http://jbcp.shahed.ac.ir 10.22070/jbcp.2019.4098.1110

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol.7, No.1, March 2019

A Semiannual Publication of
Shahed University Press

The effect of quercetin on learning and memory deficit, lipid peroxidation, and cholinesterase activeity following lipopolysaccharide in the rat

Zohreh Kaedi[1], Jamshai Narenjkar[2], Mehrdad Roghani[3*]

1. School of Medicine, Shahed University, Tehran, Iran.
2. Department of Pharmacology, School of Medicine, Shahed University, Tehran, Iran.
3. Neurophysiology Research Center, Shahed University, Tehran, Iran

Abstract

Background and Objective: Lipolysaccharide (LPS) is a large molecule isolated from bacteria such as the enterobacteriaceae family with a negative effect on memory and learning through disturbing the balance of free radicals and creating oxidative stress conditions. In this study, we evaluated the effect of quercetin on oxidative stress and LPS-induced memory impairment in the rat.

Materials and Methods: Male rats (n=40) were randomly divided into 5 groups: control, control under treatment with quercetin at a dose of 50 mg/kg, LPS, and LPS groups treated with quercetin at doses of 10 or 50 mg/kg. For induction of inflammation, LPS dissolved in normal saline (500 µg/kg) was injected intraperitoneally. After one week, the passive avoidance behavior was tested in the shuttle box and hippocampal homogenate was prepared. Acetylcholinesterase (AChE) activity and lipid peroxidation (malondialdehyde, MDA) were measured using specific kits. Data were analyzed by SPSS software (version 16).

Results: Step-through latency (STL) in quercetin50-treated LPS group was significantly greater than control group ($p<0.05$). In addition, AChE activity and level of MDA was significantly lower in quercetin50-treated LPS group versus LPS group ($p<0.05$). Meanwhile, quercetin at a dose of 10 mg/kg did not have such a significant effect.

Conclusion: Quercetin at a dose of 50 mg/kg has a protective effect on learning and memory impairment due to LPS and part of its beneficial effect is mediated via attenuation of lipid peroxidation and AChE.

Key words: Lipolysaccharide, Quercetin, Neuroinflammation, Lipid peroxidation, Cholinesterase.

1. Introduction

Development of systemic inflammation is as an important risk factor for occurrence of cognitive decline (1). The elderly people are highly vulnerable to the adverse effects of infections on cognition (2). Greater generation of pro-inflammatory cytokines contributes to development of neuroinflammation (3, 4). Inflammation is also seen in the brains of patients with Alzheimer's disease (5). Lipopolysaccharide (LPS) is an endotoxin seperated from Gram-negative bacteria that could stimulate pro-inflammatory cascades with generation of pro-inflammatory cytokines (6). LPS challenge is associated with cognitive deficit (7). Enhanced oxidative stress (8) and increased acetylcholinesterase activity (9) are usually seen after LPS exposure.

Revised: January 2019, **Accepted:** February 2019, **Available online:** March 2019
Corresponding Author: Mehrdad Roghani **Email:** mehjour@yahoo.com

Quercetin is a naturally occurring bioflavonoid that is found in many fruits and vegetables (10). Quercetin have multiple beneficial effects such as anti-cancer, anti-inflammatory and protective effects against neurodegeneration (11-14). In addition, quercetin could inhibit neuronal apoptosis due to cytotoxic treatments (14, 15). It has shown that this flavonoid could attenuate neuroinflammation and may be beneficial for treatment of neural disorders (16-18). Furthermore, quercetin could reduce oxidative damage due to chemotherapeutic agents (19). This study was undertaken to assess the possible efficacy of quercetin against LPS-induced cognitive decline in the rat and to find out some related modes of action.

2. Materials and Methods

2.1. Experimental design

Male albino Wistar rats (Pasteur's institute, Tehran, Iran, 180-210 g) were kept under standard housing conditions (temperature: 21-23°C; humidity: 40-60%; 12:12 h lighting cycle) with food and water available *ad libitum*. Procedures involving animals and their care were conducted in conformity with the NIH guidelines for the care and use of laboratory animals.

The rats (n=40) were randomly divided into 5 groups: control, quercetin50-treated control, LPS, and LPS groups receiving quercetin at doses of 10 or 50 mg/kg. To induce systemic inflammation, LPS from Escherichia coli (SigmaAldrich, St Louis, MO, USA; 0111:B4) was injected intraperitoneally at a dose of 500 µg/kg for seven days. Systemic LPS administration is a widely-accepted model for neuroinflammation induction in rodents with elevation of brain cytokines (20, 21). All behavioral experiments were carried out from 11:00 to 16:00 by an experimenter blind to groups and treatments. All animals were sacrificed on day 7 after retention and recall trial of passive avoidance test.

