Neuroprotective Effect of *Perilla* Extracts on PC12 Cells

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Background: *Perilla frutescens (L) Britton contains some active principles which had neuroprotective actions.*
Objective: *The present study was aimed to investigate the effect of Perilla extracts on neuroprotection, antioxidation and neurite outgrowth in PC12 cells.*
Material and Method: *The neuroprotective effect of freeze-dried ethanolic extract from Perilla leaves and cold-pressed seed oil were tested on PC12 induced with beta-amyloid protein. The inhibition of tau-protein hyperphosphorylation and the antioxidant enzyme activity were analyzed. The neurite outgrowth bearing cells were investigated and MEK-1 protein production was analyzed by enzyme immunometric assay.*
Results: *In PC12 culture induced toxicity by beta-amyloid protein: (1) the decrease in cell viability was attenuated in cells pretreated with leaf extract 200 mg/ml and oil 50 mg/ml; (2) SOD activity seemed to decrease when pretreated cells with the extracts; (3) tau phosphorylation was decreased by pretreated cells with50 mg/ml of oil. Moreover, given Perilla leaf extract or seed oil to PC12 culture, the amount of neurite outgrowth bearing cells increased harmoniously with MEK-1 protein expression.*
Conclusion: *Perilla leaf extract and seed oil reversed the effect of beta-amyloid induced toxicity by decreasing oxidative stress and inhibition of tau-protein hyperphosphorylation. The enhancement of neurite outgrowth by Perilla extracts was also revealed.*

Keywords: *Perilla frutescens, Neuroprotection*

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Alzheimer's disease (AD), the neurodegenerative disorder, is characterized by loss of neurons in the brain and the appearance of neurofibrillary tangles within neurons and senile or amyloid plaques[1]. The beta-amyloid protein induced oxidative stress and neurotoxicity has been the well-known mechanism of the disease[2]. Therefore, the antioxidant activity may be one of the possible therapeutic strategies to inhibit beta-amyloid induced neurotoxicity and improve neurological symptoms in AD[3].

Perilla frutescens (L) Britton, Family LAMINACEAE consists of memory enhancer principles such as luteolin, rosmarinic acid and docosahexaenoic acid (DHA, C22: 6n-3) or Omega 3 fatty acid. Luteolin improved cognitive impairment[4] and enhanced memory in animal model[5], also enhanced cholinergic activities in PC12 cell culture[6]. Neuroprotective effect of luteolin against apoptosis and effect on β-amyloid protein (25-35)-induced toxicity in rat neuron culture were reported[7]. These effects were due to antioxidative stress which occurred within mitochondria[8]. In addition, luteolin promoted neurite out growth in neuronal culture by stimulating nerve growth factor (NGF), glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor, (BDNF)[9]. Rosmarinic acid, a polyphenol flavonoid, protected PC12 cells from beta-amyloid peptide induced neurotoxicity[10,11] and activated cholinergic activities in PC12 cells through phosphorylation of ERK1/2[12]. Many studies revealed that DHA effected neuro-protective and ameliorative actions against neuronal

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diseases, so DHA would be beneficial effect on cognitive decline in AD[13,14]. Furthermore, it had been well-known that tau-protein phosphorylation was downstream of beta-amyloid toxicity and tau-protein plaque was found in AD[15]. Any substances that can decrease beta-amyloid-induced tau phosphorylation may be useful as a therapeutic agent for the treatment or prevention of AD.

Therefore, we examined the possible neuroprotective mechanisms of *Perilla* against beta-amyloid induced toxicity in PC12 cells, including the antioxidation, the tau protein inhibition, and neurite outgrowth of the neuronal cells.

Material and Method

Chemicals and reagents

Rosmarinic acid, alpha-tocopherol or vitamin E, MTT (3, -4, 5 dimethylthiazol-2-yl-2, 5 diphenyl tetrazolium bromide), SDS (sodium dodecyl sulfate), DMSO (N, N-dimethylsulfoxide), absolute ethanol, nerve growth factor (NGF), phosphate-buffered saline (PBS), bovine serum albumin (BSA), beta-amyloid protein (1-42), superoxide dismutase, MEK-1 EIA kit catalog No. ADI-900-122A, 19160 SOD determination kit were purchased from Sigma Aldrich. Radioimmunoprecipitation assay buffer (RIPA) buffer was purchased from Amresco, USA. Dulbecco's modified Eagle's medium (DMEM), horse serum (HS) and fetal bovine (FBS), 1% penicillin-streptomycin were obtained from Gibco.

