RESEARCH ARTICLE

Neuroprotective Effect of *Bacopa Monnieri* Extract on Oxidized Low Density Lipoprotein-Induced Neurotoxicity in SH-SY5Y Neuroblastoma Cells

Panit Yamchuen[1], Narttaya Chaiwiang[1], Phakhamon Lapphanichayakool[1], Kornkanok Ingkaninan[2], Nanteetip Limpeanchob[1]

[1] *Department of Pharmacy Practice and Center of Excellence for Innovation in Chemistry, Pharmacology Research Unit, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand*

[2] *Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand*

Abstract

Oxidized low density lipoprotein (LDL)-mediates neurotoxicity and possibly plays some roles in the pathological process of Alzheimer's disease (AD). Neuroprotection of *Bacapa monerri* (L.) Wettst (BM) has been studied in various cells and animal models but not in oxidized LDL-induced toxicity. This study aimed to investigate the neuroprotective effects of BM extract and four components including three bacoside A; bacopaside I (BA-I), II (Ba-II), and bacoside A3 (Ba-A3), and one flavonoid; luteolin (Lut) in SH-SY5Y neuroblastoma cells. The results demonstrated that BM extract diminished the neurotoxicity of oxidized LDL in a dose dependent manner, potentially by suppression of cellular oxidative stress. Among four tested compounds, Lut exhibited the most effect on preventing the cell injury mediated by oxidized LDL. These compounds also suppressed the increment of cellular acetylcholinesterase (AChE) activity mediated by oxidized LDL, possibly as the consequence of oxidation suppression. These data support the beneficial effect of BM as a neuroprotective agent and this effect is possibly a combined result of its constituents.

Keywords: *Bacopa monnieri*, Alzheimer's disease, oxidized low density lipoprotein, Bacoside A, Luteolin

Address correspondence and reprint request to: Nanteetip Limpeanchob, Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand.
E-mail address: nanteetipl@nu.ac.th

ฤทธิ์ปกป้องประสาทของสารสกัดสมุนไพรพรมมิจากพิษที่ถูกเหนี่ยวนำด้วยออกซิไดซ์ไลโปโปรตีนชนิดความหนาแน่นต่ำในเซลล์ประสาทเพาะเลี้ยงชนิด SH-SY5Y blastoma

พานิชย์ ยามชื่น[1], นาตยา ไชยเวียง[1], ภคมน ลาภพาณิชยกุล[1], กรกนก อิงคนินันท์[2], นันทีทิพ ลิ้มเพียรชอบ[1]

[1] ภาควิชาเภสัชกรรมปฏิบัติ, ศูนย์ความเป็นเลิศด้านนวัตกรรมทางเคมี, และหน่วยปฏิบัติการวิจัยทางเภสัชวิทยา คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร จังหวัดพิษณุโลก

[2] ภาควิชาเภสัชเคมีและเภสัชเวช คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร จังหวัดพิษณุโลก

บทคัดย่อ

ออกซิไดซ์ไลโปโปรตีนชนิดความหนาแน่นต่ำส่งผลให้เกิดพิษต่อเซลล์ประสาทและอาจมีบทบาทต่อการกระบวนการทางพยาธิวิทยาของการเกิดโรคอัลไซม์เมอร์ ที่ผ่านมามีการศึกษาฤทธิ์ปกป้องประสาทของสมุนไพรพรมมิ (*Bacapa monerri* (L.) Wettst) ด้วยวิธีการต่าง ๆ ทั้งในเซลล์เพาะเลี้ยงและในสัตว์ทดลอง แต่ยังไม่มีการศึกษาฤทธิ์ต่อต้านพิษจากออกซิไดซ์ไลโปโปรตีนชนิดความหนาแน่นต่ำ ในการศึกษานี้จึงมุ่งเน้นทดสอบฤทธิ์ปกป้องประสาทของสารสกัดหยาบจากสมุนไพรพรมมิ และสารประกอบที่พบมากในสมุนไพรนี้อีก 4 ชนิด โดยเป็นสารกลุ่มบาโคไซด์-เอ (bacoside A) 3 ชนิด ได้แก่ bacopaside I (BA-I), II (Ba-II) และ bacoside A3 (Ba-A3) รวมทั้งสารกลุ่มฟลาโวนอยด์อีกหนึ่งชนิด ได้แก่ ลูทิโอลิน (luteolin) โดยทำการทดลองในเซลล์ประสาทเพาะเลี้ยงชนิด SH-SY5Y ผลการทดลองพบว่าสารสกัดหยาบพรมมิแสดงฤทธิ์ยับยั้งพิษที่เกิดจากออกซิไดซ์ไลโปโปรตีนชนิดความหนาแน่นต่ำได้ โดยฤทธิ์นี้ขึ้นกับความเข้มข้นและคาดว่าอาจเป็นผลมาจากฤทธิ์ในการยับยั้งภาวะเครียดออกซิเดชันภายในเซลล์ จากการทดสอบสารทั้งสี่ชนิดพบว่า ลูทิโอลินเป็นสารที่แสดงประสิทธิภาพสูงสุดในการปกป้องเซลล์จากพิษของออกซิไดซ์ไลโปโปรตีนชนิดความหนาแน่นต่ำ นอกจากนี้ยังพบว่าสารที่ทำการทดสอบทุกชนิดสามารถยับยั้งการเพิ่มขึ้นของการทำงานของเอนไซม์อะเซทิลโคลีนเอสเตอร์เลสที่เหนี่ยวนำด้วยออกซิไดซ์ไลโปโปรตีนชนิดความหนาแน่นต่ำ ซึ่งคาดว่าเป็นผลที่เกิดมาจากฤทธิ์ของสารทดสอบในการยับยั้งการเกิดออกซิเดชัน ผลจากการศึกษานี้สนับสนุนการใช้ประโยชน์ของสารสกัดพรมมิเป็นสารปกป้องประสาทโดยฤทธิ์ดังกล่าวนี้คาดว่ามาจากผลรวมของการออกฤทธิ์ของสารประกอบชนิดต่าง ๆ ในสารสกัด