2.2. Passive avoidance test

The used devise was composed of two compartments, i.e. one illuminated and one dark chamber with grid floor connected by a guillotine door. Electric shock was delivered by an isolated stimulator. On the first and second days, each rat was placed into the apparatus and left for 5 min to explore the chambers and to habituate. During the acquisition trial (third day), rats were placed in the illuminated chamber and after a 5 min habituation period, the guillotine door was opened and the latency to enter the dark chamber was recorded. After the rat entering the dark chamber, the door was closed and an electric foot shock (1 mA, 1 s) was delivered to floor grid. On the acquisition day, the initial latency (IL) of entrance into the dark chamber was recorded and rats with ILs greater than 60 s were excluded from the study. Twenty-four hours later, a retention test was done and the interval between the placement in the illuminated chamber and the entrance into the dark chamber was measured as step-through latency (STL up to a maximum of 240 s as cut-off).

2.3. Determination of hippocampal MDA and AChE

At the end of week 1, hippocampal tissue (n = 5-6 from each group) was separately dissected out and 10% homogenate was prepared in lysate buffer and in the presence of protease inhibitor cocktail and the supernatant was aliquoted and stored at -70°C for the following experiments.

Malondialdehyde (MDA) level in the supernatant was measured as described before (22). For determination of MDA concentration (thiobarbituric acid reactive substances, TBARS), trichloroacetic acid and TBARS reagent were added to supernatant, then mixed and incubated at boiling water for 90 min. After cooling on ice, samples were centrifuged at 5000×g for 10 min and the absorbance of the supernatant was read at 532 nm.

AChE activity was determined on the basis of degradation of acetylthiocholine iodide into a product that binds to 5,5-dithiobis-2-nitrobenzoic acid and turns yellow (23). The kinetics of the reaction was followed spectrophotometrically over 5 min at 412 nm. AChE activity was expressed as mM of substrate hydrolyzed/min/g protein.

2.4. Statistical analysis

All results were expressed as mean ± S.E.M. The parametric test one-way ANOVA was used for data analysis and if a significant difference was found out, pair-wise comparison was done using the Tukey *post-hoc* test. In all calculations, a difference at $p<0.05$ was regarded as significant.

3. Results

Figure 1 shows the performance of rats in passive avoidance paradigm as shown by initial (IL) and step-through (STL) latencies. With respect to IL, no significant differences were obtained amongst the groups. Meanwhile, LPS and LPS+Quercetin5050 groups exhibited a significant disturbance of retention and recall in this test ($p<0.05$-0.01), as it was evident by a lower STL and quercetin50-treated LPS group had a significantly higher STL versus LPS group ($p<0.05$).

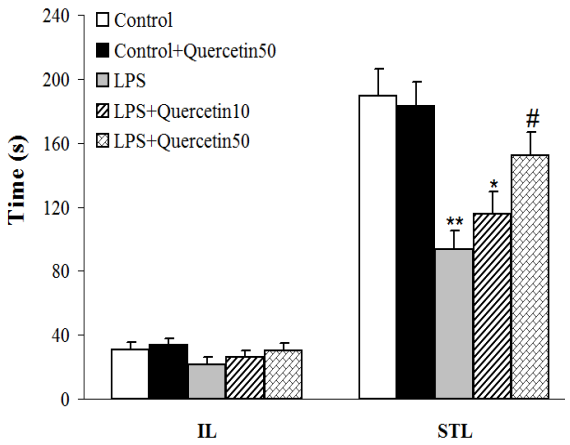

Figure 1. Initial (IL) and step-through (STL) latencies of treated-control and LPS-challenged rats in single-trial passive avoidance task (n = 8 for each group) * P<0.05, ** p<0.01 (vs. control) # p<0.05 (vs. LPS)

LPS-injected rats had a significantly elevated hippocampal level of MDA (Fig. 2) (p<0.05) and treatment of LPS-injected rats with quercetin at a dose of 50 mg/kg significantly decreased MDA (p<0.05) as compared to LPS group.