Preparation of stock solutions

The beta-amyloid (1-42) was pre-aggregated prior to use[3]. Beta-amyloid (1 mg) was dissolved in 1 ml of DMEM media and incubated in a 37°C water bath for 72 hour to induce aggregation. The aggregated beta-amyloid was then diluted to 100 µg/ml (100 µM) and stored at -20°C until use. Rosmarinic acid, vitamin E was separately dissolved in DMSO to obtain 1 mg/ml concentration, and stored at -20°C until use. NGF was dissolved in PBS and 0.1% BSA to obtain concentration of 2.5 mg/ml and stored at -20°C until use MTT was dissolved in PBS to obtain 1 mg/ml stock solution. Lysis buffer was 10% w/v of SDS in 0.01N HCl.

Culture of PC12 cells

PC12 rat pheochromocytoma cells were purchased from American type culture collection (ATCC). Cells were cultured on poly-D-lysine coated plate and routinely maintained in DMEM supplemented with 15% HS, 5% FBS and 1% penicillin-streptomycin at 37°C under 5% CO_2. Cells were utilized for experiments during exponential growth phase between passage 3 and 8. The differentiated media was DMEM supplemented with only 1.5% HS and 1% penicillin-streptomycin and was used in beta-amyloid induced toxicity testing and in cell differentiation period.

Perilla extract

Perilla frutescens were planted and harvested in Maehongson, Thailand during January 2015 and the voucher specimens were deposited at the University of Phayao, Phayao, Thailand. Ethanolic extract from *Perilla* leaves and cold pressed seed oil were prepared by the Section of Biochemistry, School of Medical Sciences, University of Phayao, Phayao, Thailand. Quality control of some ingredients and antioxidative activity of the extracts were also performed. Leaf extract and seed oil were dissolved in DMSO to obtain final concentration of 50, 100, 200 mg/ml in 1% DMSO.

Determination of Perilla extracts toxicity

PC12 cells (5 x10^4 cells per well, 100 µl) were plated in 96-well tissue culture plates to reach the exponential growth. Cells were pretreated with 100 µl of media plus either of 100 µl of tested substances as leaf extract or seed oil or rosmarinic acid for 48 hour. Cell viability was determined by MTT method[16]. Briefly, 10 µl MTT 5 mg/ml solution was added in each 100 µl medium and incubated at 37°C in CO_2 incubator for 4 hour. Then supernatant was removed and 200 µl DMSO was added. The plate was vigorously agitated then incubated in CO_2 for 40 hour. By using microplate reader (Bio Tex Model Power Wave XS, USA), the optical density of the resulting solution was colorimetrically determined at 570 nm. The percentage of viable cell was calculated.

Determining of the extracts' ability to protect PC12 cells against beta-amyloid insult

PC12 cells (4x10^4 cells/well, 100 µl) were plated in 96-well tissue culture plates and allowed to reach 70% confluent growth. Thereafter, 100 µl of DMEM media and either intervention of tested substances as 50, 100 mg/ml of leaf extract, seed oil or vitamin E 20 µM as positive control was replaced in each well and incubated for 2 hour. To induce toxicity that resulted in PC12 cell viability of 60%, the differentiated media was used and the pre-aggregated beta-amyloid 20 µM/well was added. Then cell viability was assayed after 24 hour of incubation[3].

Measurement of antioxidant effects of the extracts

Measurement of SOD enzyme activity

PC12 cells were pretreated with either intervention as same as in 2.6 for 48 hour then incubated with beta-amyloid 24 hour. The cells in the absence or presence of those interventions were washed 2 times with cold PBS, then collected onto ice cold PBS (0.1 M containing 0.05 mM EDTA) and homogenized. The homogenized was then centrifuged at 4°C at 12,000 g for 10 min[3]. The supernatant was applied to measure of superoxide dismutase (SOD) according to the instruction for the reagent kits and detected the absorbance by using spectrophotometer (Shimadzu, UV-1601, Japan).

