuseppe, Placido & Emanuela, 2018). However, the assumption is that antioxidants may exert beneficial effects against the oxidant/antioxidant imbalance-induced brain aging, because they have a wide range of reversals of negative responses.

Abelmoschus Esculentus (L.) Moench, or Okra fruit, is an annual plant mainly grown in tropical countries. It has long been used as a vegetable, dietary supplement, and traditional medicine against gastritis. The chemical constituents in Okra are mainly polysaccharides, polyphenols, flavonoids, isoquercitrin, and quercetin-3-O-gentiobiose. Recently, pharmacological studies of Okra have revealed several pieces of evidence including anti-oxidant effects, anti-fatigue, and neuroprotection activities, among others (Islam, 2019; Xia *et al*., 2015). However, its potential effect on aging neurons are not well understood. Therefore is seems likely that Okra, which capitalizes on all these strengths, would be a good candidate food supplement for resistance to age-related diseases. Therefore, the aim of the study was to clarify the effects of Okra extract on reducing cell stress, senescence, and synaptic plasticity impairment. To examine the hypothesis, the SK-N-SH cells were treated with H_2O_2 to establish an aging neuronal model and then the effects of Okra on the aging neurons were explored.

2. Materials and Methods

2.1. Reagents

Hydrogen peroxide (H_2O_2), N-acetyl cysteine (NAC), DTNB (5, 5′-Dithiobis-(2-Nitrobenzoic acid), and Isoquercetrin were purchased from Sigma Aldrich Co. (St. Louis, MO,USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and 2′,7′-dichlorofluorescein diacetate were purchased from Merck Millipore. BetaRed™ β-Gal assay kit was from Millipore (MA, USA). Formic acid, Acetonitrite, and column Hypersil ODS (250mmx4.6mm, 5µm) were from RCI Labscan (Thailand).

2.2. Preparation of extraction

Okra was collected from the Nakhon Pathom province, Thailand, and was authenticated by Bangkok herbarium: BK No. 171158, Department of Agriculture, Chatuchak, Bangkok, Thailand. Firstly, the whole fresh okra fruit (*Abelmoschus Esculentus* L.) was blanched in 0.1% NaCl solution (the ratio of fresh okra to 0.1% NaCl at 1:5). Then, it was dried in a hot air oven (UM 400, Memmert, Germany) at 55 ^oC for 24 hours. The dried okra was ground into powder using a blender (HR2115⁄02, Philips, Thailand). Next, to extract the mucilage of dried okra, the okra powder was mixed with 85% ethanol solution (the ratio of okra powder to 85% ethanol at 1:4) The mixture was then centrifuged at 4 ^oC, 5000 rpm for 20 minutes. The mucilage sediment was separated and dried in a hot air oven at 55 ^oC for 24 hours. The final extract was kept away from light and moisture for further use. The percentage yield of the Okra ethanolic extract (OKR) was 9.58% (w⁄w) from the crude starting material.

2.3 High performance liquid chromatography (HPLC)

HPLC was applied to investigate the content of Quercetin-3-0-glucoside in Okra fruit, using a diode array detector, and the extract was injected into a Hypersil ODS column (250 x 4.0 mm i.d.; 5 µm particle size). The mobile phase consisted of methanol and 0.2% formic acid in deionized water. The running time with gradient elution was started from 0-60 min in condition at 30-60% and flow rate for all analyses was 1.0 mL⁄min. The absorbance was measured at a wavelength of 370 nm. The content of Quercetin-3-O-glucoside was determined by comparing the peak area of the extract solution with the peak area standard curve. The standard Quercetin-3-O-glucoside solutions were prepared in the range of 1-100 µg⁄mL. The extract solutions were prepared by dissolving 1 mg of the extract with 1 mL of methanol and sonication.

2.4 Cell culture

Human neuroblastoma (SK-N-SH) cell lines were purchased from the American Type Culture Collection (Mannassas, VA, USA). SK-N-SH cells were cultured in minimum essential medium (MEM) supplemented with 10% FBS, 100 U⁄mL penicillin, and 100 µg⁄mL streptomycin (GIBCO-BRL, Gainthersburg, MD) at 37°C in humidified 5% CO_2 and 95% air. The cells confluence were cultured to 80-90% and passed every 2-3 days by trypsinization with 0.025% Trypsin⁄EDTA.

