Neuroprotective mechanisms of 3-n-butylphthalide in neurodegenerative diseases (Review)

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Received April 7, 2019; Accepted September 19, 2019

DOI: 10.3892/br.2019.1246

Abstract. Since 3-n-butylphthalide (NBP) was approved by the China Food and Drug Administration for the treatment of acute ischemia stroke in 2002, a number of studies have investigated NBP worldwide. In recent years, NBP has also demonstrated potential as treatment of several neurodegenerative diseases, which has increased the interest in its mechanisms of protection and action. Clinical studies and studies that used cell or animal models, have directly demonstrated neuroprotective effects of NBP via the following mechanisms: i) Inhibiting the inflammatory reaction; ii) reducing mitochondrial oxidative stress; iii) regulating apoptosis and autophagy; iv) inducing resistance to endoplasmic reticulum stress; and v) decreasing abnormal protein deposition. Therefore, NBP may be a potential drug for neurodegenerative diseases, and it is particularly important to identify the mechanism of NBP as it may assist with the development of new drugs for neurodegeneration. The present review summarizes the neuroprotective mechanisms of NBP and discusses new perspectives and prospects. The aim of the current review is to provide a new summary regarding NBP and its associated mechanisms.

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1. Introduction

3-n-butylphthalide (NBP), approved by the China Food and Drug Administration for the treatment of acute ischemic stroke, is a type of compound isolated from the seeds of Chinese celery (1). The molecular structure of NBP is presented in Fig. 1. Therapy using NBP has been recommended by Chinese guidelines for acute ischemic stroke (2). A randomized double-blind trial (clinical trial no. ChiCTR-TRC-09000483) reported that NBP significantly improves clinical outcomes, including the modified Rankin Scale (3) and National Institute of Health Stroke Scale scores (4), of patients who experienced ischemic stroke (5). In addition, a study demonstrated that NBP therapy persistently increases the level of endothelial progenitor cells in peripheral blood, ameliorate cerebral blood flow and improve neuronal functions (6). Furthermore, NBP has been reported to be a safe treatment for cerebral ischemia stroke (5-7). A study has indicated that NBP exhibits protective effects in several neurodegenerative diseases (8). However, to the best of our knowledge, the neuroprotective mechanism of NBP remains unclear. Therefore, the present review discusses the potential mechanism of neuroprotective effects of NBP. The aim of the current review is to provide further understanding regarding the advances of NBP.

2. NBP inhibits the inflammatory reaction

Inflammation, a complex biological response to injury, is associated with neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease (PD), multiple sclerosis, amyotrophic lateral sclerosis, traumatic brain injury (TBI) and more (9-11). NBP has exhibited anti-inflammatory effects in various models of these diseases and certain mechanisms have been identified. NBP has been reported to reduce...
the inflammatory reaction by inhibiting nucleotide binding oligomerization domain like receptor protein 3-inflammation some microglia activation and mitigating the Alzheimer's-like pathology via the nuclear factor erythroid-2-related factor 2-thioredoxin-interacting protein-TXNIP-thioredoxin axis in an APP/PS1 mouse model (12,13). Furthermore, NBP inhibited the inflammatory reaction in lipopolysaccharide (LPS)-induced rats via inhibition of c-Jun N-terminal kinase activation and the NF-κB pathway (14,15). NBP was reported to improve dyskinesia in a LPS-induced PD mouse model via a reduction in the loss of dopaminergic neurons, activation of mouse microglia, an increase in TNF-α levels and α-synuclein deposition in the black substantia of the mouse midbrain (16). Additionally, NBP-treatment reduces NF-κB activation following TBI (17), and NBP also inhibits the inflammatory reaction via the same pathway in spontaneously hypertensive rats (18). Notably, a number of studies have indicated that NBP inhibits the inflammatory reaction in other neuroassociated experimental models, such as an experimental model of autoimmune encephalomyelitis of microglia or autoimmune myositis in guinea pigs (19,20). In addition, NBP-treatment has been demonstrated to significantly ameliorate cerebral ischemia reperfusion-induced brain injury of Sprague-Dawley (SD) rats by inhibiting toll like receptor 4/NF-κB-associated inflammation (21). NBP attenuates advanced glycation end products-induced endothelial dysfunction by ameliorating inflammatory responses (22). In summary, there is some understanding regarding the mechanism of NBP in the inhibition of inflammation.