Determination of tau protein phosphorylation

For western blotting assay, after the treatments as 2.7.1, cells were collected, washed twice with cold phosphate buffer saline and lysed on ice with RIPA buffer containing with protease inhibitor cocktail. The soluble cell lysate was obtained by centrifugation at 14,000 g for 20 min at 4°C. The concentration of the total proteins was determined by the Bradford assay (Bio-Rad, USA). Then, protein samples (45 μg/lane) were separated by 10% SDS-PAGE gels and transferred to a nitrocellulose membrane[17]. Membrane was treated with blocking solution and then incubated with the following rabbit polyclonal primary antibodies: 1:2,000 for beta-actin (Cell signaling Technology, Beverly, MA), 1:1,000 for total-Tau (Santa Cruz Biotechnology, CA, USA) and 1:200 for p-tau (Thr 205) and p-tau (Ser 396) (Santa Cruz Biotechnology, CA, USA), at 4°C for overnight. Blots were then incubated with goat anti-rabbit LI-COR IRDye 680 antibody (dilution 1:10,000). The specific bands were visualized and densitometrically analyzed using LI-COR 2800 Odyssey Fc. Imaging system (LI-COR Bioscienecs, USA).

Determination of neurite outgrowth

In 24-well plates, PC12 cells ($8x10^4$ cells/well, 2 ml) were cultured in differentiated media plus 2 ng/ml NGF together with leaf extract or with seed oil then incubated for 48 hour. Cells cultured in differentiated media plus 50 ng/ml NGF was used as positive control. As observed by inverted microscope (Nikon, Eclipse TS 100-F, Japan), the cell images were captured using Nikon ACT-1C for DXM 1200 C. Cells with outgrowths longer than the cell body diameter were scored positive for neurite-bearing cell. Count these cells/100 total cells/well and 3 wells/plate and 3 plates/experiment and expressed as percentage of neurite-bearing cells in 100 cells[18,19].

Determination of Mitogen-activated protein kinase (MEK 1)

After counting neurite-bearing cells, each culture was determined for MEK-1 according to the method of total MEK-1, enyzme immunometric assay kit, catalog No. 900-122A, assay designs Inc., Stressgen, USA. Briefly, cells were scraped, washed with DMEM and centrifuged at 800 rpm, 5 min. Washed cells again with PBS and counted cells. Fresh lysis buffer was added according to the instruction for the reagent kits (125 μl/10^{-6}-10^{-7} cells/ml). Cell solution was sonicated on ice and centrifuged at 12,000 g, 4°C for 30 min. The supernatant was kept at -80°C until the determination for total MEK1 was performed[20].

Data analysis

All data are expressed in means $\pm$ SEM of at least three independent experiments. A one-way analysis of variance (ANOVA) followed by Duncann post hoc test was performed to compare group. Differences were considered statistically significant at $p<0.05$.

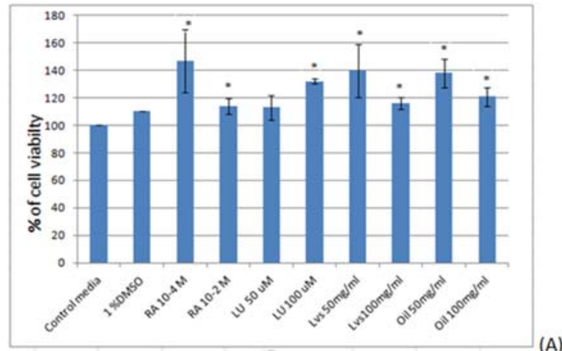
Results

Perilla extracts had no toxicity to PC12 cells

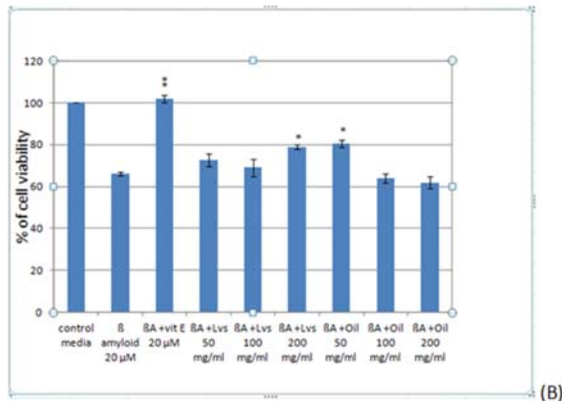
DMEM was used as control group and supposed to show 100% survival. *Perilla* leaf extract and seed oil at dose of 100 mg/ml had no toxic to PC12 cells. At a dose of 50 mg/ml, both leaf extract and seed oil increased cell viability more than dose of 100 mg/ml. Rosmarinic acid, as positive control, at dose of 10^{-4} M showed viable cells as well as luteolin at dose of 100 mg/ml (Fig. 1A).