2.5 Cell viability assay

The cell viability in SK-N-SH cells was evaluated for cytotoxic dose of ethanolic extract of Okra (OKR) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, SK-N-SH cells at the density of the 2×10^4 cells⁄mL were seeded in 96-well microplate at

37°C for 24 h, followed by treating with the several concentrations of OKR (0-500 µg/mL) for 24 h. Thereafter, 10 µg/ml of the MTT solution was added and maintained further for 1 h at 37°C. Finally, DMSO was added to dissolve formazan produced. For assessing of therapeutic effects of OKR on H_2O_2–induced aging, cells were prior treated with 10 µM of H_2O_2 for 4 h and then discard and wash the media with H_2O_2 3 times before treating with (10, 25, 50, or 75 µg/mL) of OKR for 24 h. Dosing 20 µM NAC, which is an antioxidant, was used as the positive control. Afterwards, the MTT assay was performed. The absorbance was measured at 570/600 nm by using a spectrophotometer (Synergy HT microplate reader, Biotek, VT, USA).

2.6 ROS assay

The density of 2×10^4 cells/mL of SK-N-SH cells was maintained in 96-black well plates at 37°C for 24 h. Thereafter, the cells were pre-incubated with 10 µM H_2O_2 for 4 h, and then incubated with/without 10, 25, 50, or 75 µg/mL of OKR for 24 h. Dosing 20 µM of H_2DCF-DA in phosphate saline buffer (PBS) was then incubated further at 37ºC for 2 h. The fluorescence density was then measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm by using a Synergy HT microplate reader (Biotek, VT, USA).

2.7 SA-β-galactosidase enzyme assay

The β-galactosidase activity was used for assessing the state of cellular senescence in SK-N-SH cells. Briefly, the 1×10^5 cells/mL of SK-N-SH cells were cultured in 6-well plates at 37°C for 24 h, the cells were pretreated with 10 µM H_2O_2 for 4 h, and then treated with/without of 10, 25, 50, or 75 µg/mL of OKR for 24 h. According to the manufacturer's standard protocol, the media was discarded and then the cells were incubated with a fixed solution for 15 min at room temperature. Thereafter, SA-β-Gal detection solution was incubated in the assay at 37ºC for 24 h and finally the cells were investigated under phase contrast microscopy for counting the SA-β-gal positive cells (blue) as a percentage of the total cell population.

2.8 Acetyl cholinesterase activity (AChE) assay

The AChE activity in SK-N-SH cells was assessed following Ellman's method. Briefly, the supernatant from cell lysate by High Ionic Strength (HIS) buffer containing 10 mM $NaHPO_4$, pH 7.0-8.0, 1 M NaCl, 10% Triton X-100, and 1 mM EDTA was pre-incubated with Ellman's solution (3 mM DTNB in 100 mM sodium phosphate buffer, pH 7.0-8.0) at 4ºC for 20 min. Then, 1 mM acetylthiocholine iodide was mixed in the assay at 37ºC for 20 min. Finally, the reaction mix was immediately read for absorbance at 405 nm at regular intervals of 2 min by using a spectrophotometer (Synergy HT microplate reader, Biotek, VT, USA).

2.9 Statistical Analysis

Data are expressed as mean ± SEM of four independent experiments. The significance was analyzed using one-way analysis of variance (ANOVA) followed by Post Hoc Dunnett's test for determining the significance of differences between individual groups. $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 The cytotoxicity of OKR in SK-N-SH cells

The cytotocixity results for OKR demonstrated that cell viability slightly decreased at 100 µM (96.31±2.8%) and significantly decreased at 250 µg/mL (87.04±3.4%) (p<0.01) compared to the control group (Figure 1). In accordance to these results, the optimal concentrations of OKR at 10, 25, 50, and 75 µg/mL were then used in subsequent experiments and NAC (positive control) was used only at 20 µg/mL.

