Boswellia serrata has promising impact on glutamate and quinolinic acid-induced toxicity on oligodendroglia cells: In vitro study

Article *in* Acta Poloniae Pharmaceutica · January 2017

CITATIONS
7

READS
316

4 authors, including:

Vafa Baradaran Rahimi
Mashhad University of Medical Sciences
31 PUBLICATIONS **270** CITATIONS
SEE PROFILE

Vahid Reza Askari
Mashhad University of Medical Sciences
53 PUBLICATIONS **482** CITATIONS
SEE PROFILE

Acta Poloniae Pharmaceutica – Drug Research, Vol. 74 No. 6 pp. 1803–1811, 2017 ISSN 0001-6837
Polish Pharmaceutical Society

BOSWELLIA SERRATA HAS PROMISING IMPACT ON GLUTAMATE AND QUINOLINIC ACID-INDUCED TOXICITY ON OLIGODENDROGLIA CELLS: *IN VITRO* STUDY

VAFA BARADARAN RAHIMI[1], VAHID REZA ASKARI[1], AKRAM MEHRDAD[2] and HAMID R. SADEGHNIA[1-3]*

[1]Student Research Committee, Department of Pharmacology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Neurocognitive Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Pharmacological Research Center of Medicinal Plants, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract: Excessive release of glutamate and quinolinic acid (QA) can lead to neuroglial inflammation and death, and may cause many acute and chronic neurological disorders including multiple sclerosis (MS). *Boswellia serrata* (BS) has been reported to exert anti-oxidant and anti-inflammatory activities. In this study, we investigated the oligoprotective effect of BS on oxidative OLN-93 cells injury induced by glutamate and QA. The protective effect of different increasing concentrations of BS was evaluated using cell viability, malondialdehyde (MDA) as lipid peroxidation index, and the assessment of intracellular reactive oxygen species (ROS) in OLN-93 cells. Different concentrations of BS (0-40 µg/mL) had no significant effect on OLN-93 cell viability. The increasing concentrations of glutamate and QA (more than 4 mM) led to cell death in a concentration dependent manner ($p < 0.01$) and significantly increased ROS and MDA levels ($p < 0.001$ for both). Different concentrations of BS (10-40 µg/ml) significantly improved cell viability and mitigated MDA and ROS generation following glutamate and QA oligotoxicity ($p < 0.05$ and $p < 0.001$, respectively). Our results provide the first evidence of oligoprotective effects of BS against glutamate and QA-induced toxicity in OLN-93 cells through diminishing oxidative stress, and can be subjected to further studies in preclinical models of neuroinflammatory diseases, including MS.

Keywords: glutamate, quinolinic acid, oligotoxicity, *Boswellia serrata*, oxidative stress, OLN-93 cells

Multiple sclerosis (MS) is a chronic inflammatory and potentially disabling disease of central nervous system (CNS) that caused by progressive damage to myelin sheaths and/or oligodendrocytes (1). Oligodendrocytes are considered myelin forming cells of the CNS and need a lot of energy for lipid biosynthesis. Therefore, they generate large amounts of reactive oxygen species (ROS) that can cause oxidative damage. Oxidative damage has an important role in demyelization disorders including MS (2).

Glutamate is characterized as one of the main excitatory neurotransmitters in the mammalian CNS (3) that has an important role in neural transmission, differentiation, development and plasticity (4). Although it's important role, uncontrolled release and high concentrations of this excitatory amino acid can lead to neuronal and glial cells death and can cause many neurological disorders including ischemia, Alzheimer's disease, and MS (5). Glutamate toxicity occurs through two main mechanisms including glutamate receptor-induced cytotoxicity and ROS-mediated oxidative damage (6).

Quinolinic acid (QA, 2, 3-pyridine dicarboxylic acid), an endogenous NMDA glutamatergic receptor agonist (7), is a toxic metabolite of tryptophan produced *via* kynurenine pathway. It has been demonstrated that QA has an important role in multi-etiological disorders, such as neuroinflammatory and neurodegenerative disorders including MS (8).

Boswellia serrata (BS) is a moderate to large branching tree belonging to Burseraceae family and

* Corresponding author: e-mail: sadeghniahr@mums.ac.ir; phone: +98 513 882 8566; fax: +98 513 882 8567

is found in India, Northern Africa and the Middle East (9). The phytochemical content of *Boswellia serrata* oleo-gum resin consists of monoterpenes (α-thujone), diterpenes (such as incensole and its derivatives), triterpenes (such as α- and β-amyrins), pentacyclic triterpenic acids (boswellic acids), and tetracyclic triterpenic acids (tirucall-8,24-dien-21-oic acids). The 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-β-boswellic acid (AKBA) have been considered as the main active constituents (10). In previous studies, many pharmacological properties of BS oleo-gum resin has been mentioned including anti-inflammatory, analgesic (6), anti-viral (11), anti-neoplastic (12), anti-oxidant, memory improvement (13) and anti-microbial activities (14). Also, BS has promising effects in some chronic inflammatory diseases including rheumatoid arthritis (15), bronchial asthma (16), osteoarthritis (17), ulcerative colitis and Crohn's disease (18).