คำสำคัญ: สมุนไพรพรมมิ, โรคอัลไซม์เมอร์, ออกซิไดซ์ไลโปโปรตีนชนิดความหนาแน่นต่ำ, สารบาโคไซด์-เอ, ลูทิโอลิน

Introduction

Alzheimer's disease (AD) is the most common cause of dementia and pathologically characterized by accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles containing hyperphosphorylated tau.[1] AD neurodegeneration carries multiple risks including hypercholesterolemia.[2] The role of cholesterol is controversial[1,3] but a key link to AD is oxidized cholesterol (oxysterols)[4] leading to microvascular dysregulation and blood brain barrier (BBB) leakage. Thus, cerebrospinal fluid (CSF) of AD patients contain increased oxidized lipoprotein[5] and has antibodies against oxidized low density lipoprotein (LDL)[6,7], suggesting links between oxidative stress, oxidized cholesterol/lipoprotein, and AD pathogenesis.

In cultured neurons, oxidized LDL induced cell death by generating reactive oxygen species (ROS)[8], caspase-3 activation[9], and glutathione depletion.[3] Oxidized LDL also activated BACE-1, thereby promoting Aβ accumulation[3] while killing cells via toll-like receptor-4 (TLR4).[10] The increased ROS via oxidized LDL promoted another AD pathology by enhancing acetylcholinesterase (AChE) activity in culture neurons.[8] Thus, oxidized LDL-mediated neurotoxicity provides an accessible model on which interventions leading to reduced AD pathogenesis can be tested.

Bacapa monerri (L.) Wettst (BM) is used as a nootropic agent in Ayurvedic medicine[11], an idea supported its antiepileptic, anxiolytic, antidepressant, and sedative actions.[12] Furthermore, BM reduced β-amyloid levels[13] and protected neurons against Aβ-induced toxicity.[14] Limited clinical trials suggest some potential to improve cognition[15] possibly through reducing AChE activity and β-amyloid, increasing antioxidant function, improving cerebral blood flow, and neurotransmitter modulation.[11]

BM extract contains tetracyclic triterpenoid saponins called bacoside A, which is a mixture of saponins including bacoside A3 (Ba-A3), bacopaside I (Ba-I), bacopaside II (Ba-II), bacopasaponin C and jujubogenin isomer of bacosaponin C (bacopaside X) and two common flavonoids, luteolin (Lut) and apigenin.[16] These compounds are considered to provide the neuroprotective and precognitive functions of BM by testing in various *in vitro* and *in vivo* models[17-23], while Lut also prevented Aβ-induced neuronal death[24]. BM enjoys global commercialisation as a dietary supplement by promoting its perceived pro-cognitive effects, but there lacks a clear understanding about its cellular mechanism of action.

In this study, we aimed to show that BM extract and four components (Ba-I, Ba-II, Ba-A3 and Lut) can improve the viability of neurons (SH-SY5Y neuroblastoma cells), by reducing intracellular ROS, lipid peroxidation, and downregulating AChE provoked by oxidized LDL.

Materials and Methods

Materials

Dulbecco's modified eagle's medium (DMEM)/F12, retinoic acid, 5,5'-dithio-bis (2-nitrobenzonic acid) (DTNB), acetylthiochloine iodide (ATCI), 2',7'-dichlorofluorescein diacetate (DCFH-DA), neostigmine, and luteolin were purchased

from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), trypsin/EDTA and penicillin-streptomycin were purchased from Gibco (Grand Island, NY). Amicon® Ultra-4 centrifuge filters were purchased from Merck Millipore (Darmstadt, Germany). The BCA protein assay kit was purchased from Thermoscience (Rockford, IL). Ba-I, Ba-II, and Ba-A3 were purchased from Natural Remedies Private Limited (Bangalore, India).