3.2 OKR reduced cell death in H_2O_2-induced senescence of SK-N-SH cells

According to our previous results, 10 µM of H_2O_2 significantly reduced cell viability so that approximately 20% of the cells that were assessed in the study represented aging neurons, following Nopparat's protocol (Nopparat, Sinjanakhom, & Govitrapong, 2017). Importantly, they found that the administration of toxic-generated free radicals, such as H_2O_2, could promote the cellular senescence response in brain tissue, leading to morphological changes, dysfunction, and cell death (Schliebs & Arendt, 2011). This study demonstrated that the exposure to H_2O_2 caused SK-N-SH cells to have significantly decreased viability (81.46±4.0%) (Figure 2). Recent discoveries in molecular biology have revealed that the reduction of cell viability in aging neurons may be caused by the disturbance to cell recycling process or autophagy (Chakraborty *et al*., 2019; Costas & Rubio, 2017). The impairment of autophagy is notably an issue affecting cell recovery, leading to suppressed cellular function in many organelles such as mitochondria and endoplasmic reticulum (Lapierre *et al*., 2015). In the case of disabled cell repair, it may accelerate impairment of cell division or cell cycle arrest prior to mediating the aging response (Leidal, Levine, & Debnath, 2018).

To elucidate the inhibitory effects of OKR on H_2O_2-induced senescence in SK-N-SH cells, the cells were pretreated with 10 µM H_2O_2 for 4 h, and then treated with/without OKR (10, 25, 50, or 75 µg/mL) or NAC (20 µM) for 24 h. The results demonstrated that H_2O_2 treatment group

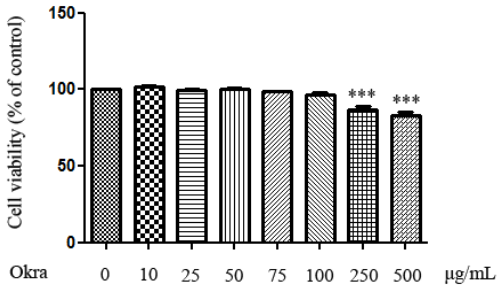

Figure 1. The cytotoxicity concentrations of OKR treatment. The SK-N-SH cells were treated with Okra for 24 h and analyzed by using MTT assay**.** The values present the mean ± SEM from 4 independent experiments. ***p< 0.001, in comparison with the control.

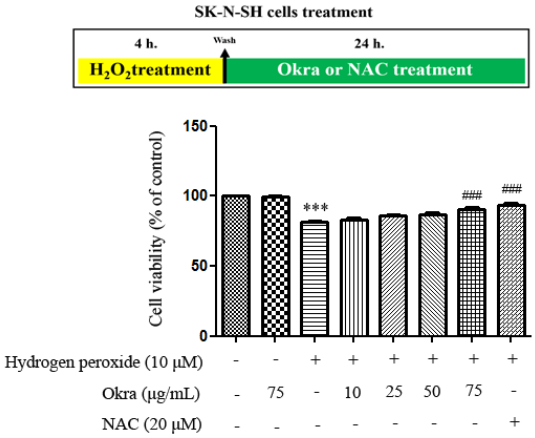

Figure 2. OKR promoted cell survival in H_2O_2 treated SK-N-SH cells. The SK-N-SH cells were treated with 10 µM H_2O_2 for 4 h and then the media were discarded and replaced with OKR (10-75 µg/mL) or 20 µM NAC treatment for 24 h. The cell viability was analyzed by using MTT assay. The values are represented as mean ± SEM from 4 independent experiments. ***p< 0.001, in comparison with the control; ###p < 0.001, in comparison with the H_2O_2 alone.

had significantly decreased cell viability (81.46±4.0%) (p<0.001). On the other hand, OKR significantly reversed these effects at 75µg/mL (90.24±3.1%) compared to H_2O_2 treatment alone (Figure 2). Interestingly, the highest dose of OKR has a high potency effect similar to NAC (positive control) in the promotion of cell viability. Therefore, we suggest that the promotion of cell viability by OKR in aging SK-N-SH cells might be caused by its antioxidant properties.