In the present study, the oligoprotective effect of BS was evaluated on oxidative OLN-93 (oligodendroglia) cells injury induced by glutamate and QA.

Figure 1. *Boswellia serrata* oleo gum resin

EXPERIMENTAL

Preparation of the ethanolic extract of BS oleo-gum resin

The oleo-gum resin of BS (Fig. 1) was provided from Medicinal Plants Division of Imam Reza Pharmacy (herbarium No. 92083). The plant material was washed, dried, and ground to fine powder with a blender. Then, the powder (10 g) was soaked in 100 mL of 56% ethanol, for 48 h at 40°C under gentle shaking. The resulting BS was filtered and the solvent was removed using rotary evaporator. The residue (yield 30% w/w) was kept at -20°C until use.

Cell culture

The OLN-93 cell line was purchased from Pasture Institute (Tehran, Iran) and cultured in Dulbecco's modified Eagle's medium (DMEM)

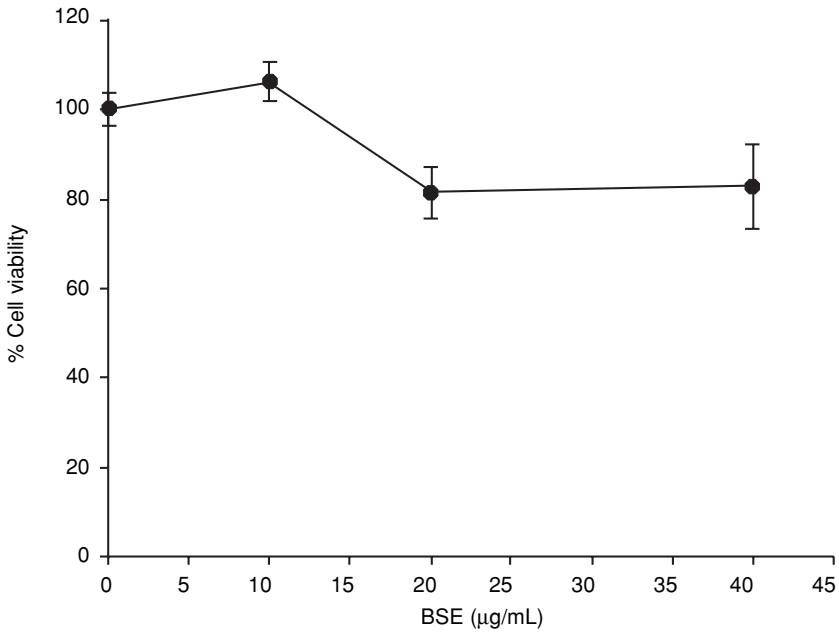

Figure 2. The effect of BS on viability of OLN-93 cells after 24 h incubation. Data were presented as the mean ± SEM (n = 9)

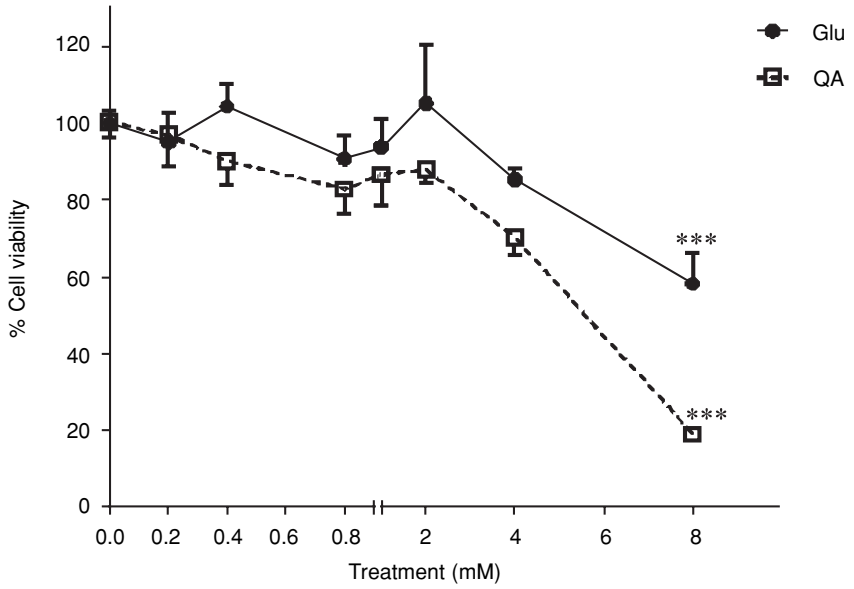

Figure 3. The effect of glutamate and quinolinic acid (QA) on viability of OLN-93 cells after 24 h incubation. Data were presented as the mean ± SEM (n = 9). $^{***}p < 0.001$ as compared to the corresponding control values (concentration 0)

enriched with 10% fetal calf serum (FCS) and 100 units/mL of penicillin/streptomycin. The cells were incubated in humidified atmosphere at 5% CO_2 and 37^{o}C.

