ejpmr, 2018,5(11), 337-341

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

BACOSIDE-A ATTENUATED *IN VITRO* ACTIVATION OF PRIMARY ASTROCYTE AND MICROGLIAL CULTURES

Krishnadas Madhu and Prakash T.*

Department of Pharmacology, Acharya & BM Reddy College of Pharmacy, Bengaluru, India.

***Corresponding Author: Prakash T.**

Department of Pharmacology, Acharya & BM Reddy College of Pharmacy, Bengaluru, India.

Article Received on 27/08/2018 **Article Revised on 17/09/2018** **Article Accepted on 07/10/2018**

ABSTRACT

Activation of microglia and astrocytes is regarded as the initial phase in neuroinflammatory disorders like multiple sclerosis. The current study focus on the role of Bacoside-A on activated microglia and astrocyte. The action evaluated with LPS induced primary cultures of microglia and astrocyte. It reduced the production of NO and TNF-α in LPS stimulated microglia and astrocyte cultures. The present study emphasizes on the potential of Bacoside-A in neurodegenerative disorders like multiple sclerosis.

KEYWORDS: Bacoside, neuroinflammation, multiple sclerosis, microglia, astrocyte.

Abbreviations: MS, Multiple Sclerosis; CNS, Central Nervous System; LPS, Lipopolysaccharide; TNFα, Tumor Necrosis Factor α.

INTRODUCTION

Multiple Sclerosis is a neurodegenerative disorder primarily due to the inflammation of myelin resulting in demyelination, axonal damage, and neuronal apoptosis. These are initiated by the promotion of Th1 cells to CNS, resulting in the activation of microglia and astrocyte. These lead to the production of NO, pro-inflammatory cytokines and chemokines (Havenith CE *et. al*., 1998; Dong Y & Benveniste EN, 2001; De Keyser J *et. al*., 2003). This promotes the peripheral macrophages into CNS resulting in demyelination and neuron damage (Matyszak MK *et. al*., 1999). So, molecules can ably inhibit the production of NO and TNF-α which markedly reduce the progression of MS. This will improve the quality of life of patients.

Bacoside-A consists of compounds like bacoside-A3, bacopaside-II, jujubogenin, and bacopasaponin-C. It possesses anti-stress (Chowdhuri DK *et. al*., 2002) and inhibits aging-related neurodegeneration (Rastogi M *et. al*., 2012). Its mode of action is still unexplored in conditions like neuro-inflammation. In this study, we focus on the role of Bacoside-A on activated astrocyte and microglia in neuroinflammation. This study will help in the expansion of the use of herbal compounds in the treatment of neuroinflammatory disorders like MS.

MATERIALS AND METHODS

Materials used

Bacoside-A 98% (Finetech Industry Limited, China), LPS (Sigma Aldrich, St. Louis, MO), Poly D Lysine Hydrobromide (Sigma Aldrich, St. Louis, MO), Dulbecco Modified Eagle's Media [DMEM (Himedia)], Fetal Bovine Serum [FBS (Himedia)], Trypsin (Himedia), OPI Media (Sigma Aldrich, St. Louis, MO), mouse GM-CSF (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany).

Animals

B6 mouse pups, 1day old were obtained from Biogen Laboratory Animal Facility, Bengaluru, India. All experiments were implemented according to the guidelines of CPCSEA, India and approved by institutional ethics committee of Acharya & BM Reddy College of Pharmacy, Bengaluru, India. (Ref. no: IAEC/ABMRCP/2015-2016/05).