3. NBP reduces mitochondrial oxidative stress

Mitochondria, the site of oxidative metabolism in eukaryotes, produce energy through the oxidation of carbohydrates, fats and amino acids (23). Therefore, mitochondrial dysfunction in the form of oxidative stress may contribute to the pathogenesis of various neurodegenerative diseases (24). Oxidative stress is considered a condition that is caused by an imbalance between pro- and antioxidant factors, which leads to molecular and cellular damage (25). Oxidative stress serves an essential role in the development of age-related diseases (26). NBP exhibits a cumulative beneficial effect on the process of mitochondrial damage (27). This section will discuss the mechanisms involved in mitochondrial oxidative stress.

Recently, NBP exhibited a powerful effect on antioxidant stress in some different models. NBP inhibited oxidative stress in K141N-induced SH-SY5Y cells and in LPS-induced rats through activation of the Kelch-like ECH-associating protein 1 Nrf2-related factor 2-antioxidant response element signaling pathway (15,28). Similarly, NBP reduced oxidative damage to provide neuroprotection in mice following TBI and in rats following carbon monoxide poisoning (29,30). In addition, NBP protects against cerebral ischemia-reperfusion injury by decreasing antioxidant stress via the ERK signaling pathway (31). NBP also protects against H2O2-induced injury in neural stem cells by activation of the PI3K/Akt and the Mash1 signaling pathways (32). Furthermore, NBP has been reported to increase superoxide dismutase and catalase activity, and reduce malondialdehyde activity in the experimental autoimmune myositis (EAM) model, NBP directly protects muscle mitochondria and muscle cells from oxidative damage (33). However, the protective effect of NBP on mitochondrial function is not only limited to neurodegeneration, but also appears in cardiovascular diseases. A study suggested that NBP exerts a cardioprotective effect on cardiac ischemic injury via the regulation of mitochondrial function both using in vivo and in vitro experiments (34). In summary, the antioxidant effect of NBP has been widely recognized.

4. NBP regulates apoptosis and autophagy

Apoptosis and autophagy are basic biological phenomena of cells, which serve essential roles in removing abnormal cells in multicellular organisms. Disorders in the apoptosis and autophagy processes may cause the occurrence of neuropathy (35). The neuroprotective effect of NBP via the regulation...
Table I. Neuroprotective mechanisms of 3-n-butylphthalide.