3.3. OKR markedly alleviated ROS production in H_2O_2-induced aging of SK-N-SH cells

The ROS production plays a key role as mediator of the aging process and death. Therefore, the promotion of cell viability by OKR needs to be assessed for whether it is involved with ROS. The results showed that H_2O_2 treatment group had markedly increased ROS production compared to the control group (233.69±11.0%) (p<0.001). In the OKR treatment, there was reduced ROS production in a dose-dependent manner compared to H_2O_2 treatment alone (Figure 3). Interestingly, the OKR at 50-75 µg/mL (132.13±9.6% and 130.96±5.5%) had a similar effect as the NAC treatment (121.53±11.9%). The OKR treatment alone did not show any effect in SK-N-SH cells (98.96±2.1%). Thus, the promotion of cell viability in aging neurons by OKR might be strongly associated with the reduction of ROS.

Based on the above data, we speculate that OKR therapies that target cellular senescence and negative response may constitute a strategy for the treatment of aging neurons. Several pieces of evidence have also revealed that fruit and vegetables are important sources of natural antioxidants for attenuating intracellular ROS in degenerative disease, in both *in vivo* and *in vitro* studies (Durazzo & Lucarini, 2019; Serafini & Peluso, 2016) Therefore, the decrease of ROS content by OKR may be caused by the activity of phenolic and flavonoid compounds that generally tend to be strong natural bioactive antioxidants (Patel, Rogers, & Huang, 2008).

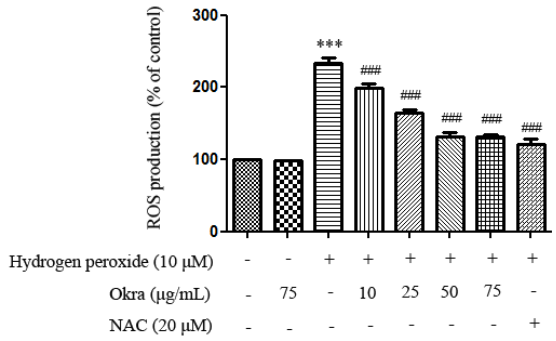

Figure 3. The inhibitory effect of OKR on ROS production in SK-N-SH cells treated with H_2O_2. The level of intracellular ROS production was determined by ROS assay. The values present the mean ± SEM from 4 independent experiments. ***p< 0.001, in comparison with the control; ###p < 0.001, in comparison with the H_2O_2 alone.

In addition, the reduction of ROS has been closely implicated with the promotion of cell viability in SK-N-SH cells (Figure 2) (p<0.001). The up-regulation of cell viability was widely accepted because it was controlled by the cell cycle process. In particular, it appears that ROS may directly impair the cell cycle mechanism in a manner that increases the cell proliferating inhibitory protein (Li, Chen, Xiong, & Zhu, 2016; Zhou, Shao, & Spitz, 2014). This role suggests that properly used antioxidants might be able to promote cell viability or inhibit cell death response. Interestingly, the highest concentration of OKR had a high potency similar to the standard synthetic antioxidant NAC.

3.4. OKR reversed senescence in H_2O–induced aging of SK-N-SH cells

The reduction of cell viability both in normal and cell stress conditions is commonly associated with the cell senescence process. According to previous results, we then investigated the therapeutic role of OKR on cellular senescence using SA-β-gal assay. The results showed that H_2O_2 treatment induced a large number of SA-β-gal positive cells, approximately 50% (Figure 4). Treatment with OKR at the concentrations of 50 or 75 µg/mL significantly decreased SA-β-gal positive cells (37.67±5.1% or 34.81±3.2%) compared to H_2O_2 treatment alone (p<0.001). However, the highest concentration of OKR showed a lesser effect than the NAC treatment (18.9±3.6%).

An oxidant/antioxidant imbalance could have profound implications for the cellular senescence and aging brain disorders, and previous research has pointed out this abnormality with SA-β-gal up-regulation (Cai *et al*., 2020). Our study has employed H_2O_2, a strong free radical, to mediate cellular senescence in a neuronal cell culture, to have a test model of aging, and this model has been used to examine the mechanistic evidence of a new test compound on aging (Wang, Wei, & Xiao, 2013). Here, we discuss the evidence related to senescent cells in H_2O_2 treatment, and the mechanistic contribution of these cells that may actively contribute to a low cell proliferation (Figure 2). Additionally, the cells in H_2O_2 treatment had morphological changes, including cell shrinkage, shortened neurite, and cell deformation, that have recently been linked to characteristics