Cell culture

Mouse microglia and astrocyte cultures obtained according to the previous protocol (Storer PD *et. al*., 2005) Concisely, brain cortices of 3 mouse pups were excised and cut into pieces. The tissues were trypsinized, filtrated using 70 µm cell strainer and centrifuged at 1200 rpm for 5 min to get the pellet. The collected pellet was resuspended in the media [DMEM, 10% FBS, 1.4 mM glutamine, Penicillin-Streptomycin, OPI media and mouse GM-CSF (10 ng/mL)], plated into a T25 flask previously coated with Poly D Lysine (10µg/mL) and allowed to grow confluence at 37^0C/5% CO_2. Microglia and astrocyte were separated from the mixed glial culture using orbital shaker at 37^0C at 180 rpm for two h (for microglia) and 260 rpm for 6h (for separating oligodendrocytes from astrocytes). Microglia were seeded into a T25 flask with the same media, while the

astrocytes were cultured with a media devoid of mouse GM-CSF and incubated at 37^0C/5% CO_2. The obtained cells were plated into 96 well plates for 24 h and activated using 0.5 ng/mL of LPS for 24 h except in control wells. All evaluations carried out after 24 h of treatment with various concentrations of Bacoside-A except in control and LPS treated wells.

Cell Proliferation assay

Cell proliferation was evaluated using 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) in primary microglia and astrocyte cells as described previously. The plates read at 570 nm and references at 655 nm using Tecan M200.

Nitric oxide Production

Griess reaction was used to detect the production of NO as described previously. The microplates read at 550 nm (Tecan M200). The results were obtained in triplicates and reported as the average molar amount of nitrite released into the supernatant.

Estimation of TNF-α

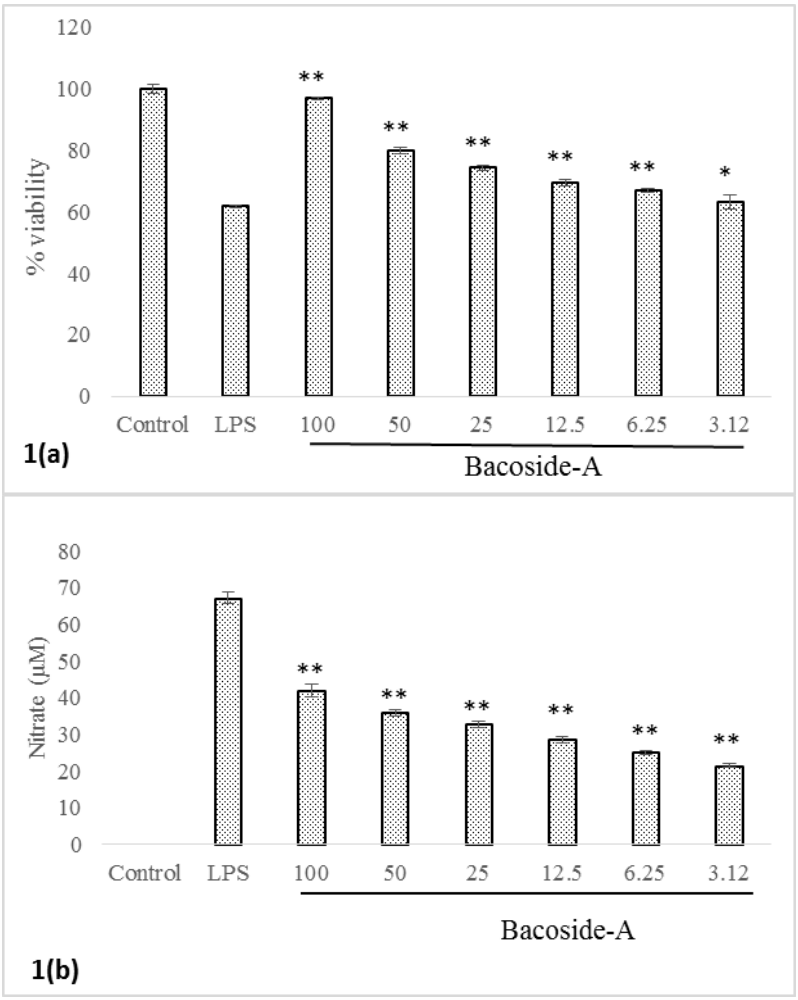
TNF-α evaluated from the cell supernatant obtained from the wells using a mouse TNF α kit (Cayman Chemical).