| Author, year | Study subject | Method | Molecular mechanism | Refs. |
|--------------|---------------|--------|---------------------|-------|
| A, Inflammation inhibition |
| Wang et al, 2018 | APP/PS1 mice A172, SH-SY5Y | Transgenic LPS induced | NLRP3 inflammasome activation inhibition | (13) |
| Yang et al, 2018 | SD rats | LPS induced | NF-κB pathway inhibition | (14) |
| Zhao et al, 2016 | C57BL/6 mice | LPS induced | Downregulation of JNK activation | (15) |
| Zhao et al, 2017 | C57BL/6 mice | Traumatic brain injury | NF-κB pathway inhibition | (17) |
| Wang et al, 2018 | EAE | Neuroantigen-specific proinflammatory T cells induced | Suppression of PGAM5 | (19) |
| Yang et al, 2018 | SD rats LPS induced | | Increased HGF expression | (21) |
| Zhao et al, 2016 | C57BL/6 mice | Traumatic brain injury | NF‑κB pathway inhibition | (17) |
| Wang et al, 2018 | EAE Neuroantigen‑specific | Suppression of PGAM5 | | (19) |
| Zhang et al, 2016 | SD rats | Cerebral ischemia reperfusion induced | Enhanced Nrf2 expression | (28) |
| Liu et al, 2017 | HUVECs | Advanced glycation end product induced | RAGE/NF-κB pathway inhibition | (22) |
| B, Reduction of mitochondrial oxidative stress |
| Yang et al, 2017 | SH-SY5Y Missense mutations | | Increased Nrf2 expression | (28) |
| Liu et al, 2017 | ICR mice | Traumatic brain injury | Nrf2-ARE pathway activation | (29) |
| Li et al, 2015 | SD rats | Carbon monoxide poisoned | Keap1/Nrf2 pathway activation | (30) |
| Zhu et al, 2018 | ICR mice | Cerebral ischemia reperfusion injury | ERK signaling inhibition | (31) |
| Wang et al, 2018 | NSCs from SD rats | Hydrogen peroxide induced | PI3K/Akt and Mash1 pathway activation | (32) |
| Chen et al, 2017 | Guinea pigs | Experimental autoimmune myositis | Enhanced Na⁺-K⁺ and Ca²⁺-Mg²⁺ ATPase activities | (33) |
| Tian et al, 2017 | H9C2 | Hydrogen peroxide induced | Enhanced Nrf-1 and TFAM expression | (34) |
| C, Regulation of apoptosis and autophagy |
| Zhao et al, 2017 | C57BL/6 mice | Traumatic brain injury | Downregulated caspase-3 and -9 expression | (17) |
| Liu et al, 2017 | HUVECs | Advanced glycation end product induced | Regulation of Bcl-2 expression | (22) |
| Lei et al, 2014 | SH-SY5Y | β-amyloid induced | Regulation of Bcl-2, caspase-3 and -9 expression | (37) |
| Xu et al, 2017 | C57BL/6 mice | Repeated cerebral ischemia reperfusion | Bel-2/Bax elevation | (38) |
| Xiang et al, 2014 | APP/PS1 mice | Transgenic | BDNF/TrkB/PI3K/Akt pathway regulation | (39) |
| D, Resistance to endoplasmic reticulum stress |
| Liao et al, 2018 | SD rats | Doxorubicin induced | GRP78, CHOP and caspase-12 expression regulation | (41) |
| Niu et al, 2018 | SD rats | Bilateral surgical ligation of common carotid arteries | GRP78, CHOP and caspase-12 expression regulation | (42) |
| Zheng et al, 2017 | SD rats | Laminecetomy performed at T9 | ATF-4, ATF-6, XBP-1, PDI, GRP78, CHOP and cleaved-caspase 12 attenuation | (43) |
| HBMECs | Thapsigargin induced | | | |
of apoptosis and autophagy has been demonstrated. Treatment with NBP has been reported to reduce apoptotic cell death by increasing the levels of cleaved caspase-3 and caspase-9 following TBI (17). Furthermore, NBP blocks neural apoptosis in areas surrounding cortical contusions on the brain that are induced by TBI (29). The neuroprotective mechanism of NBP involves the mitochondrial apoptotic pathway. NBP inhibits HSPB8 K141N mutation-induced neurotoxicity, attenuates β-amyloid-induced toxicity in SH-SY5Y cells, and protects rat cardiomyocytes from ischemia or reperfusion through regulating mitochondrial-mediated apoptosis (28,36,37). Furthermore, certain studies have demonstrated the inhibition of apoptosis by NBP via the Akt pathway. One study reported that NBP activates Akt/mTOR signaling to inhibit neuronal apoptosis and autophagy in mice with repeated cerebral ischemia reperfusion injury (38). Another study demonstrated that NBP improves cognitive impairment of APP/PS1 mice by inhibiting apoptosis via the PI3K/AKT pathway (39). Additionally, NBP reduces the number of apoptotic cells by regulating Bcl-2 in HUVECs and an EAM model (22,33).

5. NBP resists endoplasmic reticulum stress

ERS is characterized by incorrect folding and aggregation of unfolded proteins in the endoplasmic reticulum lumen and a disturbance of the calcium balance, which can activate the unfolded protein response and lead to disturbance of the cell function and cell death (40). In recent years, certain studies have reported an anti-ERS effect of NBP. One study demonstrated that NBP inhibits doxorubicin-induced ERS in SD rats (41). In addition, NBP alleviates vascular cognitive impairment by regulating ERS and the Sonic hedgehog/Patched homolog 1 signaling pathway in SD rats (42). Both of these studies agreed that NBP attenuates ERS through regulating the expression of 78-kDa glucose-regulated protein (GRP78), CCAAT-enhancer binding protein homologous protein (CHOP) and caspase-12. Furthermore, NBP also inhibits ERS by attenuating activation transcription factor (ATF)-4, ATF-6, X-box-binding protein 1 (PDI), protein disulfide isomerase; APP, amyloid precursor protein; ATF, activating transcription factory; CHOP, CCAAT-enhancer binding protein homologous protein; MPP+, 1-methyl-4-phenylpyridinium.

Table I. Continued.