Cell viability assay

Cell viability was detected using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. Briefly, 10^4 cells were seeded in 96-well plate for 24 h. Different increasing concentrations of BS (10-40 µg/mL) were then added to OLN-93 cells and after 24 h, the cell survival rates were measured. In another sets of experiments, the OLN-93 cells were first pretreated with BS (10-40 µg/mL) for 24 h and then exposed to glutamate (8 mM) or QA (8 mM) for another 24 h, in which the same treatments were applied. After that, 10 µL of MTT solution (5 mg/mL in final volume) was added to the cells and the absorbance of each well was read using StatFAX 2100 ELISA plate reader (Awareness Inc, USA) at 570 nm in referencing 620 nm. The assay was performed in triplicate and the cell viability level was expressed as percent of survival cells (19).

Lipid peroxidation assay

Malonedialdehyde (MDA) was measured as lipid peroxidation marker using thiobarbituric acid (TBA) method. Briefly, 10^6 OLN-93 cells were seeded in 12 well plates for 24 h. Next, different concentrations of BS (10-40 µg/mL) were added to OLN-93 cells for 24 h. Then, the cells were exposed to glutamate (8 mM) and QA (8 mM). After 24 h, the cells were scraped in two volumes of TBA reagent (TBA/TCA/HCl) and boiled for 20 min at 100^{o}C. After cooling, the cells were centrifuged at 3000 rpm for 5 min and the absorbance of each supernatant was measured using fluorescent microplate reader at excitation wavelength of 530 nm and emission wavelength of 550 nm. The assay was performed in triplicate (19). Protein content was determined using bicinchoninic acid (BCA) protein assay kit (Sigma-Aldrich, St. Louis, MO).

Assessment of intracellular reactive oxygen species (ROS)

Intracellular ROS was determined by spectroflurimetry using 2',7'-dichlorofluorescin diacetate (DCFH-DA). 10^4 OLN-93 cells were cultured in 96 well plates for 24 h. Next, different concentrations of BS (10-40 µg/mL) were added to OLN-93 cells. After 24 h, the cells were exposed to glutamate (8 mM) and QA (8 mM) for 24 h. After that, DCFH-DA (20 µM) was added to cells and incubated for 30 min. Then, the florescence intensity of each well was read using fluorescent microplate reader at excitation wavelength 485 nm and emission wavelength 520 nm. The assay was performed in triplicate (19).

Statistical analysis

Data analysis was done using one-way analysis of variance (ANOVA) with Tukey-Kramer's *post hoc* test by GraphPad Prism 6.01 (La Jolla, CA) software. $p < 0.05$ was considered significant.

RESULTS

BS exhibits no toxic potential towards OLN-93 cells

Different concentrations of BS (0-40 µg/mL) caused no significant alterations on OLN-93 cells