Statistical analysis

All the data reported as Mean ± SEM, n=3. One-way ANOVA followed by Dunnet's post hoc test (GraphPad Prism 5.0) used in this study. $p < 0.01$ is considered significant.

RESULTS

Inflammation in primary microglial and astrocyte cultures were achieved by induction with LPS. The extent of inflammation predicted by the production of NO and TNF-α in cultures. LPS induction resulted in the decline of cell viability and had risen the levels of NO and TNFα in microglial cultures. The treatment with various concentrations of Bacoside-A showed marked reduction ($p<0.01$) in NO and TNF-α in LPS induced microglial culture as demonstrated in Figure 1. LPS induction did not exhibit any change in cell viability of astrocytes. But, LPS treatment produced elevated levels of nitrate and TNF-α in astrocyte culture. Meanwhile, treatment with various concentrations of Bacoside-A significantly ($p<0.01$) lowered the levels of NO and TNFα in LPS treated astrocyte cultures as described in Figure 2.



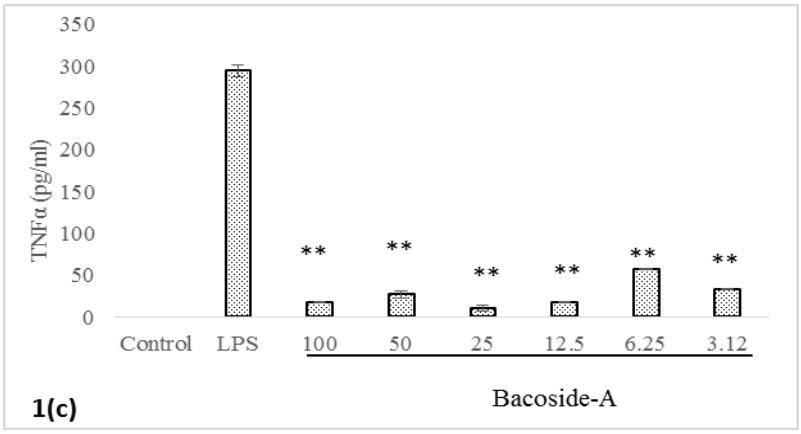


Figure 1: Action of Bacoside-A on LPS activated microglial culture. Each bar represents Mean ± SEM, n=3. Statistical analysis was determined using ANOVA followed by Dunnet's post hoc test. ** represents p<0.01, when compared to LPS treated culture.