D. Resistance to endoplasmic reticulum stress

| Author, year | Study subject | Method | Molecular mechanism | Refs. |
|--------------|--------------|--------|---------------------|-------|
| He et al, 2017 | SD rats | Laminectomy performed at T9 | ATF-4, ATF-6, XBP-1, PDI, GRP78, CHOP and cleaved-caspase 12 attenuation | (44) |
| | PC12 | Thapsigargin induced | ATF-4, ATF-6, XBP-1, PDI, GRP78, CHOP and cleaved-caspase 12 attenuation | |

E. Reduced abnormal protein deposition

| Author, year | Study subject | Method | Molecular mechanism | Refs. |
|--------------|--------------|--------|---------------------|-------|
| Peng et al, 2010 | 3xTg-AD mice | Transgenic | Direction of APP processing towards a non-amyloidogenic pathway | (47) |
| Peng et al, 2012 | AβPP/PS1 mice | Transgenic | Tau hyperphosphorylation inhibition | (48) |
| Chen et al, 2018 | C57BL/6 mice | LPS induced | Reduction of α-synuclein deposition | (16) |
| Huang et al, 2010 | PC12 | MPP+ toxicity induced | Reduction of α-synuclein deposition | (49) |

LPS, lipopolysaccharide; SD, Sprague Dawley; JNK, c-Jun N-terminal kinase; HGF, hepatocyte growth factor; PGAM5, PGAM family member 5; RAGE, receptor for advanced glycation end-product; Nrf, nuclear respiratory factor; ARE, antioxidant response element; Keap1, Kelch-like ECH-Associating protein 1; Mash1, mammalian achaete scutep homolog-1; TFAM, human mitochondrial transcription factor A; ICR, Institute of Cancer Research; NSC, neural stem cell; BDNF, brain derived neurotrophic factor; TrkB, Tyrosine receptor kinase B; GRP78, 78-kDa glucose-regulated protein; XBP-1, X-box-binding protein 1; PDI, protein disulfide isomerase; APP, amyloid precursor protein; ATF, activating transcription factory; CHOP, CCAAT-enhancer binding protein homologous protein; MPP+, 1-methyl-4-phenylpyridinium.
umion-induced cellular model and a LPS-induced mice model of PD via reducing the accumulation of α-synuclein (16,49). However, the molecular mechanisms of how NBP reduces the accumulation of α-synuclein and inhibits tau hyperphosphorylation remain unclear. Furthermore, to the best of our knowledge, there is no associated study that provides the clinical evidence that NBP is effective in multiple sclerosis or Lewy body dementia via attenuating abnormal protein deposition. Potentially, new findings can be revealed in additional neurodegenerative diseases.

7. Conclusion

In summary, current studies suggest that NBP serves a neuroprotective role through inhibiting inflammation, protecting mitochondrial function, alleviating oxidative stress, regulating apoptosis, resisting ERS and decreasing the abnormal protein deposition (Fig. 2). Details on specific molecular mechanisms are presented in Table 1. Taken together, it is suggested that NBP provides a promising therapeutic strategy for neurodegenerative diseases. In further studies, the mechanism of action of NBP may be further clarified, and the understanding regarding its potential uses may be expanded.

Acknowledgements

Not applicable.

Funding

This study was supported by grants from the Natural Science Foundation of China (grant no. 81371442), the Training program for outstanding young teachers in higher education institutions of Guangdong Province (grant no. YQ2015024) and the Fundamental Research Funds for the Central Universities (grant no. 21617482).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