Preparation and standardization of BM extract

BM was collected from Phitsanulok province, Thailand. It was identified by Associate Professor Wongsatit Chuakul, Faculty of Pharmacy, Mahidol University, Thailand. The voucher specimen, Phrompittayarat001, was kept at the Pharmaceutical Botany Mahidol Herbarium, Mahidol University, Thailand. BM was cut into small pieces and dried in hot-air oven at 50ºC for 12 h. The dried plant was coarsely powdered and percolated twice with circulating 95% ethanol for 8 h. The extract was then filtrated and dried under reduced pressure. Constituent analysis of BM extract was analyzed by HPLC with some modification from previously described method.[25] Briefly, the separation was performed using a Shimadzu LC-20AT liquid chromato-graphy equipped with a SPD-20A UV/VIS detector. The mobile phase consisted of 0.2% orthophosphoric acid and acetonitrile (65:35 V/V) adjusted to pH 3.0 with 5M NaOH, and chromatography was run at 1.0 mL/min flow rate. All peaks were integrated at detector wavelength 205 nm and were assigned by retention times compared with standards. Calibration curves of seven standards (Ba-A3, Ba-I, Ba-II, bacopasaponin C isomer, bacosaponin C, Lut, and apeginin) were prepared based on peak areas of seven concentrations (7.8-500 µg/mL).

Isolation and oxidation of human LDL

LDL isolation and oxidation were performed as previously described.[8] The protocol was approved by Naresuan University Institutional Review Board (IRB No. 148/56). Briefly, LDL was isolated from plasma provided by Blood Bank, Naresuan University Hospital by density gradient ultracentrifugation. LDL was collected and dialyzed in 1×PBS containing 10 µM EDTA for 24 h at 4°C. LDL (1 mg/mL) was oxidized by 10 µM $CuSO_4$ at 37ºC for 2 h and the reaction was terminated by adding EDTA (0.3 mM final concentration). The EDTA and $CuSO_4$ were removed by passing LDL sample through a 30 kDa cut-off Amicon filter. The degree of LDL oxidation was measured as lipid peroxidation product using thiobarbituric acid reactive substance (TBARs) assay.

Cell culture preparation

The human neuroblastoma cell line (SH-SY5Y) (American Type Culture Collection, Rockville, MD) was cultured in DMEM/F12 containing 10% FBS and 0.01% penicillin-streptomycin at 37ºC in 95 % air and 5% CO_2 incubator. Cells were seeded into 96-well plates (2×10^4 cells/well), allowed to attach for 24 h, and differentiated in low serum culture medium (2% FBS) containing 10 µM retinoic acid for 6 days. Before treatment, cells were incubated in serum-free medium for 24 h.

Determination of cell viability by MTT assay

Differentiated SH-SY5Y cells were treated with BM extract or tested compounds for 2 h. Then oxidized LDL was added (200 µg/mL final concentration) and incubated for 24 h. MTT (5 mg/mL in PBS) was added to each well 2 h prior to the end of treatment. The medium was then removed and 200 µL of DMSO:ethanol (1:1) was added to dissolve formazan crystals. The absorbance was measured at 595 nm by a microplate reader.

Determination of intracellular reactive oxygen species (ROS)

Cells were incubated with 10 µM DCHF-DA in serum- and phenol red-free DMEM/F-12 for 30 min and culture medium was then removed. Cells were then pretreated with BM extract or tested compounds for 2 h before adding 200 µg/mL oxidized LDL and then incubated for 24 h. The fluorescence intensity was measured by a microplate reader with excitation wavelength at 485 nm and emission wavelength at 535 nm. Data from each experiment were normalized by number of viable cells.

Determination of lipid peroxidation by TBARs assay

At the end of treatment, 100 µL of TBARs reagent containing 0.4% TBA, 1.4% TCA, and 8% HCl (1:2:1) was directly added to each well. The mixtures were incubated at 90ºC for 1 h and cooled to room temperature. The fluorescence intensity was measured by a microplate reader with excitation wavelength at 535 nm and emission wavelength at 595 nm.

Cellular acetylcholinesterase (AChE) activity assay

Cells were lysed by lysis buffer containing 15 mM tris (pH 7.4), 150 mM NaCl and 1% triton X-100. AChE activity was determined by Ellman's colorimetric method with minor modification.[26] The yellow reaction is formed by the reaction of DTNB (1.5 mM) and thiocholine released from ATCI by enzymatic hydrolysis, and measured by a microplate reader at 405 nm. Neostigmine, an AChE inhibitor, was used as a positive control. Data were normalized by numbers of viable cells.

Ferric iron reducing antioxidant power (FRAP) assay

Reducing activities were assessed by their ability to reduce Fe^{3+} to Fe^{2+} using the FRAP assay. FRAP reagent (180 µL) containing 3 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ (10:1:1) was mixed with 20 µL of BM extract (250 µg/mL) or tested compounds (250 µM). The absorbance was measured at 595 nm.

DPPH radical scavenging capacity assay

DPPH solution (180 µL of 0.2 mM in methanol) was mixed with 20 µL of BM extract (250 µg/mL) or tested compounds (250 µM). The absorbance was measured by a microplate reader at 510 nm. Data were expressed as percent of radical scavenging activity.

Statistical analysis

Data from all experiments were presented as mean ± standard errors of mean (SEM). The data were analyzed by analysis of variance (ANOVA) with a LSD test between groups. Comparisons between two groups used student's *t*-test and differences were considered to be significant when $p \leq 0.05$.

Results

Chemical constituents of BM extract and their cytotoxicity

BM ethanolic extract used in this study contained at least five saponin glycosides and two flavonoids (Table 1). Four standard compounds including Ba-I, Ba-II, Ba-A3 and Lut were selected to study their neuroprotective actions and compare with BM extract. At concentrations ranging from 0.1 to 25 µM, most compounds showed no toxicity to differentiated SH-SY5Y cells (Table 2), except Ba-II which induced cell death at 25 µM. BM extract at 25 µg/mL (containing approximately 1 µM bacoside A mixture) and lower were not toxic to cells. So the extract at 25 µg/mL and standard compounds at 10 µM were used for further studies.