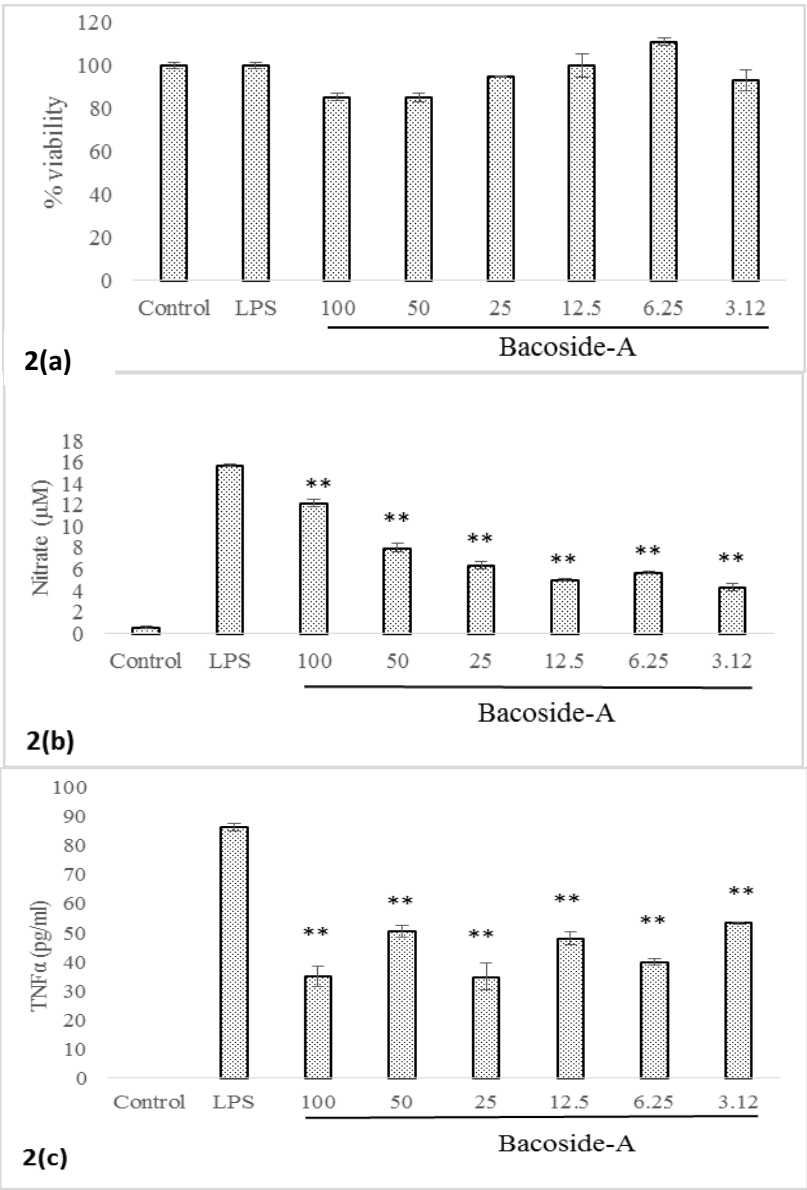


Figure 2: Action of Bacoside-A on LPS activated astrocyte culture. Each bar represents Mean ± SEM, n=3. Statistical analysis was determined using ANOVA followed by Dunnet's post hoc test. ** represents p<0.01, when compared to LPS treated culture.

DISCUSSION

The infiltration and proliferation of CD4+ T cells into the CNS, followed by the activation of microglia and astrocytes result in the pathological initiation of disease. The activation of microglia and astrocytes leads to the generation of inflammatory mediators such as pro-inflammatory cytokines like IL-6, IL-1β, TNF-α and NO in CNS. These inflammatory mediators cause the mass recruitment of macrophages, resulting in the progression of MS. Therefore, the treatment strategies that inhibit the activation of microglia and astrocyte may reduce the progression of disease thereby improving the quality of life of patients.

The inflammation induced release of NO has a crucial role in the apoptosis of oligodendrocyte than that of astrocyte and microglia (Mitrovic B *et. al*., 1994). NO cause faster demyelination due to the reduction in myelin formation and more rapid myelin depletion. The release of NO supported vasodilation and increased the permeability of BBB in active lesions (Mayhan WG *et. al*., 2000), enhances the entry of inflammatory cells and mediators into the CNS (Shukla A *et. al*., 1996), induce conduction block. It also increases the axonal degeneration in demyelinated neurons (Shrager P *et. al*., 1998) and results in neuronal damage. Treatment with Bacoside-A in LPS induced microglia and astrocyte culture decreased the levels of NO, which explicitly specify the potential of Bacoside-A to counteract the neuroinflammation.

During inflammation, TNFα contributes to the injury mediated activation of astrocytes and microglia (Merrill JE *et. al*., 1991). It enhances BBB permeability (Sedgwick JD *et. al*., 2000), promotes excitatory synaptic transmission, reduces inhibitory neurotransmission (Pickering M *et. al*., 2005) and synaptic plasticity (Beattie EC *et. al*., 2002). More elevated levels of TNF-α were observed near the active MS lesions (Hofman FM *et. al*., 1989) and other neuroinflammatory disorders. Bacoside-A at various concentrations could ably inhibit the production of TNF-α in the LPS induced microglia and astrocyte cultures. Bacoside-A may reduce the progression of inflammation during activation of microglia and astrocyte in the CNS.