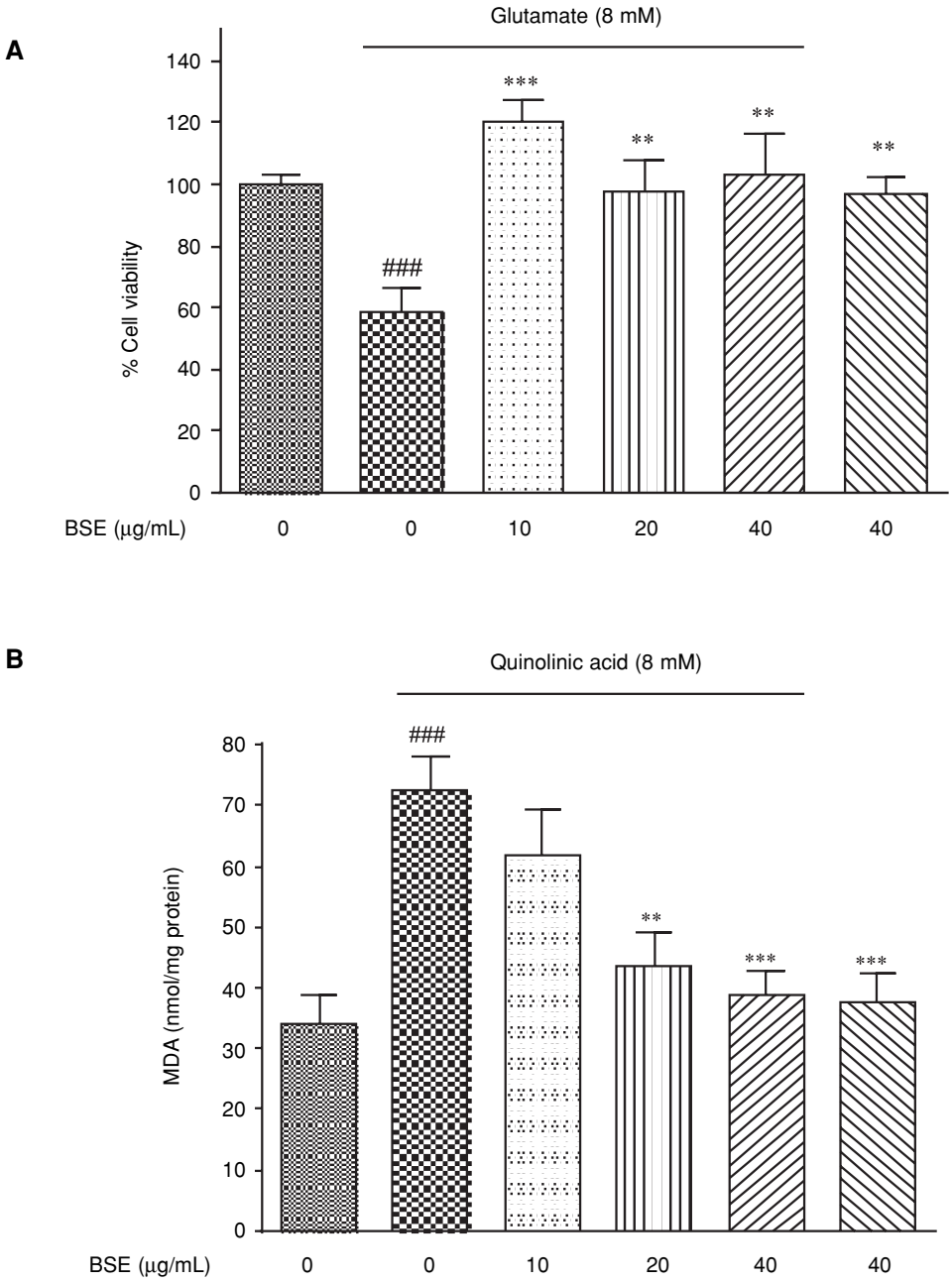

Figure 4. **(A).** The effect of BS on viability of OLN-93 cells following glutamate-induced toxicity. Data were presented as the mean ± SEM (n = 9). $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to glutamate group. $^{###}p < 0.001$ compared to control value. **(B).** The effect of BS on viability of OLN-93 cell following quinolinic acid (QA)-induced toxicity. Data were presented as the mean ± SEM (n = 9). $^{***}p < 0.001$ as compared to QA group. $^{###}p < 0.001$ as compared to control value