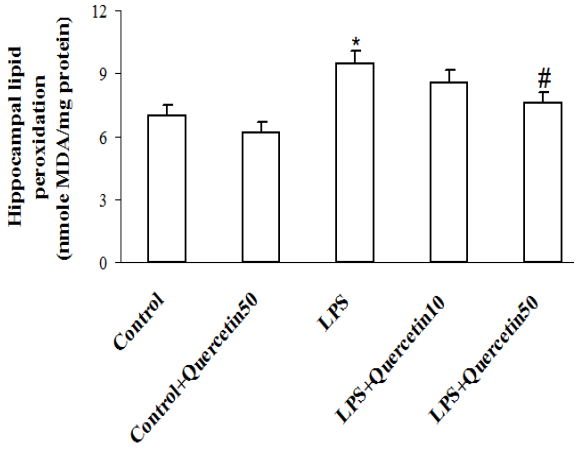

Figure 2. MDA concentration as a marker of lipid peroxidation in hippocampal lysate from different groups. * p<0.05 (in comparison with control), # *P*<0.05 (in comparison with LPS). (n = 6 for each group).

Figure 3 presents AChE activity in hippocampal lysate from different groups. This activity was significantly elevated in LPS (p<0.01) and LPS+quercetin10 (p<0.05) groups and this increase was non-significant in quercetin50-treated LPS group as compared to control group. In addition, quercetin treatment of LPS-injected rats at a dose of 50 mg/kg significantly lowered AChE activity relative to LPS group (p<0.05).

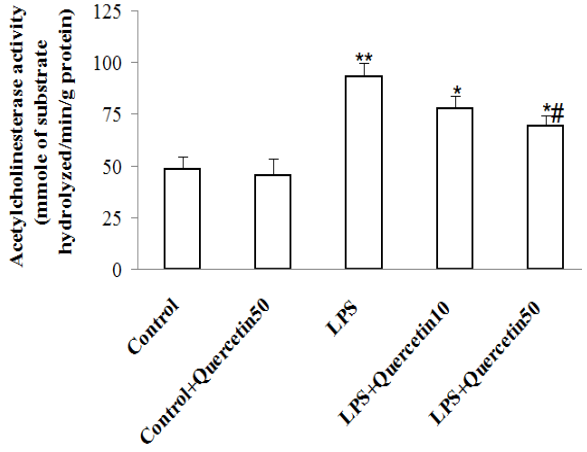

Figure 3. AChE activity in hippocampal lysate from different groups. * p<0.05, ** p<0.01 (in comparison with control), # *P*<0.05 (in comparison with LPS). (n=6 for each group).

4. Discussion

The obtained results from our study clearly confirmed that quercetin treatment dose-dependently improves LPS-induced cognitive deficits via attenuation of lipid peroxidation and AChE activity.

Earlier investigations have shown that passive avoidance test using the shuttle box in rodents involves two independent memory types, short-term memory when retention test is conducted 90 min after training and long-term memory when retention test is conducted 24 h and 7 days after training (24, 25). Since retention and recall trial in passive avoidance task in our study was performed 24 h after the acquisition trial, thus, it is considered a long-term memory that needs protein synthesis process in related synapses. In our study, quercetin treatment may have improved retention and recall in passive avoidance task via affecting synaptic organization, however, there is still no report on this topic that itself warrants more researches. In contrast to our results, one study showed that quercetin targets cysteine string protein (CSPalpha) and could impair synaptic transmission (26).

LPS challenge is also associated with increased production of reactive oxygen species (ROS) and appearance of an imbalance between the oxidant and antioxidant status (27). In this study, we showed a marked elevation of hippocampal MDA level in LPS-challenged group. The increased MDA content of these rats clearly indicate that enhanced lipid peroxidation is involved in the development of cognitive impairment. MDA level significantly decreased in quercetin50-treated LPS group. This may indicate that quercetin at its higher dose may have exerted antioxidant activity and protected the hippocampal tissue against lipid peroxidation.

Brain cholinergic system has a pivotal role in learning and memory processes (28). Part of cognitive dysfunction in metabolic (29, 30) and/or inflammatory (31, 32) diseases is ascribed to reduced cholinergic transmission and its impact on retention and recall of stored information. One of the important regulators of cholinergic signaling pathway is the degrading enzyme AChE. There are conflicting and contrasting reports on the effect of LPS-induced inflammation on AChE activity, some showing its increase (31, 33) and some demonstrating its reduction (34, 35) in the brain region.

In conclusion, quercetin at a dose of 50 mg/kg has a protective effect on learning and memory impairment due to LPS and part of its beneficial effect is mediated via attenuation of lipid peroxidation and AChE.

Acknowledgement

This research project was the result of MD thesis project, approved and financially supported in part by Shahed University.