CONCLUSION

Bacoside-A counteracts the LPS induced activation of microglia and astrocyte in primary culture by inhibiting the generation of NO species and TNF-α. These effects recommend that it has a prominent role in reducing the neuroinflammation, produced during MS. Further studies are essential to unravel the mechanism of action of Bacoside-A in neuroinflammation.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Divakar Goli, B Premnath Reddy and Dr. D Giles for providing facilities for this research.

Conflict of interest

We confirm that there are no known conflicts of interest related to this publication.

REFERENCES

1. Beattie EC, Stellwagen D, Morishita W, et al., Control of synaptic strength by glial TNF alpha, Sci, 2002; 295: 2282-2285.
2. Chowdhuri DK, Parmar D, Kakkar P, et al., Antistress effects of bacosides of Bacopa monnieri: modulation of Hsp70 expression, superoxide dismutase and cytochrome P450 activity in rat brain, Phytother. Res, 2002; 16: 639–645. doi:10.1002/ptr.1023
3. De Keyser J, Zeinstra E, Frohman E, Are astrocytes central players in the pathophysiology of multiple sclerosis? Arch. Neurol, 2003; 60: 132– 136.
4. Dong Y, Benveniste EN, Immune function of astrocytes, Glia, 2001; 36: 180–190.
5. Havenith CE, Askew D, Walker WS, Mouse resident microglia: isolation and characterization of immunoregulatory properties with naive CD4+ and CD8+ T-cells, Glia, 1998; 22: 348–359.
6. Hofman FM, Hinton DR, Johnson K, et al., Tumor necrosis factor identified in multiple sclerosis brain, J. Exp. Med, 1989; 170: 607-612.
7. Matyszak MK, Denis-Donini S, Citterio S, et al., Microglia induce myelin basic protein-specific T cell anergy or T cell activation, according to their state of activation, Euro. J. Immunol, 1999; 29: 3063– 3076.
8. Mayhan WG, Nitric oxide donor-induced increase in permeability of the blood-brain barrier, Brain Res, 2000; 866: 101–108.
9. Merrill JE, Effects of interleukin-1 and tumor necrosis factor alpha on astrocytes, microglia, oligodendrocytes, and glial precursors in vitro, Develop. Neurosci, 1991; 13: 130-137.
10. Mitrovic B, Ignarro LJ, Montestruque S, et al., Nitric oxide as a potential pathological mechanism in demyelination: its differential effects on primary glial cells in vitro, Neurosci, 1994; 61: 575–585.
11. Pickering M, Cumiskey D, O'Connor JJ, Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system, Exp. Physiol, 2005; 90: 663-670.
12. Rastogi M, Ojha RP, Prabu PC, et al., Prevention of age-associated neurodegeneration and promotion of healthy brain aging in female Wistar rats by long-term use of bacosides, Biogerontol, 2012; 13: 183. https://doi.org/10.1007/s10522-011-9367-y
13. Sedgwick JD, Riminton DS, Cyster JG, et al., Tumor necrosis factor: a master-regulator of leukocyte movement, Immunol. Today, 2000; 21: 110-113.

14. Shrager P, Custer AW, Kazarinova K, et al., Nerve conduction block by nitric oxide that is mediated by the axonal environment, J. Neurophysiol, 1998; 79: 529–536.
15. Shukla A, Dikshit M, Srimal RC, Nitric oxide-dependent blood-brain barrier permeability alteration in the rat brain, Experientia, 1996; 52: 136–140.
16. Storer PD, Xu J, Chavis J, Drew PD, Peroxisome proliferator-activated receptor-gamma agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis, J. Neuroimmunol, 2005; 161: 113-122.