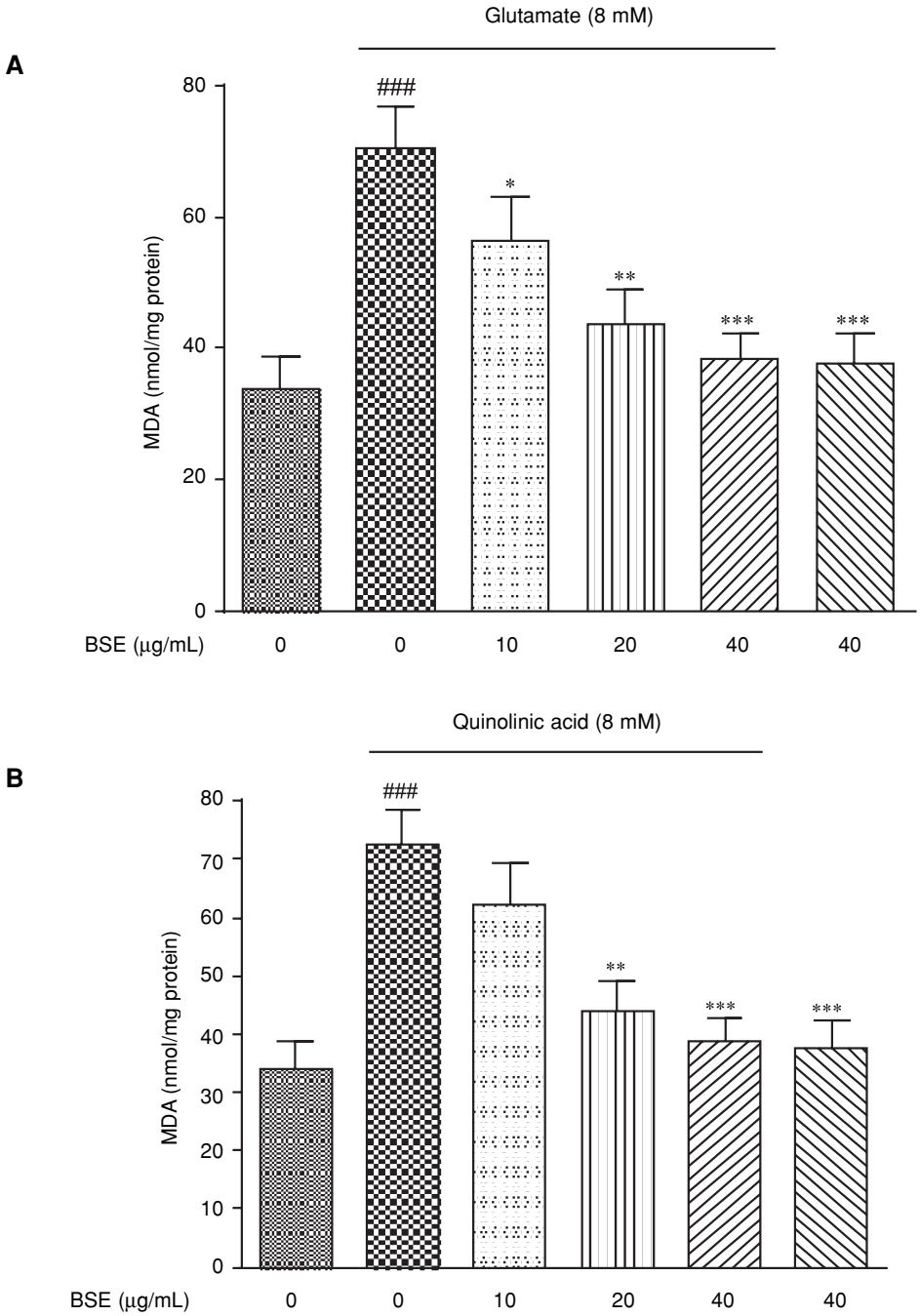

Figure 5. **(A)**. The effect of BS on lipid peroxidation level following glutamate-induced OLN-93 cells toxicity. Data were presented as the mean ± SEM (n = 9). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to glutamate group. $^{###}p < 0.001$ as compared to control value. **(B).** The effect of BS on lipid peroxidation levels following quinolinic acid (QA)-induced OLN-93 cells toxicity. Data were presented as the mean ± SEM (n = 9). $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to QA group. $^{###}p < 0.001$ compared to control value

viability (Fig. 2). As demonstrated in Fig. 3A, the concentrations more than 4 mM of glutamate induced significant toxicity in OLN-93 cells ($p < 0.001$). QA at concentrations of 4 and 8 mM had significant toxicity for OLN-93 cells in a concentration-dependent manner and maximum toxicity was observed at 8 mM ($p < 0.01$ and $p < 0.001$, respectively; Fig. 3B).

BS significantly improved the viability of glutamate and QA injured cells

As illustrated in Figure 4A, glutamate (8 mM) significantly decreased the viability of OLN-93 cell to approximately 60% of control ($p < 0.001$). In comparison with glutamate group, BS significantly improved the viability of glutamate injured cells with approximately 50% (at 10 µg/mL, $p < 0.001$) and 40% (for 20 and 40 µg/mL, $p < 0.01$) in comparison to glutamate group.

As described in Figure 4B, QA (8 mM) significantly reduced the viability of OLN-93 cells to approximately 20% of control group ($p < 0.001$). In contrast, BS enhanced the viability of QA injured cells with approximately 80% (at 10 µg/mL, $p < 0.001$), and 75% (for 20 and 40 µg/mL, $p < 0.05$, Fig. 4B).