RL was a major contributor in writing the manuscript. RL, RW, LZ and WB contributed to researching data, discussing content and editing the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Xu QZ, Zhou Y, Shao BZ, Zhang JJ and Liu C: A Systematic Review of Neuroprotective Efficacy and Safety of DL-3-N-Butylphthalide in Ischemic Stroke. Am J Chin Med 47: 507-525, 2019.
2. Chinese Society of Cerebral Blood Flow and Metabolism: The Chinese guidelines for the evaluation and management of cerebral collateral circulation in ischemic stroke (2017). Zhonghua Nei Ke Za Zhi 56: 460-471, 2017 (In Chinese).
3. Banks JL and Marotta CA: Outcomes validity and reliability of the modified Rankin scale: implications for stroke clinical trials: a literature review and synthesis. Stroke 38: 1091-1096, 2007.
4. Heldner MR, Zuberl C, Mattie HP, Schroth G, Weck A, Mono ML, Gralla J, Jung S, El-Koussy M, Lüdi R, et al: National Institutes of Health stroke scale score and vessel occlusion in 2152 patients with acute ischemic stroke. Stroke 44: 1153-1157, 2013.
5. Cui LX, Zhu YC, Gao S, Wang JM, Peng B, Ni J, Zhou LX, He J and Ma XQ: Ninety-day administration of dl-3-n-butylphthalide for acute ischemic stroke: A randomized, double-blind trial. Chin Med J (Engl) 126: 3405-3410, 2013.
6. Zhao H, Yun W, Zhang Q, Cai X, Li X, Hui G, Zhou X and Ni J: Mobilization of Circulating Endothelial Progenitor Cells by dl-3-n-Butylphthalide in Acute Ischemic Stroke Patients. J Stroke Cerebrovasc Dis 25: 752-760, 2016.
7. Zhang C, Zhao S, Zang Y, Gu F, Mao S, Feng S, Hu L and Zhang C: The efficacy and safety of DI-3-n-butylphthalide on progressive cerebral infarction: A randomized controlled STROBE study. Medicine (Baltimore) 96: e7257, 2017.
8. Huang L, Wang S, Ma F, Zhang Y, Peng Y, Xing C, Feng Y, Wang X and Peng Y: From stroke to neurodegenerative diseases: The multi-target neuroprotective effects of 3-n-butylphthalide and its derivatives. Pharmacol Res 135: 201-211, 2018.
9. Skaper SD, Facelli L, Zuzso M and Giusti P: An Inflammation-Centric View of Neurological Disease: Beyond the Neuron. Front Cell Neurosci 12: 72, 2018.
10. Liu J and Wang F: Role of Neuroinflammation in Amyotrophic Lateral Sclerosis: Cellular Mechanisms and Therapeutic Implications. Front Immunol 8: 1005, 2017.
11. Niu F, Sharma A, Feng L, Ozkizilcik A, Muresanu DF, Luaufente JV, Tian ZR, Nozari A and Sharma HS: Nanowired delivery of DL-3-n-butylphthalide induces superior neuroprotection in concussive head injury. Prog Brain Res 245: 89-118, 2019.
12. Zhang Y, Huang LJ, Shi S, Xu SF, Wang XL and Peng Y: L-3-n-butyphthalide Rescues Hippocampal Synaptic Failure and Attenuates Neuropathology in Aged APP/PS1 Mouse Model of Alzheimer's Disease. CNS Neurosci Ther 22: 979-987, 2016.
13. Wang CY, Xu Y, Wang X, Guo C, Wang T and WangZY: DL-3-n-Butylphthalide Inhibits NLRP3 Inflammasome and Mitigates Alzheimer’s-Like Pathology via Nrf2-TXNIP-TrX Axis. Antioxid Redox Signal 30: 1411-1431, 2018.
14. Yang M, Dang R, Xu P, Guo Y, Han W, Liao D and Jiang P: DL-3-n-Butylphthalide improves lipopolysaccharide-induced depressive-like behavior in rats: Involvement of Nrf2 and NF-κB. Neurosci Lett 681: 1-6, 2018.
15. Zhao CY, Lei H, Zhang Y, Li L, Xu SF, Cai J, Li PP, Wang L, Wang XL and Peng Y: L-3-n-Butylphthalide attenuates neuro-inflammatory responses by downregulating JNK activation and upregulating Heme oxygenase-1 in lipopolysaccharide-treated mice. J Asian Nat Prod Res 18: 289-302, 2016.
16. Chen Y, Jiang M, Li L, Ye M, Yu M, Zhang L, Ge B, Xu W and Wei D: DL-3-n-butylphthalide reduces microglial activation in lipopolysaccharide-induced Parkinson’s disease model mice. Mol Med Rep 17: 3884-3890, 2018.
17. Zhao Y, Lee JH, Chen D, Gu X, Caslin A, Li J, Yu SP and Wei D: DL-3-n-butylphthalide induced neuroprotection, regenerative repair, functional recovery and psychological benefits following traumatic brain injury in mice. Neurochem Int 111: 82-92, 2017.
18. Zhu J, Zhang Y and Yang C: Protective effect of 3-n-butylphthalide against hypertensive nephropathy in spontaneously hypertensive rats. Mol Med Rep 11: 1448-1454, 2015.
19. Wang Y, Bi Y, Xia Z, Shi W, Li B, Li B, Chen L and Guo L: Butylphthalide ameliorates experimental autoimmune encephalomyelitis by suppressing PGAM5-induced necroptosis and inflammation in microglia. Biochem Biophys Res Commun 497: 80-86, 2018.
20. Chen J, Wang J, Zhang J and Pu C: Effect of butylphthalide intervention on experimental autoimmune myositis in guinea pigs. Exp Ther Med 15: 152-158, 2018.
21. Zhang P, Guo ZF, Xu YM, Li YS and Song JG: N-Butylphthalide (NBP) ameliorated cerebral ischemia reperfusion-induced brain injury via HGF-regulated TLR4/ NF-κB signaling pathway. Biomed Pharmacother 83: 658-666, 2016.