Table 1. Content of certain saponin glycosides and flavonoids in BM extract.

Constituents	Mean ± SD (%w/w)
Glycosides	
Ba-A3	0.74 ± 0.06
Ba-II	0.98 ± 0.06
Bacopaside X	0.53 ± 0.04
Bacopasaponin C	1.00 ± 0.06
Ba-I	0.93 ± 0.06
Flavonoids	
Lut	0.11 ± 0.01
Apigenin	0.07 ± 0.00

Table 2. Effect of BM and constituent bacosides on SH-SY5Y cell viability.

Compounds	Cell viability (% of control)			
	0.1 µM	**1 µM**	**10 µM**	**25 µM**
Ba-I	100.9 ± 2.0	100.4 ± 3.5	95.8 ± 3.7	95.6 ± 4.2
Ba-II	103.4 ± 3.1	106.7 ± 5.0	91.9 ± 5.3	26.2 ± 8.9*
Ba-A3	104.4 ± 10.9	96.9 ± 7.7	101.2 ± 5.5	89.6 ± 2.0
Lut	99.6 ± 4.2	102.4 ± 4.5	131.0 ± 2.3*	102.0 ± 6.6
	1 µg/mL	**10 µg/mL**	**25 µg/mL**	**50 µg/mL**
BM extract	102.1 ± 4.1	107.3 ± 4.2	111.0 ± 3.7	72.4 ± 6.5*

Note: Values are means ± SEM, * $p \leq 0.05$ compared to control (untreated cells)

BM extract and its active components protected cells from oxidized LDL-induced oxidative stress

Oxidized LDL (200 µg/mL) mediated approximately 60% death of SH-SY5Y cells and BM extract, particularly at 10 and 25 µg/mL, could effectively prevent cell death (Figure 1A). Oxidized LDL generated tremendous oxidative stress in cultured neurons expressing as many folds increased intracellular ROS and lipid peroxidation (Figure 1B, C). BM extract effectively suppressed both intracellular ROS and lipid peroxidation in dose-dependent patterns (Figure 1B, C). Vitamin E analog, trolox (25 µM), could also prevent those effects induced by oxidized LDL.

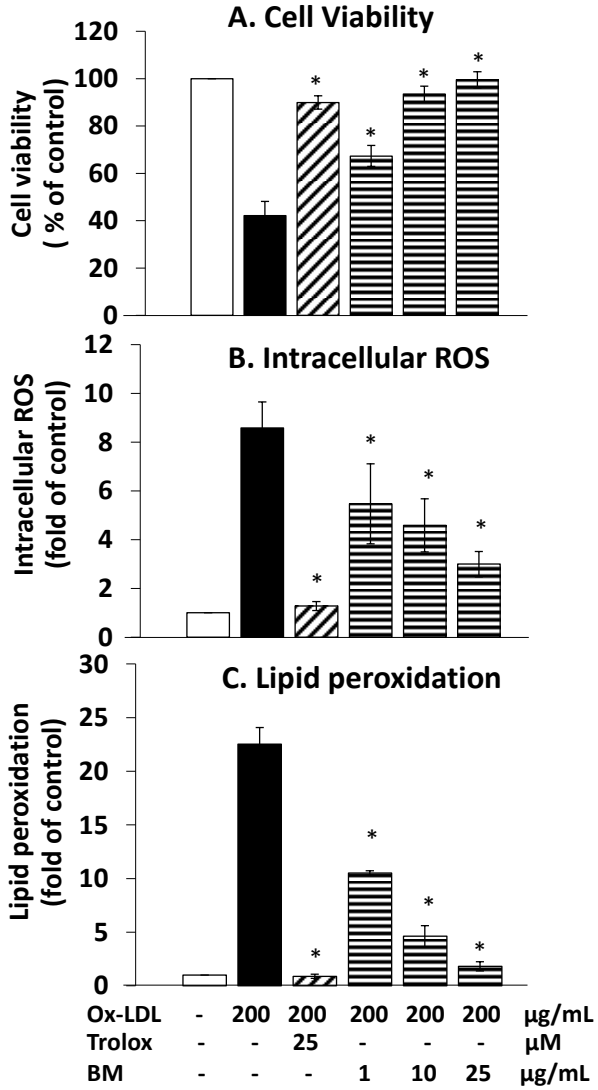

Figure 1. Protection against oxidative actions of oxidized LDL by BM extract. Cells were treated BM extract 2 h prior to incubating with oxidized LDL (ox-LDL) for 24 h. Cell viability by MTT assay (A), intracellular ROS (B), and lipid peroxidation (C) were determined. Data are presented as means±SEM from at least four different batches of cells. * $p \leq 0.05$, compared to cells treated with ox-LDL.

The effects of four BM active compounds on oxidized-LDL induced cell damage were showed in Figure 2. Compared to trolox (25 µM) and BM extract (25 µg/mL), all three saponins (10 µM); Ba-I, Ba-II, and Ba-A3, moderately prevented cellular oxidative stress mediated by oxidized-LDL. A flavonoid, Lut exhibited better neuroprotection in comparison with saponins at the same concentration (Figure 2).