Perilla leaf extract and seed oil protected PC12 cells against beta-amyloid induced toxicity

Cell viability was expressed as percentage of control media group. Treatment of PC12 cells with 20 μM beta-amyloid protein (1-42) for 24 hour induced approximately 40% cell death. Pretreated cells with 200 mg/ml leaf extract or 50 mg/ml seed oil for 48 hour, then treated with beta-amyloid, significantly increased cell viability to 79-80%. Meanwhile the positive control, vitamin E 20 μM increased cell viability significantly versus the beta-amyloid group. The leaf extract and seed oil at dose of 200 and 50 mg/ml respectively also decreased amyloid toxicity by showing cell survival of



(A)



(B)

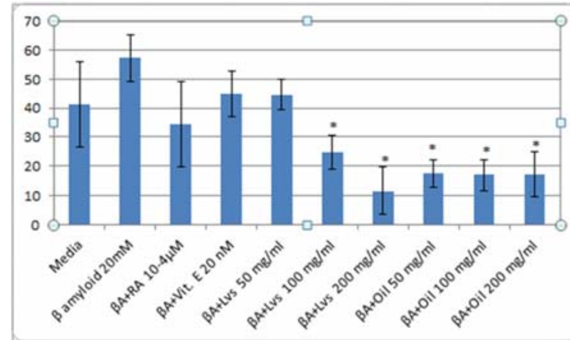
* $p<0.05$, ** $p<0.01$ versus control media (A) and beta-amyloid group (B).

Fig. 1 Effect of tested substances on cell viability of PC12 determined by MTT assay. Cells were incubated with rosmarinic acid (RA), Luteolin (LU), *Perilla* leaf extracts (Lvs), seed oil (Oil) at different concentrations. Cell viability was expressed as percentage of untreated cells (control media) (A). Cells were treated with each of tested substances for 2 hour before incubated with beta-amyloid for 24 hour (B). Results expressed as means ± SEM of four independent experiments.

80% (Fig. 1B).

Perilla leaf extract and seed oil decreased the oxidative stress induced by beta-amyloid

Treatment of PC12 cells with beta-amyloid increased SOD level but the result was not significantly when compared to the media group. Pretreatment of cells with either of rosmarinic acid $2x10^{-4}$M, vitamin E 20 µM or leaf extract at dose of 50 mg/ml then treated with beta-amyloid also increased SOD activity. At dose of 100, 200 mg/ml leaf extract and 50, 100, 200 mg/ml seed oil significantly decreased SOD activity when compared to both of beta-amyloid and media group. (Fig. 2)



* $p<0.05$ versus beta-amyloid group

Fig. 2 The enzyme superoxide dismutase (SOD) activity in PC12 cells after pretreatment cells with rosmarinic acid (RA), vitamin E (vit E), *Perilla* leaf extracts (Lvs) and seed oil (Oil) at difference concentrations for 2 hour then incubated with beta amyloid protein 24 hour. Data represented the means ± SEM of three independent experiments.

Perilla seed oil decreased tau protein accumulation induced by beta-amyloid in PC12 cells

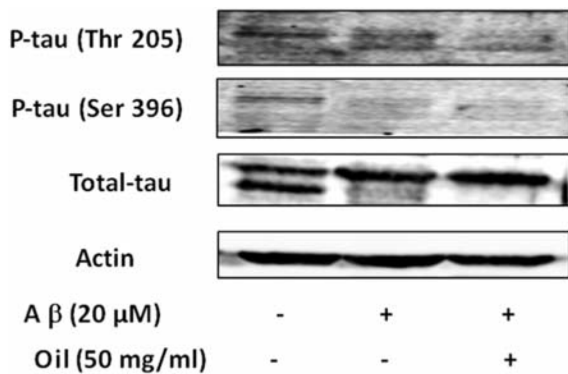
The effect of seed oil treatment on beta-amyloid induced tau protein hyperphosphorylation was investigated by western blotting assay. Our data showed that tau protein hyperphosphorylation at Thr 205 and Ser 396 was increased after β-amyloid induction for 6 hour on PC12 (Fig. 3). However, pretreatment with seed oil at dose of 50 mg/ml before beta-amyloid induction could inhibit the tau protein hyperphosphorylation at two phosphorylation sites.