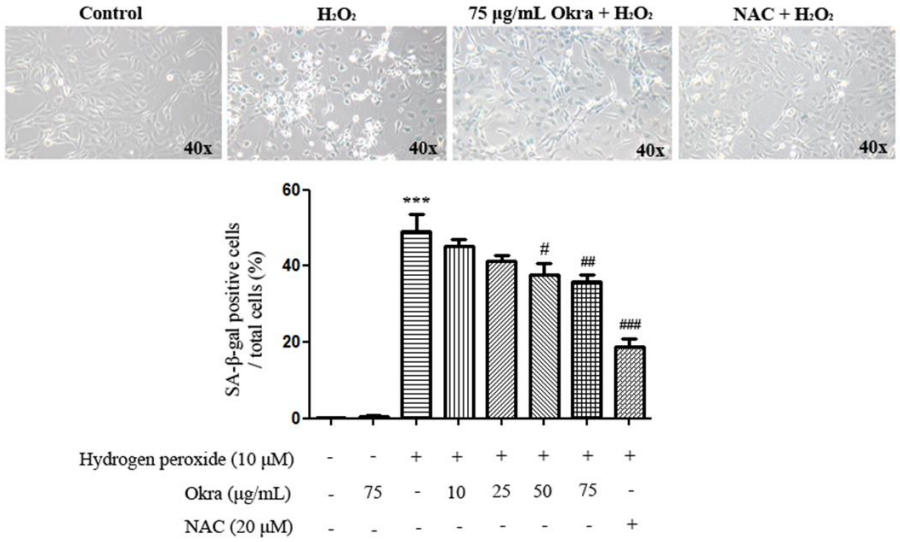

Figure 4. The anti-senescence effect of OKR on H_2O_2–induced aging of SK-N-SH cells. The β-galactosidase assay was used to examine the percent of β-Galactosidase positive cells in SK-N-SH cell population. The values present the mean ± SEM from 4 independent experiments. ***p< 0.001, in comparison with the control;$^{\#}p < 0.05$, $^{\#\#}p < 0.01$,$^{\#\#\#}p < 0.001$, in comparison with the H_2O_2 alone

of aging neurons (Mattson & Arumugam, 2018). Previous findings have demonstrated that the administration of a strong (or a high concentration of) antioxidant such as flavonoid or melatonin can contribute the cell proliferation and reverse cellular senescence process (Nopparat, Sinjanakhom, & Govitrapong, 2017). The reduction in SA-β-gal positive cells by OKR in H_2O_2 treatment may be caused by both phenolic and flavonoid compounds, mediated by the Sirt-1 protein or autophagy mechanism that has been commonly reported in previous studies (Cetrullo *et al*., 2016; Hayakawa *et al*., 2013). However, the highest dose of OKR (75 µg/mL) did not show the critical reduction of SA-β-gal positive cells similar to NAC. So, we suggest that the OKR might not have enough antioxidant content, such as flavonoids and the microelements of free radical scavengers, which are commonly reported to easily degrade from exposure to atmospheric oxygen, hyperthermal conditions, or light, in the alleviation of SA-β-gal compared to NAC.

3.5. OKR inhibited AChE activity in H_2O_2-induced aging of SK-N-SH cells

In accordance with previous results, OKR has potency to reduce aging and negative responses in SK-N-SH cells. We then explored the role OKR in synaptic plasticity of aging neurons. The results showed that H_2O_2 group had markedly promoted AChE activity compared to the control treatment (1.7±0.06) (p<0001) (Figure 5). Treatment with OKR significantly alleviated to AChE activity (p<0.05) compared to the aging group. Interestingly, at the highest dose the OKR treatment (1.43±0.12) had a similar effect as the NAC (1.44±0.1). The OKR treatment alone did not have any effect on SK-N-SH cells. Thus, we suggest that OKR has potency to reduce the AChE activity in SK-N-SH cells which promoted the synaptic plasticity. Notwithstanding, we suggest that the antioxidant properties of both OKR and NAC may not be the main factors involved in the reduction of AChE activity. Previous studies have reported that anti-oxidants can