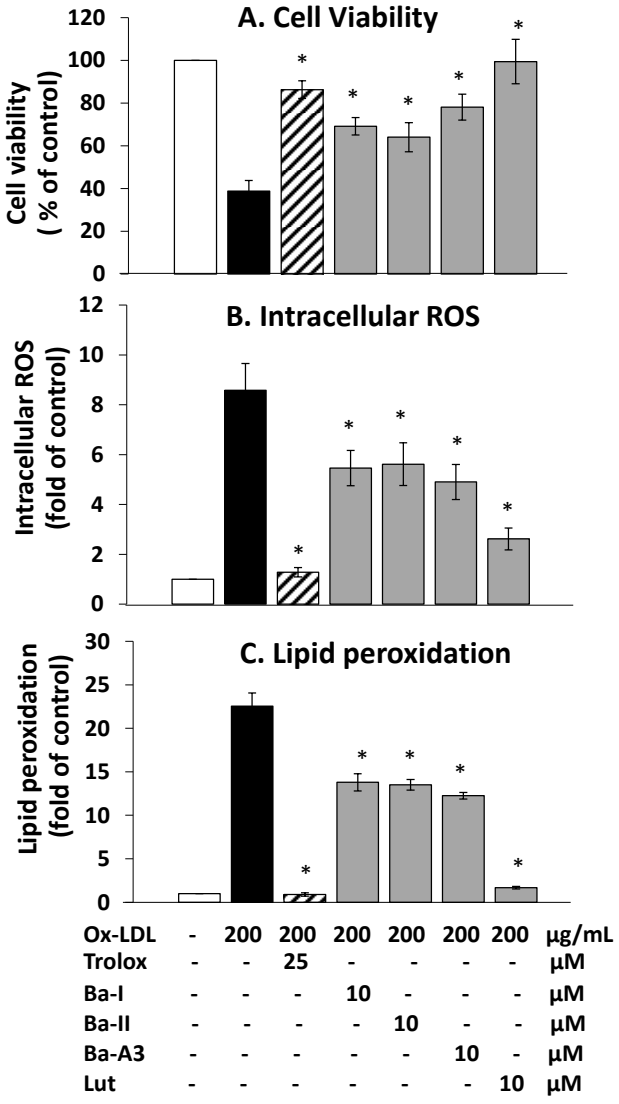

Figure 2. Neuroprotection by Ba-I, Ba-II, Ba-A3 and Lut on oxidized LDL-induced SH-SY5Y cells damage.

Cells were treated with tested compounds for 2 h prior to ox-LDL and incubated for 24 h. Cell viability by MTT assay (A), intracellular ROS (B), and lipid peroxidation (C) were determined. Data are presented as means±SEM from at least four different batches of cells. * $p \leq 0.05$, compared to ox-LDL-treated cells

BM extract and its active components reduced cellular AChE activity

SH-SY5Y cells treated with oxidized LDL exhibited approximately two folds AChE activity higher than that of control cells (Figure 3). BM extract, Ba-I, Ba-II, Ba-A3, and Lut, significantly diminished such effect of oxidized LDL. Lut showed slightly higher inhibitory activity than the three saponins. We also determined AChE inhibitory activity in the *in vitro* enzymatic assay of BM extract and four active compounds. The results showed that all tested compounds did not directly inhibit the activity of AChE (data not shown). Taken all data together, the effect of BM and its components on cellular AChE activity might possibly be the consequence of their ability to suppress cellular oxidative stress.

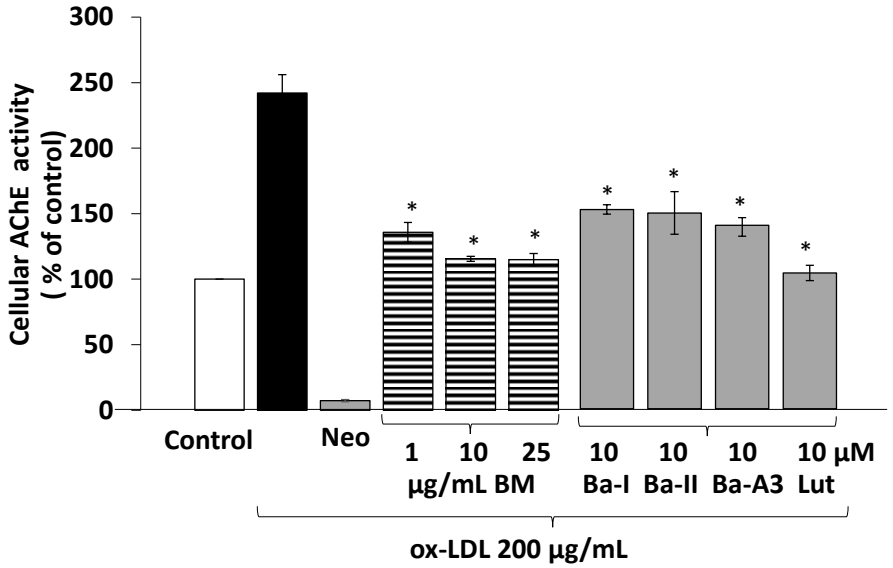

Figure 3. Effect of BM extract and four constituents on cellular AChE activity. Cells were treated with BM and tested compounds for 2 h prior to ox-LDL and incubated for 24 h. Cells were then lysed and measured for AChE activity. Data are presented as means±SEM from at least four different batches of cells. Neo = neostigmine (100 µM), * $p \leq 0.05$, compared to ox-LDL-treated cells