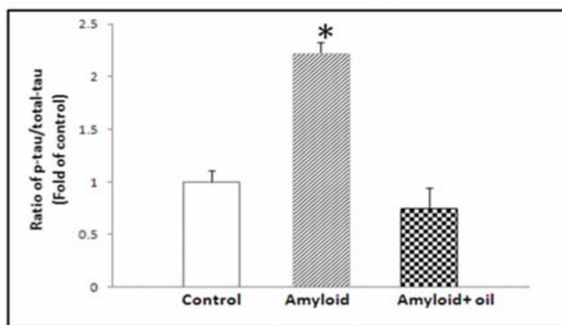
Perilla leaf extract and seed oil promote neurite out growth in PC12 cells by stimulating MEK-1

In differentiated media plus either of NGF, romarinic acid, leaf extract or seed oil, PC12 cells could differentiate neurite outgrowth (Fig. 4) At dose of 50, 100 mg/ml leaf extract and seed oil 100 mg/ml promoted neurite outgrowth as well as 50 ng/ml of NGF (Fig. 5) MEK-1, one of key protein synthesized within cells whenever cells differentiated, increased significantly and correspondingly to the amount of neurite-bearing cells. Rosmarinic acid rapidly stimulated neurite outgrowth when observed in 24 hour and seemed to stop increasing the amount of neurite outgrowth cells in 48 hour. This group also exhibited Mek-1 protein in 48 h less than the other groups (Fig. 6).

Discussion

Perilla leaf extract and *Perilla* seed oil at dose



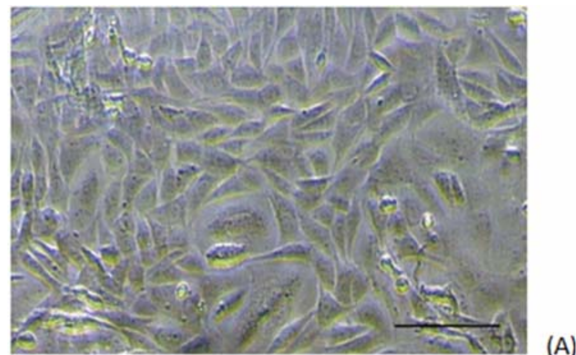


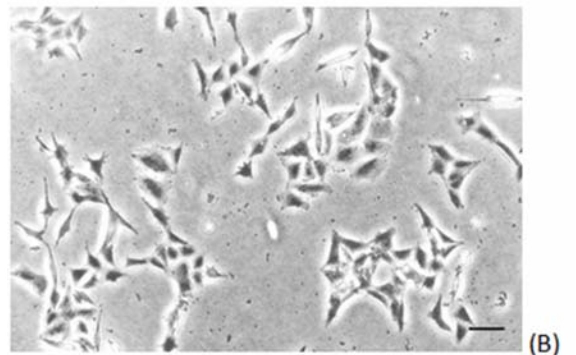
* $p<0.05$ versus control and the amyloid + oil group

Fig. 3 Western bolt analysis of tau protein phosphorylation (at Thr 205 and Ser 396 sites) in PC12 cells pretreated with 50 mg/ml of *Perilla* seed oil (Oil) 2 hour then treated with beta-amyloid for 6 hour. Graph of densitometer values showing changes in the levels of phosphorylated tau/ total tau (% of control).

of 100 mg/ml had no toxic effect on PC12 cells. This proliferative effect was in agreement to rosmarinic acid at dose of 10^{-4}M[10]. Luteolin, another active substance found in *Perilla* leaves, also had proliferative effect on PC12 at low concentration of 1 and 10 μM[21]. Therefore, the proliferate effect of leaf extract might be due to rosmarinic and luteolin. It was noticeable that the leaf extract and seed oil at dosage of 50 mg/ml had proliferative effect more than 100 mg/ml. So, there might be any substances in the crude extracts which might against to cell growth.