Conflict of interest

The authors declare that they have no competing interests.

References

1. Bettcher BM, Kramer JH. Longitudinal inflammation, cognitive decline, and Alzheimer's disease: a mini-review. Clinical Pharmacology and Therapeutics 2014;96(4):464-9.
2. Metti AL, Cauley JA. How predictive of dementia are peripheral inflammatory markers in the elderly?. Neurodegenerative Disease Management 2012;2(6):609-22.
3. Brites D, Fernandes A. Neuroinflammation and Depression: Microglia Activation, Extracellular Microvesicles and microRNA Dysregulation. Frontiers in Cellular Neuroscience 2015;9:476.
4. Von Bernhardi R, Eugenin-von Bernhardi L, Eugenin J. Microglial cell dysregulation in brain aging and neurodegeneration. Frontiers in Cellular Neuroscience 2015;7:124.
5. DeLegge MH, Smoke A. Neurodegeneration and inflammation. Nutrition in Clinical Practice 2008;23(1):35-41.
6. Sun JS, Yang YJ, Zhang YZ, Huang W, Li ZS, Zhang Y. Minocycline attenuates pain by inhibiting spinal microglia activation in diabetic rats. Molecular Medicine Reports 2015;12(2):2677-82.
7. Hsing CH, Hung SK, Chen YC, Wei TS, Sun DP, Wang JJ, et al. Histone Deacetylase Inhibitor Trichostatin A Ameliorated Endotoxin-Induced Neuroinflammation and Cognitive Dysfunction. Mediators Inflamm 2015;2015:163140.
8. Jangra A, Sriram CS, Lahkar M. Lipopolysaccharide-Induced Behavioral Alterations Are Alleviated by Sodium Phenylbutyrate via Attenuation of Oxidative Stress and Neuroinflammatory Cascade. Inflammation 2016.
9. Ming Z, Wotton CA, Appleton RT, Ching JC, Loewen ME, Sawicki G, et al. Systemic lipopolysaccharide-mediated alteration of cortical neuromodulation involves increases in monoamine oxidase-A and acetylcholinesterase activity. Journal of Neuroinflammation 2015;12:37.
10. Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy: facts and fancies. Biochemical Pharmacology 2012;83(1):6-15.
11. Abdel-Diam MM, Samak DH, El-Sayed YS, Aleya L, Alarifi S, Alkahtani S. Curcumin and quercetin synergistically attenuate subacute diazinon-induced inflammation and oxidative neurohepatic damage, and acetylcholinesterase inhibition in albino rats. Environmental Science and Pollution Research International 2018.
12. Halder S, Kar R, Chandra N, Nimesh A, Mehta AK, Bhattacharya SK, et al. Alteration in cognitive behaviour, brain antioxidant enzyme activity and their gene expression in F1 generation mice, following Cd exposure during the late gestation period: modulation by quercetin. Metabolic Brain Disease 2018;33(6):1935-43.
13. Khan K, Najmi AK, Akhtar M. A Natural Phenolic Compound Quercetin Showed the Usefulness by Targeting Inflammatory, Oxidative Stress Markers and Augment 5-HT Levels in One of the Animal Models of Depression in Mice. Drug Research (Stuttg) 2018.
14. Wang Q, Liu C. Protective effects of quercetin against brain injury in a rat model of lipopolysaccharide-induced fetal brain injury. International Journal of Developmental Neuroscience 2018;71:175-80.

15. Du G, Zhao Z, Chen Y, Li Z, Tian Y, Liu Z, et al. Quercetin protects rat cortical neurons against traumatic brain injury. Molecular Medicine Reports 2018;17(6):7859-65.

16. Bahar E, Kim JY, Yoon H. Quercetin Attenuates Manganese-Induced Neuroinflammation by Alleviating Oxidative Stress through Regulation of Apoptosis, iNOS/NF-kappaB and HO-1/Nrf2 Pathways. International Journal of Molecular Sciences 2017;18(9).

17. Gao FJ, Zhang SH, Xu P, Yang BQ, Zhang R, Cheng Y, et al. Quercetin Declines Apoptosis, Ameliorates Mitochondrial Function and Improves Retinal Ganglion Cell Survival and Function in In Vivo Model of Glaucoma in Rat and Retinal Ganglion Cell Culture In Vitro. Frontiers in Molecular Neuroscience 2017;10:285.

18. Li X, Wang H, Wen G, Li L, Gao Y, Zhuang Z, et al. Neuroprotection by quercetin via mitochondrial function adaptation in traumatic brain injury: PGC-1alpha pathway as a potential mechanism. Journal of Cellular and Molecular Medicine 2018;22(2):883-91.