Free radical scavenging and reducing activities of BM extract and its active components

From the *in vitro* antioxidant assays of BM extract and its active components, Lut at 25 µM exhibited the highest DPPH free radical scavenging capacity when compared to either trolox at the same concentration or BM extract at 25 µg/mL (Figure 4A). On the other hand, ferric reducing activity of trolox (25 µM) was higher than other compounds (Figure 4B). It should be noted that these three saponins demonstrated very low radical scavenging and ferric reducing activities whereas BM extract generously established those antioxidant activities.

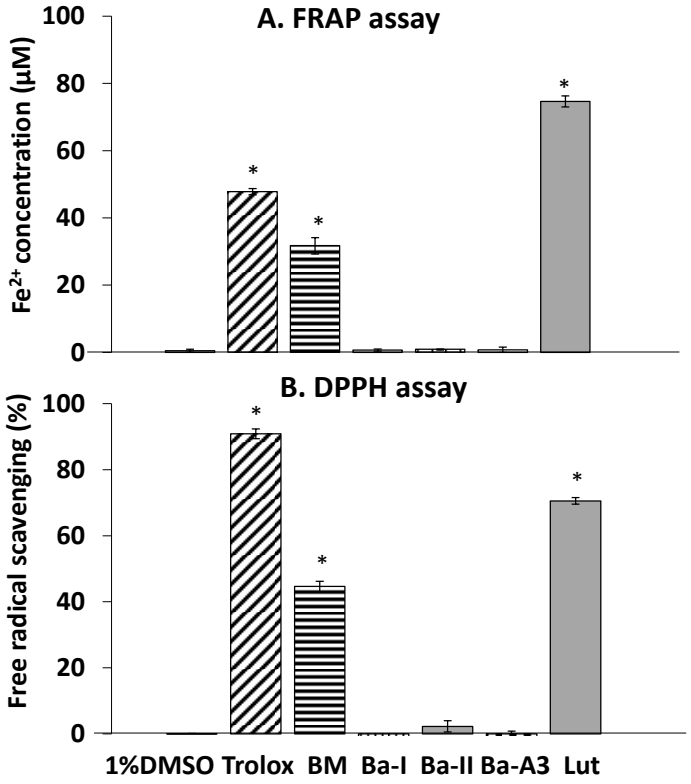

Figure 4. Antioxidant activities of BM extract and its constituents. Metal ion reducing activity by FRAP (A) and free radical scavenging by DPPH assay (B) were measured. BM extract at 25 µg/mL and saponins and Lut at 25 µM were tested. Data are presented as means±SEM from three experiments. * $p \leq 0.05$, versus 1%DMSO

Discussion

Of the BM extract and four tested constituents, luteolin (Lut) was the most effective compound to prevent a spectrum of oxidative effects of oxidized LDL and 10 µM of Lut had almost the same effect as 25 µM Trolox. Since BM extract contained only low content of Lut, other untested antioxidant compounds might play some roles in this pathway of neuroprotection. While the saponins appeared to be redox inactive, yet they still prevented some of the adverse oxidized LDL action. This suggested that their protection was not directly redox dependent but might promote endogenous anti-oxidant enzyme expression. Previous studies have demonstrated the indirect protective effect of BM extract on certain cell lines through upregulating antioxidant enzymes and glutathione.[22, 27] This may occur *in vivo* where catalase, glutathione peroxidase, and superoxide dismutase are increased.[21, 27-29]

Our study provided the first evidence describing protective effect of BM extract against oxidized LDL-mediated oxidative pathologies. Similar effects of BM and its saponins have been extensively studied both *in vitro* and *in vivo* models

including preserving mitochondrial function after challenging with H_2O_2 in neuroblastoma cells[30], rotenone-induced oxidative stress a dopaminergic cell-line[27], and lung epithelial cells (L123) challenged by reactive nitrogen species[22]. Oxidized LDL-induced neurotoxicity is mediated through elevated cell ROS and Ca^{2+}, depletion of cellular glutathione, caspase-apoptosis[3, 8-10] and lipid peroxidation as shown here.

The protective effect of BM constituents is less clear but a mixture of saponin/bacoside A was considered responsible[18, 20, 21, 23] although the effect of each purified bacoside A saponin has not been tested. The bacosides tested in this study (Ba-I, Ba-II, and Ba-A3) were effective at 10 µM but the BM extract contained only 1 µM bacoside which unable to reduce DPPH or Fe^{3+} at this concentration. All three standard bacoside A compounds demonstrated very low antioxidant activities, suggesting other mechanisms involved in neuroprotection.