In the present experiment, beta-amyloid (1-42) 20 μM was pre-aggregated at 37°C for 72 hour to obtain optimum result that caused PC12 cells death at 40% of control media group. Pretreatment PC12 with leaf extract (200 mg/ml) and seed oil (50 mg/ml) increased the percentage of cell viability. Interestingly, *Perilla* seed oil significantly exhibited the SOD activity but





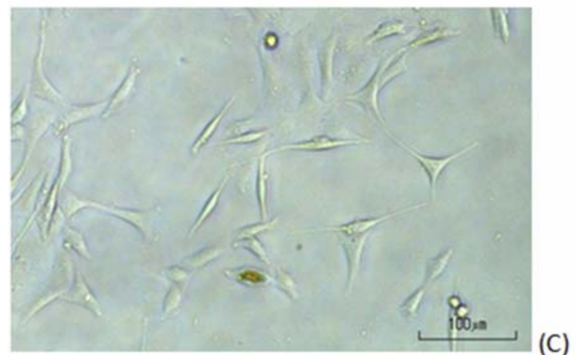
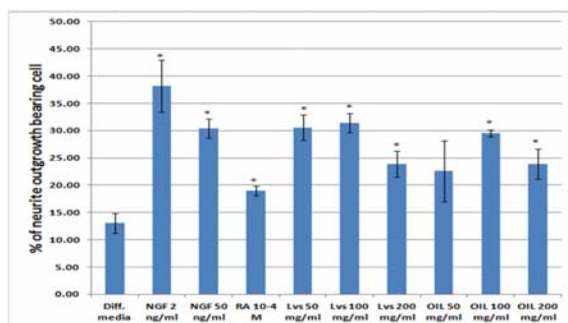


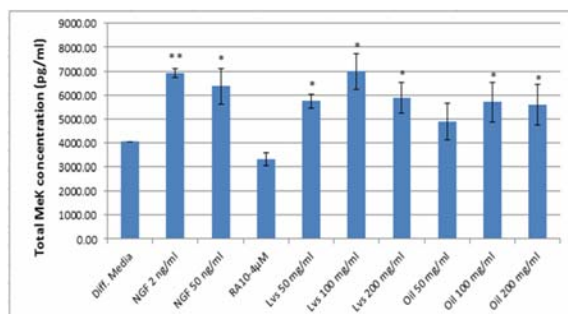
Fig. 4 PC12 cells were cultured in different media. (A) PC12 cells in DMEM; (B) PC12 in differentiated media (DMEM + 1.5% HS + 1% penicillin-streptomycin) + nerve growth factor 50 ng/ml; (C) PC12 in differentiated media (DMEM + 1.5% HS + 1% penicillin-streptomycin) + nerve growth factor 2 ng/ml + leaf extract 100 mg/ml. Cells were observed with inverted contrast microscopy. Scale bar is 100 μm.

not in a dose-related manner. At doses of 100 and 200 mg/ml seed oil did not increase cells survival significantly. There might be some unknown substances in seed oil at higher dose that antagonized cell viability. Analysis of *Perilla* seed oil was reported to contain an abundance of omega-3, omega 6 essential



* $p<0.05$ versus the differentiated media group

Fig. 5 The percentage of neurite-bearing cells when PC12 were cultured in differentiated media (DMEM + 1% FBS+ 5% HS+2 ng/ml of nerve growth factor, NGF) together with tested substances at various concentrations as *Perilla* leaf extract (Lvs); *Perilla* seed oil (Oil). Data represent the means $\pm$ SEM of three different experiments.



* $p<0.05$ versus the differentiated media group

Fig. 6 The amount of MEK-1 protein (pg/ml) synthesized within PC12 cells when the cultured media were added with rosmarinic acid (RA), NGF (nerve growth factor), Lvs (*Perilla* leaf extract) or Oil (*Perilla* seed oil) at various concentrations. Data represent the means $\pm$ SEM of three different experiments.

fatty acids in the ratio of 5.94:1 and also to contain carbohydrate, protein, magnesium and vitamin E[22].