22. Liu CY, Zhao ZH, Chen ZT, Che CH, Zou ZY, Wu XM, Chen SG, Li YX, Lin HB, Wei XF, et al: DL-3-n-butylphthalide protects endothelial cells against advanced glycation end product-induced injury by attenuating oxidative stress and inflammation responses. Exp Ther Med 14: 2241-2248, 2017.

23. Schapira AHV: Mitochondrial diseases. Lancet 379: 1825-1834, 2012.

24. Arun S, Liu L and Donmez G: Mitochondrial Biology and Neurological Diseases. Curr Neuropharmacol 14: 143-154, 2016.

25. Hybertson BM, Gao B, Bose SK and McCord JM: Oxidative stress in health and disease: The therapeutic potential of Nrf2 activation. Mol Aspects Med 32: 234-246, 2011.

26. Tan BL, Norhaizan ME, Liew WP and Sulaiman Rahman H: Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front Pharmacol 9: 1162, 2018.

27. Abdoulaye IA and Guo YJ: A Review of Recent Advances in Neuroprotective Potential of 3-N-Butylphthalide and Its Derivatives. BioMed Res Int 2016: 5012341, 2016.

28. Yang XD, Cen ZD, Cheng HP, Shi K, Bai J, Xie F, Wu HW, Li BB and Luo W: L-3-n-Butylphthalide Protects HSPB8 K141N Mutation-Induced Oxidative Stress by Modulating the Mitochondrial Apoptotic and Nrf2 Pathways. Front Neurosci 11: 402, 2017.

29. Liu Z, Wang H, Shi X, Li L, Zhou M, Ding H, Yang Y, Li X and Ding K: DL-3-n-Butylphthalide (NBP) Provides Neuroprotection in the Mice Models After Traumatic Brain Injury via Nrf2-ARE Signaling Pathway. Neurochem Res 42: 1375-1386, 2017.

30. Li Q, Cheng Y, Bi M, Lin H, Chen Y, Zou Y, Liu Y, Kang H and Guo Y: Effects of N-butylphthalide on the activation of Keap1/Nrf-2 signaling pathway in rats after carbon monoxide poisoning. Environ Toxicol Pharmacol 40: 22-29, 2015.

31. Zhu BL, Xie CL, Hu NN, Zhu XB and Liu CF: Inhibiting of GRASPD65 Phosphorylation by DL-3-N-Butylphthalide Protects against Cerebral Ischemia-Reperfusion Injury via ERK Signaling. Behav Neurol 2018: 5701719, 2018.

32. Wang S, Huang L, Zhang Y, Peng Y, Wang X and Peng Y: Protective Effects of L-3-n-Butylphthalide Against H2O2-Induced Injury in Neural Stem Cells by Activation of PI3K/Akt and Mst1 Pathway. Neuroscience 393: 164-174, 2018.

33. Chen J, Wang J, Zhang J and Pu C: 3-n-Butylphthalide reduces the oxidative damage of muscles in an experimental autoimmune myositis animal model. Exp Ther Med 14: 2085-2093, 2017.

34. Tian X, He W, Yang R and Liu Y: DL-3-n-Butylphthalide protects the heart against ischemic injury and H9c2 cardiomyoblasts against oxidative stress: Involvement of mitochondrial function and biogenesis. J Biomed Sci 24: 38, 2017.

35. Booth LA, Tavaill S, Hamed HA, Cruickshanks N and Dent P: The role of cell signalling in the crosstalk between autophagy and apoptosis. Cell Signal 26: 549-555, 2014.