The neuroprotective effects of Lut were investigated in various neuronal damage models in primary and neuronal cell lines[31-34] as well as animal models.[35-37] From our study, among four standard compounds, Lut was the strongest antioxidant and exhibited the highest activity to suppress oxidative stress in cultured neurons. However, the antioxidative activity of Lut at 10 µM was comparable to that of BM extract at 25 µg/mL which contained only low amount of Lut (approximately 0.1 µM). This suggested that other untested BM components in addition to Lut might provide the redox-mediated neuroprotection. Taken all data together, neuroprotective activity of BM could possibly be the result of synergistic effect of its major and minor active constituents via several pathways.

Oxidized LDL also upregulated AChE activity and all the intervention suppressed this augmentation. Although BM was reported to directly inhibit AChE activity tested by *in vitro* enzymatic assay[28], BM extract and all tested compounds did not change basal cellular AChE activity. Thus their ability to reduce AChE activity was possibly associated with suppression of cellular oxidative stress.

Conclusion

BM ameliorated neurotoxicity induced by oxidized LDL, partly by bacoside saponins and luteolin. The cellular oxidative stress and AChE were attenuated by BM extract and all four standard components (Ba-I, Ba-II, Ba-A3, and Lut). By using oxidized LDL-mediated neuron damage in SH-SY5Y cells, a cellular model never been tested with BM, our results still support the neuroprotection of BM. This plant displays the potential to prevent the adverse effects induced by various types of toxic agents and possibly can be used in various neurological disorders.

Acknowledgements

The authors thank Dr. Norman Scholfield for help in manuscript preparation. This work was supported by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0018/2555), the Center of Excellence for Innovation in Chemistry and Naresuan University (Grant No. R2558B027)

References

1. Reitz C, Mayeux R. Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem Pharmacol. 2014;88(4):640-51.
2. Imtiaz B, Tolppanen AM, Kivipelto M, Soininen H. Future directions in Alzheimer's disease from risk factors to prevention. Biochem Pharmacol. 2014;88(4):661-70.
3. Dias IHK, Mistry J, Fell S, Reis A, Spickett CM, Polidori MC, et al. Oxidized LDL lipids increase β-amyloid production by SH-SY5Y cells through glutathione depletion and lipid raft formation. Free Radic Biol Med. 2014;75: 48-59.
4. Marwarha G, Ghribi O. Does the oxysterol 27-hydroxycholesterol underlie Alzheimer's disease–Parkinson's disease overlap? Exp Gerontol. 2015;68:13-8.
5. Schippling S, Kontush A, Arlt S, Buhmann C, Stürenburg H-J, Mann U, et al. Increased lipoprotein oxidation in alzheimer's disease. Free Radic Biol Med. 2000;28(3):351-60.
6. Papassotiropoulos A, Lütjohann D, Bagli M, Locatelli S, Jessen F, Buschfort R, et al. 24S-hydroxycholesterol in cerebrospinal fluid is elevated in early stages of dementia. J Psychiatr Res. 2002;36(1):27-32.
7. Kankaanpää J, Turunen SP, Moilanen V, Hörkkö S, Remes AM. Cerebrospinal fluid antibodies to oxidized LDL are increased in Alzheimer's disease. Neurobiol Dis. 2009;33(3):467-72.
8. Yamchuen P, Aimjongjun S, Limpeanchob N. Oxidized low density lipoprotein increases acetylcholinesterase activity correlating with reactive oxygen species production. Neurochem Int. 2014;78:1-6.
9. Keller JN, Hanni KB, Markesbery WR. Oxidized low-density lipoprotein induces neuronal death: implications for calcium, reactive oxygen species, and caspases. J Neurochem. 1999;72(6):2601-9.
10. Nowicki M, Muller K, Serke H, Kosacka J, Vilser C, Ricken A, et al. Oxidized low-density lipoprotein (oxLDL)-induced cell death in dorsal root ganglion cell cultures depends not on the lectin-like oxLDL receptor-1 but on the toll-like receptor-4. J Neurosci Res. 2010;88(2):403-12.
11. Aguiar S, Borowski T. Neuropharmacological review of the nootropic herb *Bacopa monnieri*. Rejuvenation Res. 2013;16(4):313-26.
12. Russo A, Borrelli F. *Bacopa monniera*, a reputed nootropic plant: an overview. Phytomedicine. 2005;12(4):305-17.
13. Holcomb LA, Dhanasekaran M, Hitt AR, Young KA, Riggs M, Manyam BV. *Bacopa monniera* extract reduces amyloid levels in PSAPP mice. J Alzheimers Dis. 2006;9(3):243-51.
14. Limpeanchob N, Jaipan S, Rattanakaruna S, Phrompittayarat W, Ingkaninan K. Neuroprotective effect of *Bacopa monnieri* on beta-amyloid-induced cell death in primary cortical culture. J Ethnopharmacol. 2008;120(1):112-7.