It was proved that plant contained rosmarinic acid protected PC12 cells from beta-amyloid induced neurotoxicity. Down regulation of SOD may protect cells from apoptosis or necrosis when cells insulted by oxidative stress. Rosmarinic at dose of 10^{-4}M given 10 min before beta-amyloid to PC12 cells could decrease oxidative stress and tau hyperphosphorylation induced by beta-amyloid. When PC12 cells were in oxidative stress condition, the ROS formation was detectable at 1.5 hour after beta-amyloid treatment and the maximal effect was observed at 24 hour[10]. Afterward, the excess SOD within cells stimulated other oxidative enzymes to balance the physical condition. Therefore, the present study pointed to only the SOD activity at 24 hour. This leaf extract was qualified to have antioxidant activity in vitro with IC_{50} of 21.95 µg/ml (IC_{50} of BHT as positive control = 19.27 µg/ml). While other researchers reported high antioxidant activities of *Perilla* seed oil[23]. In the beta-amyloid treated group, the average SOD activity increased from 44 to 67 U/mg protein but this change was not significant. Rosmarinic acid also had tendency to decrease this enzyme while *Perilla* leaf extract and seed oil clearly decreased the SOD level in dose related activity. Therefore, there might be some antioxidants in the crude leaf extract and seed oil.

Nowadays, mechanism of action underlying the neuroprotection of pharmaceutical compounds is not only by antioxidation but also by their interactions with signaling-related transporters, receptors or key emzymes[24]. There were evidences that luteolin mediated neurotrophic and neuroprotective activity in PC12 via the ERK signaling pathway not antioxidation[25]. Luteolin should be found in the leaf extract which was not tested in this experiment due to the green color of the extract that might interfere with visual band. Therefore, further purification of this extract should be performed. Given seed oil at dose of 50 mg/ml before treated with beta-amyloid, the inhibition of tau phosphorylation in PC12 cells was detected. This was preliminary study of tau phosphorylation of *Perilla* seed oil at only one concentration but seed oil showed possibly trend to prevent amyloid plaque accumulation.

Neurotrophic factors are important mechanisms in survival, growth, and function of neurons. Regulation of neurotrophic factors such as neurite outgrowth has been considered as one of the targets in developing drug or therapy against neuronal disorders. Rosmarinic acid stimulated neurite out growth, when observed in 24 hour, with long neuronal cells but the amount of neurite outgrowth bearing cells did not increase in 48 hour. Therefore, Mek-1 protein seemed to be less than the other groups by the time of detection. *Perilla* leaf extract and seed oil promoted neurite outgrowth of PC12 cells when cultured in differentiated media supplemented with 2 ng/ml of NGF. These effects might be due to luteolin, one of active principle in *Perilla* leaves and other substances in seed oil[9]. These findings were in agreement to the amount of MEK-1 or MAPK, mitogen-activated protein kinase, protein synthesized within cells whenever proliferation,

transformation or differentiation occurred[24].

Besides the antioxidant activity that protected cell damage, the multiple neuroprotective mechanisms by receptor-mediated action and parallel signaling pathways were reported. For example, citrus flavonoids possess moderate antioxidant but could be neuroprotective against oxidative damage in brain[24]. Triggering the mitogen-activated protein kinase (MEK), extracellular signal-regulated kinase (ERK) 1/2-cAMP response element-binding protein, (CREB) pathway could protect neuronal cells from damage[19,26]. In addition, ERK and TrkA (Tyrosine kinase A) were found more in AD's patient brain than normal brain. In the same way, this *Perilla* seed oil could also possibly exhibit neuroprotective effect via any signaling pathway. Therefore, *Perilla* extracts would be promising for the development of general food-based neuroprotection.

Conclusion

The present study found that in the PC12 culture induced by beta-amyloid 20 µM: (1) the cell viability decreased to 60% of control; (2) the cell viability increased in culture pretreated with *Perilla* leaf extract 200 mg/ml and seed oil 50 mg/ml; (3) given *Perilla* leaf extract at dose of 100, 200 mg/ml and each dose of seed oil to cells seemed to decrease SOD activity; (4) tau phosphorylation at Thr 205 and Ser 396 was decrease when pretreated with seed oil at dose of 50 mg/ml. In DMEM culture supplemented with *Perilla* leaf extract or seed oil and, also, nerve growth factor 2 ng/ml, the neurite outgrowth of PC12 cells increased in step harmoniously with MEK-1 synthesized within cells. These studies indicated the neuroprotective action of *Perilla* extracts by decreasing oxidative stress as well as inhibition of tau-protein hyperphosphorylation. Moreover, the enhancement of neurite outgrowth by *Perilla* extracts by activated MEK-1 was also revealed. These preliminary results imply the neuroprotective effect of *Perilla* as a functional food for AD.