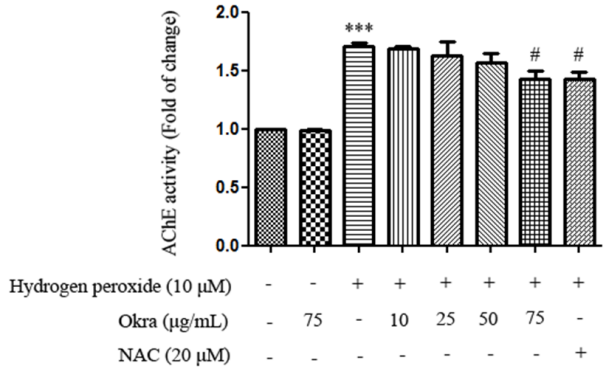

Figure 5. OKR reversed H_2O_2–mediated AChE activity in SK-N-SH cells**.** The SK-N-SH cells were first treated with H_2O_2 for 4 h before discarding the media and replacing it with OKR (10-75 µg/mL) or 20 µM NAC treatment for 24 h. The activity of AChE in the cells was determined by AChE assay. The values present the mean ± SEM from 4 independent experiments. ***p< 0.001, in comparison with the control; $^{\#\#}p < 0.01$, in comparison with the H_2O_2 alone.

slightly inhibit AChE activity, while the AChE inhibitors and anti-inflammatory compounds are principally recommended to treat this condition (Adewusi, Moodley, & Steenkamp, 2011; Xanthos & Sandkühler, 2014). Because the AChE activity is the result from both oxidative damage and inflammatory response, inhibiting AChE activity by antioxidants in OKR had little effect on aging neurons.

3.6 Determination of isoquercetrin (Quercetin-3-O-glucoside) in OKR

In our previous study, the results obviously indicated that the ethanolic extract of Okra (OKR) contained high amounts of total phenolics (8.86±0.21 mg GAE/g

extract) and flavonoids (47.71±0.07 mg QE/g extract). The antioxidant activity of OKR was also found in extract, which showed potential for scavenging free radicals including DPPH, ferric ions, and superoxide anions. In the present study, we also found that the antioxidant properties of OKR may be an effect of Quercetin-3-O-glucoside, the active flavonoid ingredient confirmed by HPLC (Figure 6). The standard curves of Quercetin-3-O-glucoside standard solutions were plotted for the peak area versus concentration, as shown in Figure 6A. Retention time of the standard Quercetin-3-O-glucoside peak was 27.5 min. HPLC chromatogram for the OKR extract solution is shown in Figure 6B. The results show Quercetin-3-O-glucoside content in the OKR extract at 0.999 mg/g extract. In accordance with previous studies, *Abelmoschus Esculentus* (L.) Moench (Family: Malvaceae), or Okra fruit, is a well-known tropical vegetable that provides a high amount of flavonoids, isoquercitrin, quercetin-3-O-glucoside, and elements that play important roles in slowing down degenerative diseases. This plant was recorded to have biologically active antioxidants and it has also received great attention, since it is effective in free radical scavenging and assumed to be less toxic than synthetic compounds (Gemede, Haki, Beyene, Rakshit, & Woldegiorgis, 2018). Therefore, the antioxidant properties of OKR may regulate the imbalance of oxidants/antioxidants that induces senescence response in aging neurons.

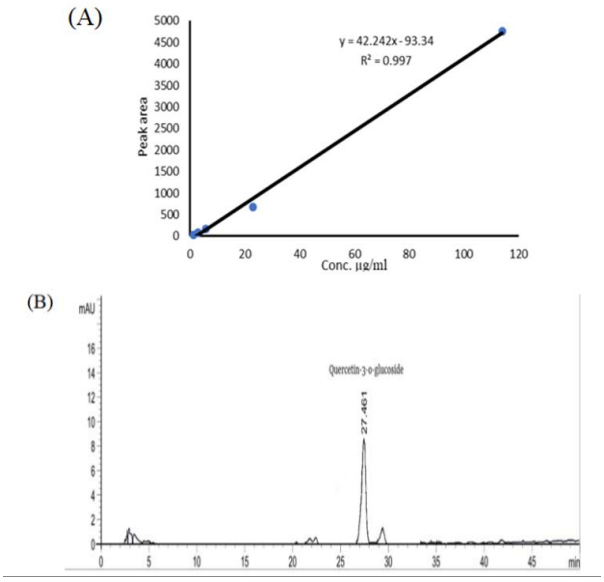

Figure 6. The constituents of OKR determined using HPLC (A) The standard curve of Quercetin-3-O-glucoside solution (Concentration 1.145-114.0 ug/ml). (B) The HPLC chromatogram of Quercetin-3-O-glucoside content in methanolic extract of OKR