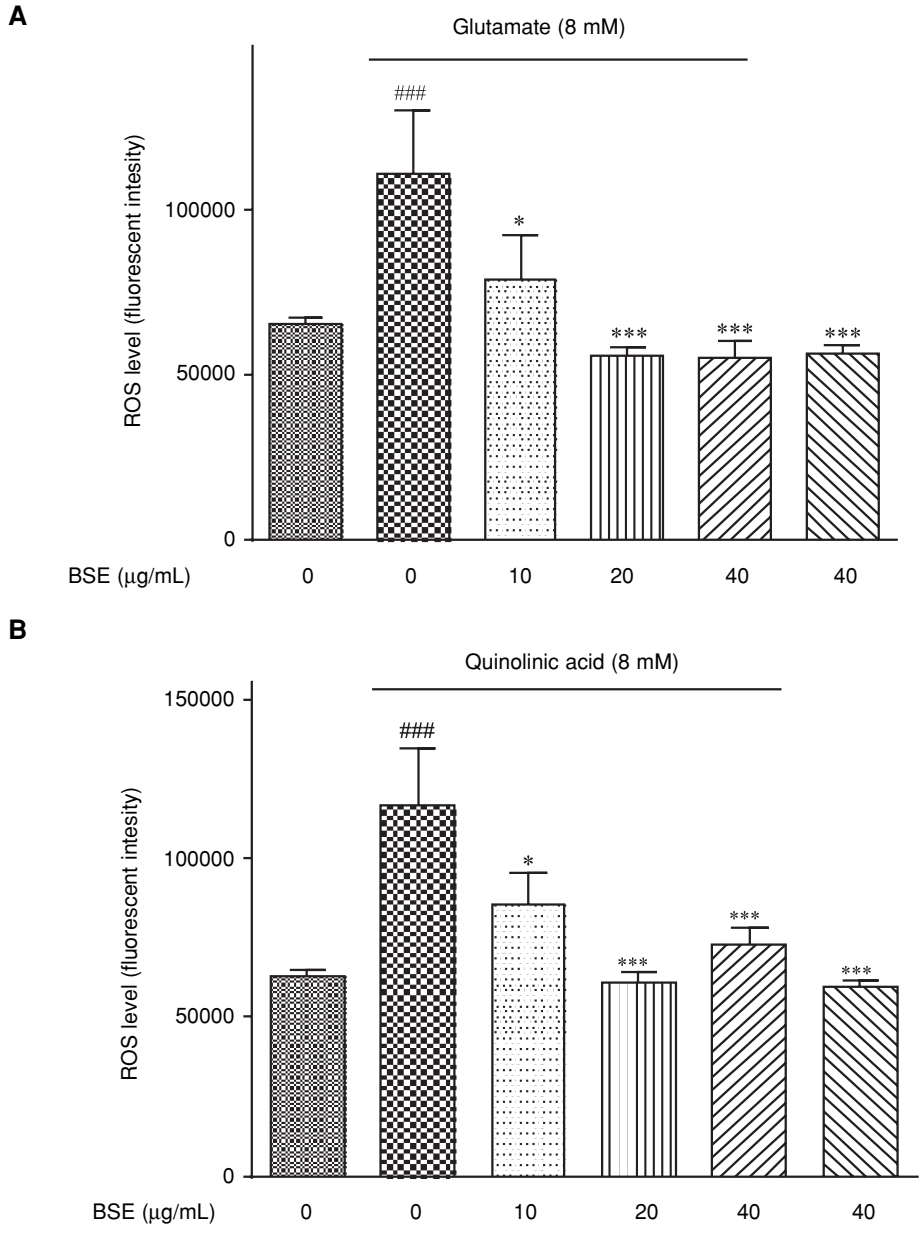

Figure 6. **(A).** The effect of BS on intracellular reactive oxygen species (ROS) following glutamate-induced OLN-93 cells toxicity. Data were presented as the mean ± SEM (n = 9). $^{*}p < 0.05$, $^{***}p < 0.001$ as compared to glutamate group. $^{###}p < 0.001$ as compared to control group. **(B).** The effect of BS on intracellular reactive oxygen species (ROS) following quinolinic acid (QA)-induced OLN-93 toxicity. Data were presented as the mean ± SEM (n = 9). $^{*}p < 0.05$, $^{***}p < 0.001$ as compared to QA group. $^{###}p < 0.001$ as compared to control group

BS significantly attenuated glutamate and QA-induced lipid peroxidation

Glutamate (8 mM) significantly raised the MDA level, as index of lipid peroxidation, to 70 ± 6.38 nM/mg protein (50% increase in comparison to control group) ($p < 0.001$, Fig. 5A). As shown in Figure 5A, different concentrations of BS (10, 20 and 40 µg/mL) significantly diminished MDA level to 57.2 ± 6.5, 44.6 ± 5.2 and 38.5 ± 3.9 nM/mg protein following glutamate-induced toxicity (20, 38 and 46% reduction in comparison to glutamate group) ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively). BS at 40 µg/mL reduced MDA level to approximately control value.

In comparison to control group, QA (8 mM) significantly increased MDA level to 73.0 ± 5.6 nM/mg (50% increase in comparison to control group, $p < 0.001$, Fig. 5B). Different concentrations of BS (20 and 40 µg/mL) significantly prevented increased level of MDA to 44.2 ± 5.3 and 39.3 ± 4.1 nM/mg protein following QA-induced toxicity (40 and 47% reduction in comparison to glutamate group) ($p < 0.01$ and $p < 0.001$, respectively, Fig. 5B). Again, BS at 40 µg/mL reduced MDA level to approximately control values.