36. Wang YG, Li Y, Wang CY, Ai JW, Dong XY, Huang HY, Feng ZY, Pan YM, Lin Y, Wang BX, et al: L-3-n-Butylphthalide protects rats’ cardiomyocytes from ischemia/reperfusion-induced apoptosis by affecting the mitochondrial apoptosis pathway. Acta Physiol (Oxf) 210: 524-533, 2014.

37. Lei H, Zhao CY, Liu DM, Zhang Y, Li L, Wang XL and Peng Y: L-3-n-Butylphthalide attenuates β-amyloid-induced toxicity in neuroblastoma SH-SY5Y cells through regulating mitochondrial-mediated apoptosis and MAPK signaling. J Asian Nat Prod Res 16: 854-864, 2014.

38. Xu J, Huai Y, Meng N, Dong Y, Liu Z, Qi Q, Hu M, Fan M, Jin W and Lv P: L-3-n-Butylphthalide Activates Akt/mTOR Signaling. Inhibits Neuronal Apoptosis and Autophagy and Improves Cognitive Impairment in Mice with Repeated Cerebral Ischemia-Reperfusion Injury. Neurochem Res 42: 2968-2981, 2017.

39. Xiang J, Pan J, Chen F, Zheng L, Chen Y, Zhang S and Feng W: L-3-n-butylphthalide improves cognitive impairment of APP/PS1 mice by BDNF/TrkB/PI3K/AKT pathway. Int J Clin Exp Med 7: 1706-1713, 2014.

40. Iurlaro R and Muñoz-Pinedo C: Cell death induced by endoplasmic reticulum stress. FEBS J 283: 2640-2652, 2016.

41. Liao D, Xiang D, Dang R, Xu P, Wang J, Han W, Fu Y, Yao D, Cao L and Jiang P: Neuroprotective Effects of dl-3-n-Butylphthalide against Doxorubicin-Induced Neuroinflammation, Oxidative Stress, Endoplasmic Reticulum Stress, and Behavioral Changes. Oxid Med Cell Longev 2018: 9125601, 2018.

42. Niu XL, Jiang X, Xu GD, Zheng GM, Tang ZP, Yin N, Li XQ, Yang YY and Lv PY: DL-3-n-butylphthalide alleviates vascular cognitive impairment by regulating endoplasmic reticulum stress and the Shh/Pcch1 signaling-pathway in rats. J Cell Physiol 234: 12604-12614, 2018.

43. Zheng B, Zhou Y, Zhang H, Yang G, Hong Z, Han D, Wang Q, He Z, Liu Y, Wu F, et al: DL-3-n-butylphthalide prevents the disruption of blood-splinal cord barrier via inhibiting endoplasmic reticulum stress following spinal cord injury. Int J Biol Sci 13: 1520-1531, 2017.

44. He Z, Zhou Y, Huang Y, Wang Q, Zheng B, Zhang H, Li J, Liu Y, Wu F, Zhang X, et al: DL-3-n-butylphthalide improves functional recovery in rats with spinal cord injury by inhibiting endoplasmic reticulum stress-induced apoptosis. Am J Transl Res 9: 1075-1087, 2017.

45. Nonaka T, Masuda-Suzukake M and Hasegawa M: Molecular mechanisms of the co-deposition of multiple pathological proteins in neurodegenerative diseases. Neuropathology 38: 64-71, 2018.

46. Goedert M: NEURODEGENERATION. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Aβ, tau, and α-synuclein. Science 349: 1255555, 2015.

47. Peng Y, Sun J, Hon S, Nylander AN, Xia W, Feng Y, Wang X and Lemere CA: L-3-n-butylphthalide improves cognitive impairment and reduces amyloid-beta in a transgenic model of Alzheimer's disease. J Neurosci 30: 8180-8189, 2010.

48. Peng Y, Hu Y, Xu S, Li P, Li J, Lu L, Yang H, Feng N, Wang L and Wang X: L-3-n-butylphthalide reduces tau phosphorylation and improves cognitive deficits in AβPP/PS1-Alzheimer's transgenic mice. J Alzheimers Dis 29: 379-391, 2012.

49. Huang JZ, Chen YZ, Su M, Zheng HF, Yang YP, Chen J and Lemere CA: L-3-n-butylphthalide protects rats' hippocampal neurons against 6-hydroxydopamine toxicity. J Alzheimers Dis 17: 1706-1713, 2007.