4. Conclusions

In summary, this study revealed the therapeutic role of OKR in alleviating the negative responses, including cell stress response, cell senescence, and loss of synapse, in H_2O_2 –treated SK-N-SH cells. We discussed the significance of targeting senescent neurons as a novel approach toward therapies for age-associated brain diseases. However, finding from *in vivo* and clinical trials are required to validate OKR as a new therapeutic agent, and to support the medicinal value of Okra as a functional ingredient in food.

Acknowledgements

The authors gratefully acknowledge the financial support and the facilities provided by Thailand Institute of Scientific and Technological Research (TISTR) and facilities provided by Faculty of Medical Science, Naresuan University, Thailand.

References

Adewusi, E. A., Moodley, N., & Steenkamp, V. (2011). Antioxidant and acetylcholinesterase inhibitory activity of selected southern African medicinal plants. *South African Journal of Botany*, *77*(3), 638-644.

Baker, D. J. & Petersen, R. C. (2018). Cellular senescence in brain aging and neurodegenerative diseases: evidence and perspectives. *The Journal of Clinical Investigation*, *128*(4), 1208-1216.

Cai, Y., Zhou, H., Zhu, Y., Sun, Q., Ji, Y., Xue, A., . . . Deng, H. (2020). Elimination of senescent cells by β-galactosidase-targeted prodrug attenuates inflam mation and restores physical function in aged mice. *Cell Research*, *30*(1), 574–589.

Chakraborty, D., Felzen, V., Hiebel, C., Stürner, E., Perumal, N., Manicam, C., . . . Behl, C. (2019). Enhanced autophagic-lysosomal activity and increased BAG3-mediated selective macroautophagy as adaptive response of neuronal cells to chronic oxidative stress. *Redox Biology*, *24*(1), 101-181.

Chen, F., Liu, Y., Wong, N. K., Xiao, J., & Kwok-Fai S. (2017). Oxidative stress in stem cell aging. *Cell Transplant*, *26*(9), 1483-1495.

Costas, M. A. & Rubio, M.F. (2017). *[*Autophagy. A strategy for cell survival*]*. *Medicina* (*B Aires*), *77*(4), 314-320.

Durazzo, A. & Lucarini, M. (2019). Fruit-based juices: Focus on antioxidant properties-Study approach and update. *Phytotherapy Research*, *33*(7), 1754-1769.

Facchini, A., Cetrulo, S., Adamo, S., Guidotti, S., Minguzzi, M., Facchini, A., Borzi, R. M., & Flammigni, F. (2016). Hydroxytyrosol prevents chondrocyte death under oxidative stress by inducing autophagy through sirtuin 1-dependent and -independent mechanisms. *Biochimica et Biophysica Acta*, *1860*(6), 1181-1191.

Fernández-Gómez, F. J., Muñoz-Delgado, E.,Montenegro, M. F., Campoy, F. J., Vidal, C. J., & Jordán J. (2010). Cholinesterase activity in brain of senescence-accelerated-resistant mouse SAMR1 and its variation in brain of senescence-accelerated-prone mouse SAMP8. *Journal of Neuroscience Research*, *88*(1), 155-166.

Gemede, H. F., Haki, G. D., Beyene, F., Rakshit, S. K., & Woldergiorgis, A. (2018). Indigenous Ethiopian okra (Abelmoschus esculentus) mucilage: A novel ingredient with functional and antioxidant properties. *Food Science Nutrition*, *6*(3), 563-571.

Giuseppe, T., Placido, B., & Emanuela, M. (2018). Overview on the effects of N-Acetylcysteine in neuro degenerative diseases. *Molecules*, *23*(12), 3305-3325.

Geng, Y. Q., Guan, J. T., Xu, X. H., & Fu, Y. C. (2010). Senescence-associated beta-galactosidase activity expression in aging hippocampal neurons. *Biochemical Biophysical Research Communication*, *396*(4), 866-879.