BS significantly mitigated intracellular ROS generation induced by glutamate and QA

As illustrated in Figure 6A, glutamate (8 mM) significantly increased ROS level to approximately 1.7 fold of control value ($p < 0.001$). Different concentrations of BS (20 and 40 µg/mL) significantly reduced ROS level (approximately 50 % of glutamate group, respectively), following glutamate-induced toxicity ($p < 0.05$ and 0.001, respectively, Fig. 6A). BS at 20 and 40 µg/mL reduced MDA level to approximately control value.

In comparison with control group, QA (8 mM) significantly ($p < 0.001$) increased ROS level (approximately 80%, Fig. 6B). Different concentrations of BS (10, 20 and 40 µg/mL) significantly made a decrement in ROS level (25, 50 and 50% of glutamate group, respectively) following glutamate-induced toxicity ($p < 0.05$ and 0.001, respectively, Fig. 6B).

DISCUSSION

In the present study, we determined the protective effect of BS on OLN-93 cells against glutamate and QA-induced oligotoxicity. The results revealed that BS (10-40 µg/mL) concentration-dependently raised cell viability and reduced oxidative stress parameters including ROS and MDA.

OLN-93, a permanent immature rat oligodendrocyte, has been used as an *in vitro* model of MS, a disease characterized by chronic demyelinating process caused by cellular injury and death in oligodendrocytes (20). MS is a relapsing-remitting disease at early stages, but overtime, it converts to a progressive disease for which existing treatments are ineffective (21). It has been suggested that oxidative stress is one of the major mechanisms for incidence of progressive MS (22). Moreover, disease progression correlates with overproduction of ROS (23). Therefore, mitigating the oxidative damage may improve the disease progression (21).

BS has been used as a potent herbal medicine for hundreds of years and known for its anti-inflammatory and anti-oxidant effects. Therefore, it can be a good remedy for many diseases including rheumatoid arthritis, ulcerative colitis, Crohn's disease and bronchial asthma (24). According to the results of this study, hydroalcoholic extract of BS has no toxicity for OLN-93 cell line at concentrations up to 40 µg/mL. In a previous study, it has been demonstrated that BS exerts no mortality and adverse effects on animal's health. It has been shown that concentrations of BS up to 500 mg/kg has no adverse effect or reduction in body weight gain in rats (25). These evidences may confirm our results.

Glutamate plays an important role as one of the excitatory neurotransmitters in the CNS and it's accumulation is highly gliotoxic (26). Glutamate-induced neurotoxicity and oligotoxicity has been proved in many studies. Glutamate (5 mM) reduced HT22 (mouse hippocampal cell line) viability to 54% compared to control group (3). Glutamate exerts toxic effects on OLCs (oligodendroglial lineage cells) in concentrations between 0.05-2 mM (27), on primary rat astrocytes in concentrations between 2-5 mM (28) and on RGC-5 (retinal ganglion cells) at concentrations between 1-25 mM (29). QA showed cytotoxic effects for most of the brain cell lines. Exposure to QA (1 mM) leads to cell death in rat oligodendrocytes (30).

In the present study, glutamate and QA (8 mM) were used to induce oligotoxicity in OLN-93 cell line. Based on our findings, different concentrations of glutamate and QA (more than 4 mM) led to cell death in a concentration-dependent manner. Moreover, glutamate and QA significantly increased the levels of ROS and MDA, as lipid peroxidation index. These results support oligotoxic potential of glutamate and QA for OLN-93 cell line.

Excessive production of ROS and oxidative stress play a major role in the pathophysiology of neuroinflammatory disorders, including MS (31). In con-

trast, antioxidants such as alpha-lipoic acid and coenzyme Q10 were able to elevate cell viability and to decrease oxidant-induced protein oxidation (31).

To our knowledge, no other reports on oligoprotective role of BS against glutamate and QA-induced toxicity have been published. However, it has been shown that BS (200, 400 mg/kg) significantly protects diabetic rats through its anti-oxidant and anti-hyperglycemia impacts (32). It has been suggested that pentacyclic triterpenes from BS gum resin inhibit 5-lipooxigenase specifically, leading to decreased leukotrine synthesis and inflammatory responses (33). It has been demonstrated that 8 weeks administration of BS (333 mg per capsule) three times a day improves rheumatoid arthritis by its anti-inflammatory and anti-oxidant activities (17). These studies emphasize the antioxidant impacts of BS and support our results leading to oligoprotective effect of BS extract through tested mechanisms following glutamate and QA- induced oligotoxicity.

CONCLUSION

The present study reported the protective impact of BS on OLN-93 cells during oligotoxicity induced by glutamate and QA. These protective effects were acting through diminishing oxidative damage. The outcome of this study supports the antioxidant and oligoprotective effects of BS and may provide new insight into management of neuroinflammatory disorders such as MS.