19. Dogan Z, Kocahan S, Erdemli E, Kose E, Yilmaz I, Ekincioglu Z, et al. Effect of chemotherapy exposure prior to pregnancy on fetal brain tissue and the potential protective role of quercetin. Cytotechnology 2015;67(6):1031-8.

20. 20. Henry CJ, Huang Y, Wynne A, Hanke M, Himler J, Bailey MT, et al. Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. Journal of Neuroinflammation 2008;5:15.

21. Czerniawski J, Miyashita T, Lewandowski G, Guzowski JF. Systemic lipopolysaccharide administration impairs retrieval of context-object discrimination, but not spatial, memory: Evidence for selective disruption of specific hippocampus-dependent memory functions during acute neuroinflammation. Brain, Behavior, and Immunity 2015;44:159-66.

22. Nasri S, Roghani M, Baluchnejadmojarad T, Rabani T, Balvardi M. Vascular mechanisms of cyanidin-3-glucoside response in streptozotocin-diabetic rats. Pathophysiology 2011;18(4):273-8.

23. Ellman GL, Courtney KD, Andres V, Jr., Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 1961;7:88-95.

24. Barros DM, Pereira P, Medina JH, Izquierdo I. Modulation of working memory and of long- but not short-term memory by cholinergic mechanisms in the basolateral amygdala. Behavioral Pharmacology 2002;13(2):163-7.

25. Ramirez MR, Izquierdo I, do Carmo Bassols Raseira M, Zuanazzi JA, Barros D, Henriques AT. Effect of lyophilised Vaccinium berries on memory, anxiety and locomotion in adult rats. Pharmacological Research 2005;52(6):457-62.

26. Xu F, Proft J, Gibbs S, Winkfein B, Johnson JN, Syed N, et al. Quercetin targets cysteine string protein (CSPalpha) and impairs synaptic transmission. PLoS One 2010;5(6):e11045.

27. Bai K, Xu W, Zhang J, Kou T, Niu Y, Wan X, et al. Assessment of Free Radical Scavenging Activity of Dimethylglycine Sodium Salt and Its Role in Providing Protection against Lipopolysaccharide-Induced Oxidative Stress in Mice. PLoS One 2016;11(5):e0155393.

28. Sohanaki H, Baluchnejadmojarad T, Nikbakht F, Roghani M. Pelargonidin Improves Passive Avoidance Task Performance in a Rat Amyloid Beta25-35 Model of Alzheimer's Disease Via Estrogen Receptor Independent Pathways. Acta Medica Iranica 2016:245-50.

29. Mao XY, Cao DF, Li X, Yin JY, Wang ZB, Zhang Y, et al. Huperzine A ameliorates cognitive deficits in streptozotocin-induced diabetic rats. International Journal of Molecular Sciences 2014;15(5):7667-83.

30. Liu ZH, Chen HG, Wu PF, Yao Q, Cheng HK, Yu W, et al. Flos Puerariae Extract Ameliorates Cognitive Impairment in Streptozotocin-Induced Diabetic Mice. Evidence-based Complementary and Alternative Medicine 2015;2015:873243.

31. Tyagi E, Agrawal R, Nath C, Shukla R. Effect of melatonin on neuroinflammation and acetylcholinesterase activity induced by LPS in rat brain. European Journal of Pharmacology 2010;640(1-3):206-10.

32. Silverman HA, Dancho M, Regnier-Golanov A, Nasim M, Ochani M, Olofsson PS, et al. Brain region-specific alterations in the gene expression of cytokines, immune cell markers and cholinergic system components during peripheral endotoxin-induced inflammation. Molecular Medicine 2014;20:601-11.

33. Joshi R, Garabadu D, Teja GR, Krishnamurthy S. Silibinin ameliorates LPS-induced memory deficits in experimental animals. Neurobiology of Learning and Memory 2014;116:117-31.

34. Abdel-Salam OM, Omara EA, Mohammed NA, Youness ER, Khadrawy YA, Sleem AA. Cerebrolysin attenuates cerebral and hepatic injury due to lipopolysaccharide in rats. Drug Discoveries & Therapeutics 2013;7(6):261-71.

35. Lykhmus O, Mishra N, Koval L, Kalashnyk O, Gergalova G, Uspenska K, et al. Molecular Mechanisms Regulating LPS-Induced Inflammation in the Brain. Frontiers in Molecular Neuroscience 2016;9:19.