15. Kongkeaw C, Dilokthornsakul P, Thanarangsarit P, Limpeanchob N, Norman Scholfield C. Meta-analysis of randomized controlled trials on cognitive effects of *Bacopa monnieri* extract. J Ethnopharmacol. 2014;151(1):528-35.
16. Deepak M, Sangli GK, Arun PC, Amit A. Quantitative determination of the major saponin mixture bacoside A in *Bacopa monnieri* by HPLC. Phytochem Anal. 2005;16(1):24-9.
17. Singh HK, Rastogi RP, Srimal RC, Dhawan BN. Effect of bacosides A and B on avoidance responses in rats. Phytother Res. 1988;2(2):70-5.
18. Mathew J, Paul J, Nandhu MS, Paulose CS. *Bacopa monnieri* and Bacoside-A for ameliorating epilepsy associated behavioral deficits. Fitoterapia. 2010; 81(5):315-22.
19. Singh R, Ramakrishna R, Bhateria M, Bhatta RS. *In vitro* evaluation of *Bacopa monniera* extract and individual constituents on human recombinant monoamine oxidase enzymes. Phytother Res. 2014;28(9):1419-22.
20. Sumathi T, Nathiya VC, Sakthikumar M. Protective effect of bacoside-A against morphine-induced oxidative stress in rats. Indian J Pharm Sci. 2011; 73(4):409-15.
21. Thomas RB, Joy S, Ajayan MS, Paulose CS. Neuroprotective potential of *Bacopa monnieri* and bacoside A against dopamine receptor dysfunction in the cerebral cortex of neonatal hypoglycaemic rats. Cell Mol Neurobiol. 2013; 33(8):1065-74.
22. Anand T, Pandareesh MD, Bhat PV, Venkataramana M. Anti-apoptotic mechanism of Bacoside rich extract against reactive nitrogen species induced activation of iNOS/Bax/caspase 3 mediated apoptosis in L132 cell line. Cytotechnology. 2014;66(5):823-38.
23. Anbarasi K, Vani G, Balakrishna K, Devi CSS. Effect of bacoside A on brain antioxidant status in cigarette smoke exposed rats. Life Sci. 2006;78(12):1378-84.
24. Cheng HY, Hsieh MT, Tsai FS, Wu CR, Chiu CS, Lee MM, et al. Neuroprotective effect of luteolin on amyloid beta protein (25-35)-induced toxicity in cultured rat cortical neurons. Phytother Res. 2010;24 Suppl 1:S102-8.
25. Phrompittayarat W, Wittaya-areekul S, Jetiyanon K, Putalun W, Tanaka H, Ingkaninan K. Determination of saponin glycosides in *Bacopa monnieri* by reversed phase high performance liquid chromatography. Thai Pharm Health Sci J. 2007;2(1):26-32.
26. Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J Ethnopharmacol. 2003; 89(2–3):261-4.
27. Shinomol GK, Mythri RB, Srinivas Bharath MM, Muralidhara. *Bacopa monnieri* extract offsets rotenone-induced cytotoxicity in dopaminergic cells and oxidative impairments in mice brain. Cell Mol Neurobiol. 2012;32(3):455-65.

28. Le XT, Pham HT, Do PT, Fujiwara H, Tanaka K, Li F, et al. *Bacopa monnieri* ameliorates memory deficits in olfactory bulbectomized mice: possible involvement of glutamatergic and cholinergic systems. Neurochem Res. 2013; 38(10):2201-15.
29. Sumathi T, Nathiya VC, Sakthikumar M. Protective Effect of Bacoside-A against Morphine-Induced Oxidative Stress in Rats. Indian J Pharm Sci. 2011; 73(4):409-15.
30. Singh M, Murthy V, Ramassamy C. Modulation of hydrogen peroxide and acrolein-induced oxidative stress, mitochondrial dysfunctions and redox regulated pathways by the *Bacopa monniera* extract: potential implication in Alzheimer's disease. J Alzheimers Dis. 2010;21(1):229-47.
31. Liu R, Meng F, Zhang L, Liu A, Qin H, Lan X, et al. Luteolin isolated from the medicinal plant *Elsholtzia rugulosa* (Labiatae) prevents copper-mediated toxicity in beta-amyloid precursor protein Swedish mutation overexpressing SH-SY5Y cells. Molecules. 2011;16(3):2084-96.
32. Wruck CJ, Claussen M, Fuhrmann G, Romer L, Schulz A, Pufe T, et al. Luteolin protects rat PC12 and C6 cells against MPP+ induced toxicity via an ERK dependent Keap1-Nrf2-ARE pathway. J Neural Transm Suppl. 2007(72): 57-67.
33. Zhao G, Yao-Yue C, Qin GW, Guo LH. Luteolin from Purple Perilla mitigates ROS insult particularly in primary neurons. Neurobiol Aging. 2012;33(1):176-86.
34. Zhu L, Bi W, Lu D, Zhang C, Shu X. Luteolin inhibits SH-SY5Y cell apoptosis through suppression of the nuclear transcription factor-kappaB, mitogen-activated protein kinase and protein kinase B pathways in lipopolysaccharide-stimulated cocultured BV2 cells. Exp Ther Med. 2014; 7(5):1065-70.
35. Lin TY, Lu CW, Wang SJ. Luteolin protects the hippocampus against neuron impairments induced by kainic acid in rats. Neurotoxicology. 2016;55:48-57.
36. Xu J, Wang H, Ding K, Zhang L, Wang C, Li T, et al. Luteolin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE pathway. Free Radic Biol Med. 2014;71:186-95.
37. Yu TX, Zhang P, Guan Y, Wang M, Zhen MQ. Protective effects of luteolin against cognitive impairment induced by infusion of Abeta peptide in rats. Int J Clin Exp Pathol. 2015;8(6):6740-7.