Conflict of interests

The authors declare no conflict of interests

REFERENCES

1. Burgmaier G., Schonrock L. M., Kuhlmann T., Richter-Landsberg C., Bruck W.: J. Neurochem. 75, 2270 (2000).
2. Brand A., Bauer N.G., Hallott A., Goldbaum O., Ghebremeskel K. et al.: J. Neurochem. 113, 465 (2010).
3. Wen X., Huang A., Hu J., Zhong Z., Liu Y. et al.: Neuroscience 303, 558 (2015).
4. Kritis A.A., Stamoula E.G., Paniskaki K.A., Vavilis T.D.: Front. Cell Neurosci. 9, 91 (2015).
5. Elphick L.M., Hawat M., Toms N.J., Meinander A., Mikhailov A. et al.: Toxicol. Appl. Pharmacol. 232, 258 (2008).
6. Park S.Y., Jung W.J., Kang J.S., Kim C.M., Park G. et al.: Int. J. Mol. Med. 35, 525 (2015).
7. Lopez Y.P., Kenis G., Rutten B.P., Myint A.M., Steinbusch H.W. et al.: J. Chem. Neuroanat. 71, 6 (2016).
8. Sundaram G., Brew B.J., Jones S.P., Adams S., Lim C.K. et al.: J. Neuroinflammation 11, 204 (2014).
9. Nikam T.D., Ghorpade R.P., Nitnaware K.M., Ahire M.L., Lokhande V.H. et al.: Physiol. Mol. Biol. Plants 19, 105 (2013).
10. von Rhein C., Weidner T., Henß L., Martin J., Weber C. et al.: Antiviral Res. 125, 51 (2016).
11. Khan M.A., Singh M., Khan M.S., Najmi A.K., Ahmad S.: Biomed. Res. Int. 2014, 294143 (2014).
12. Jalili C., Salahshoor M.R., Pourmotabbed A., Moradi S., Roshankhah S. et al.: Res. Pharm. Sci. 9, 351 (2014).
13. Raja A.F., Ali F., Khan I.A., Shawl A.S., Arora D.S. et al.: BMC Microbiol. 11, 1 (2011).
14. Devi P.S., Satyanarayana B., Naidu M.T.: Not. Sci. Biol. 6, 474 (2014).
15. Gupta I., Gupta V., Parihar A., Gupta S., Lüdtke R. et al.: Eur. J. Med. Res. 3, 511 (1998).
16. Kimmatkar N., Thawani V., Hingorani L., Khiyani R.: Phytomedicine 10, 3 (2003).
17. Shen Y., Takahashi M., Byun H.-M., Link A., Sharma N. et al.: Cancer Biol. Ther. 13, 542 (2012).
18. Ghorbani A., Sadeghnia H.R., Asadpour E.: Nutr. Neurosci. 18, 103 (2015).
19. Asadpour E., Ghorbani A., Sadeghnia H.R.: Acta Pol. Pharm. 71, 409 (2014).
20. Nadjafi S., Ebrahimi S.A., Rahbar-Roshandel N.: Int. J. Prev. Med. 5, 1153 (2014).
21. Lam M.A., Maghzal G.J., Khademi M., Piehl F., Ratzer R. et al.: Neurol. Neuroimmunol. Neuroinflamm. 3, e256 (2016).
22. Fischer M.T., Sharma R., Lim J.L., Haider L., Frischer J.M. et al.: Brain 135, 886 (2012).
23. Ratzer R., Sondergaard H.B., Christensen J.R., Bornsen L., Borup R. et al.: Mult. Scler. 19, 1841 (2013).
24. Khan M.A., Singh M., Khan M.S., Najmi A.K., Ahmad S.: BioMed. Res. Int. 2014, (2014).
25. Singh P., Chacko K.M., Aggarwal M., Bhat B., Khandal R. et al.: Toxicol. Int. 19, 273 (2012).
26. Dong X.-x., Wang Y., Qin Z.-h.: Acta Pharmacol. Sin. 30, 379 (2009).
27. Yoshioka A., Yamaya Y., Saiki S., Kanemoto M., Hirose G. et al.: Brain Res. 854, 207 (2000).
28. Obara-Michlewska M., Ruszkiewicz J., Zielińska M., Verkhratsky A., Albrecht J.: Neurochem. Int. 88, 20 (2015).

29. van Bergen N.J., Wood J.P., Chidlow G., Trounce I.A., Casson R. J. et al.: Invest. Ophthalmol. Vis. Sci. 50, 4267 (2009).
30. Cammer W.: Brain Res. Bull. 58, 587 (2002).
31. Ernst A., Stolzing A., Sandig G., Grune T.: Arch. Biochem. Biophys. 421, 54 (2004).
32. Azemi M.E., Namjoyan F., Khodayar M.J., Ahmadpour F., Padok A.D. et al.: Jundishapur J. Nat. Pharm. Prod. 7, 168 (2012).
33. Poeckel D., Werz O.: Curr. Med. Chem. 13, 3359 (2006).

Received: 16. 10. 2016