Haroon, K., Marya, S. A., Mohammad, A. K. & Seema, P. (2018).Flavanoids as acetylcholinesterase inhibitors: Current therapeutic standing and future prospects. *Biomedicine and Phamacotherapy*, *101*(1), 860-870.

Hayakawa, N., Shiozaki, M., Shibata, M., Koike, M., Uchiyama, Y., Matsuura, N., & Gotow, T. (2013). Resveratrol affects undifferentiated and differentiated PC12 cells differently, particularly with respect to possible differences in mitochondrial and autophagic functions. *European Journal Cell Biology*, *92*(1), 30-43.

Hernandez-Segura, A., Brandenburg, S. & Demaria, M. (2018). Induction and validation of cellular senescence in primary human cells. *Journal of Visualized Experimants*, *136*(1), 1-10.

Hernandez-Segura, A., Nehme, J., & Demaria, M. (2018). Hallmarks of cellular senescence. *Trends Cell Biology*, *28*(6), 436-453.

Islam, M. T. (2019). Phytochemical information and pharmacological activities of Okra (Abelmoschus esculentus): A literature-based review. *Phyto therapy Research*, *33*(1), 72-80.

Lapierre, L. R., Kumta, C., Sandri, M., Ballabio, A., & Hansen, M. (2015). Transcriptional and epigenetic regulation of autophagy in aging. *Autophagy*, *11*(6), 867-880.

Leidal, A. M., Levine, B., & Debnath, J. (2018). Autophagy and the cell biology of age-related disease. *Nature Cell Biology*, *20*(12), 1338-1348.

Li, H. G., Chen, J. X., Xiong, J. H., & Zhu, J. W. (2016). Myricetin exhibits anti-glioma potential by inducing mitochondrial-mediated apoptosis, cell cycle arrest, inhibition of cell migration and ROS generation. *Journal of BUON*, *21*(1), 182-190.

Martínez-Cué, C. & Rueda, N. (2020). Cellular senescence in neurodegenerative diseases. *Frontier in Cell Neuroscience*. doi:10.3389/fncel.2020.00016

Mattson, M. P. & Arumugam, T. V. (2018). Hallmarks of brain aging: adaptive and pathological modification by metabolic states. *Cell Metabolism*, *27*(6), 1176-1199.

Nopparat, C., Sinjanakhom, P., & Govitrapong, P. (2017). Melatonin reverses H2 O2 -induced senescence in SH-SY5Y cells by enhancing autophagy via sirtuin 1 deacetylation of the RelA/p65 subunit of NF-kappaB. *Journal of Pineal Research*, *63*(1), 1-10.

Patel, A. K., Rogers, J. T., & Huang, X. (2008). Flavanols, mild cognitive impairment, and Alzheimer's dementia. *International Journal Clinical Experimental Medicine*, *1*(2), 181-191.

Schliebs, R. & Arendt, T. (2011). The cholinergic system in aging and neuronal degeneration. *Behavioral Brain Research*, *221*(2), 555-563.

Serafini, M. & Peluso, I. (2016). Functional foods for health: The interrelated antioxidant and anti-inflammatory role of fruits, vegetables, herbs, spices and cocoa in humans. *Current Pharmaceutical Designs*, *22*(44), 6701-6715.

Wang, P. & Geng, J. (2019). Macrophage achieves self-protection against oxidative stress-induced ageing through the Mst-Nrf2 axis. *Nature Communication*, *10*(1), 755-764.

Wang, Z., Wei, D., & Xiao, H. (2013). Methods of cellular senescence induction using oxidative stress. *Methods of Molecular Biology*, *1048*(1), 135-144.

Xanthos, D. N. & Sandkühler, J. (2014). Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. *Nature Review Neuroscience*, *15*(1), 43-53.

Xia, F., Zhong, Y., Li, M., Chang, Q., Liao, Y., Liu, X., & Pan, R. (2015). Antioxidant and anti-fatigue constituents of Okra. *Nutrients*, *7*(10), 8846-8858.

Zhou, D., Shao, L., & Spitz, D. R. (2014). Reactive oxygen species in normal and tumor stem cells. *Advance Cancer Research*, *122*(2), 1-7.