What is already known on this topic?

Perilla frutescens composed of active substances such as rosmarinis acid, luteolin and linoleic acid (DHA). These substances were reported to have antioxidant activity and could protect PC12 cells from toxicity induced by beta-amyloid protein. Moreover, luteolin could promote nrurite growth of PC12 cell. *Perilla* oil was proved to enhance memory on animal models. *Perilla* seed was used as food in the north of Thailand.

What this study adds?

Perilla frutescens could be developed as functional food for neuroprotection in edldery and Alzheimer's patient. The present study found that in the PC12 culture induced by beta-amyloid: (1) the decrease in cell viability was attenuated in cells pretreated with *Perilla* leaves extract and seed oil; (2) leaves extract and seed oil seemed to decrease SOD enzyme activity in PC12 cells; (3) tau phosphorylation decreased when pretreated cells with seed oil. Moreover, given *Perilla* leaf extract or seed oil to PC12 culture, the amount of neurite outgrowth bearing cells increased in step harmoniously with MEK-1 protein expression. These studies indicated the neuroprotective action of *Perilla* extracts via antioxidant activity and stimulating neurite outgrowth.

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Potential conflicts of interest

None.

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ผลของน้ำมันงาขี้ม้อนและสารสกัดใบงาขี้ม้อนต่อการปกป้องเซลล์ประสาทเพาะเลี้ยง

พิมลวรรณ เสนะวงศ์, สุปราณี กองคำ, สุวพร แซ่ลิ้ม, วนัสยา สุอังควาทิน

ภูมิหลัง: *งาขี้ม้อนมีสารสำคัญที่มีรายงานผลวิจัยเกี่ยวกับการป้องกันภาวะสมองเสื่อมได้*

วัตถุประสงค์: *ศึกษาผลของสารสกัดจากใบและน้ำมันเมล็ดงาขี้ม้อนในการปกป้องเซลล์ประสาทเพาะเลี้ยงและการงอกใยประสาท*

วัสดุและวิธีการ: *ศึกษาในเซลล์เพาะเลี้ยง PC12 ใช้สารสกัดเอทธานอลจากใบและน้ำมันหีบเย็นจากเมล็ดงาขี้ม้อน ทดสอบผลการปกป้องเซลล์ประสาทจากการถูกทำลายด้วยโปรตีนเบต้าอะมีลอยด์ วัดผลการอยู่รอดของเซลล์ ผลการทำงานของเอนไซม์ superoxide dismutase ผลปริมาณโปรตีน Tau ผลต่อการงอกใยประสาทและปริมาณโปรตีน MEK1*

ผลการศึกษา: *สารสกัดจากใบขนาด 200 มก./มล. น้ำมันเมล็ดงาขี้ม้อนขนาด 50 มก./มล. ให้เซลล์เพาะเลี้ยงก่อนแล้วจึงเหนี่ยวนำให้เกิดภาวะเครียดจากอนุมูลอิสระด้วยโปรตีนเบต้าอะมีลอยด์ พบว่าเซลล์รอดชีวิตมากขึ้นซึ่งสอดคล้องกับการลดการทำงานเอนไซม์ SOD น้ำมันเมล็ดงาขี้ม้อนขนาด 50 มก./มล. มีผลลดการสร้างโปรตีน tau เมื่อเลี้ยงเซลล์ในอาหารที่ขาดซีรัมพบว่า สารสกัดจากใบและน้ำมันเมล็ดงาขี้ม้อนมีผลให้เซลล์งอกใยประสาทเพิ่มขึ้นซึ่งสอดคล้องกับการเพิ่มปริมาณโปรตีน MEK-1*

สรุป: *สารสกัดจากใบและน้ำมันเมล็ดงาขี้ม้อนมีผลปกป้องเซลล์เพาะเลี้ยง PC12 จากการเหนี่ยวนำความเป็นพิษโดยโปรตีนเบต้าอะมีลอยด์ได้โดยลดภาวะเครียดจากอนุมูลอิสระและยับยั้งการสร้างโปรตีน tau นอกจากนี้ยังเพิ่มการงอกใยประสาทและการสร้างโปรตีน MEK-1*