Luteolin Reduced the Traumatic Brain Injury-Induced Memory Impairments in Rats: Attenuating Oxidative Stress and Dark Neurons of Hippocampus

Zeinab Ashaari[1], Gholamreza Hassanzadeh[2,3], Tahmineh Mokhtari[4,5], Mahmoud Hosseini[1], Zakieh Keshavarzi[6], Mohsen Amini[2], Kowsar Bavarsad[1], Sahar Ijaz[2], Mousa-Al-Reza Hadjzadeh[1,7,8]

[1] Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[2] Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[3] Department of Neuroscience, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

[4] Nervous System Stem Cells Research Center, Semnan University of Medical Sciences, Semnan, Iran

[5] Department of Anatomy, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran

[6] Natural Products and Medicinal Plants Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran

[7] Department of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

[8] Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Received: 15 Aug. 2018; Accepted: 11 Sep. 2018

Abstract- Traumatic Brain Injury (TBI) is generally recognized as a major risk factor for memory impairments and Alzheimer's disease (AD). In this experimental study, our aim was to investigate the ameliorating effects of luteolin (LUT) on the memory impairments, oxidative stress, and histopathological changes induced by TBI in rats. The adult male Wistar rats were randomly divided into six groups including: Control (Co), sham, TBI, TBI+LUT (10 mg/kg), TBI +LUT (25 mg/kg), TBI +LUT (50 mg/kg). To evaluate the protective effects of LUT on the memory of the rats, passive avoidance test using shuttle box was performed. Finally, the animals were anesthetized, and the brain tissues were removed and analyzed for oxidative stress parameters. Using histological methods, dark neuron production was also evaluated. There was a significant decrease in the latency time to enter the dark compartment in passive avoidance test in TBI animals. This latency time was significantly increased in TBI+LUT (25 mg/kg) and TBI+LUT (50 mg/kg) groups along with significant increases in superoxide dismutase and catalase activity in the hippocampal zone and a decrease in malondialdehyde (MDA). The number of dark neurons in the hippocampus decreased with all three doses of LUT. In the present study, LUT showed neuroprotective effects, improvement in learning and reduction in memory impairment induced by TBI in rats. Protection against oxidative stress might be a possible mechanism behind these effects. Further works are necessary to work out if LUT is potentially a suitable therapeutic candidate for neural disorders.

Acta Med Iran 2018;56(9):563-570.

Keywords: Luteolin; Traumatic brain injury; Oxidative stress; Learning and memory; Rat

Introduction

Traumatic brain injury (TBI) as a major cause of death and disability is a common health problem among health professionals. It may occur because of motor vehicle accidents, shootings, earthquakes, and storms (1). Short and long-term cognitive impairments occur following TBI. TBI has also been recognized as an important risk factor for memory impairment and Alzheimer's disease (AD) (2). Results of previous studies have suggested that there are overlaps between pathological phases of TBI and memory impairment (3). An unusually high prevalence of dementia after TBI shows the relative sensitivity of the hippocampus and the mesial temporal lobe compared to the rest of the brain (3-5). Electrophysiological investigations have also revealed that changes occur in hippocampal circuit excitability following TBI. Furthermore, TBI brings about changes in

Corresponding Author: Mousa-Al-Reza Hadjzadeh
Department of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
Tel: +98 51 38002221, Fax: +98 51 38828564, E-mail address: HajzadehMR@mums.ac.ir

cellular homeostasis, dysregulation in the synaptic transmission and neuronal loss in different regions of the hippocampus (6). Memory impairment, particularly impairment of episodic memory, is one of the first symptoms of AD (7). According to the literature, it has shown that oxidative stress plays a key role in the pathogenesis of this disease. Production of reactive oxygen species (ROS) causes oxidative stress which plays a significant role in neuronal injury in the damaged brain tissues (8,9). Oxidative stress and chronic inflammation play a critical role in neuronal cell death in the brain tissues when exposed to different types of neuronal insults, including hypoxia, ischemia, and TBI (9-11). Oxidative stress damage has been involved in the progression of memory and cognitive impairments as well as AD (12). Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are important markers of the antioxidant system. Antioxidant enzymes act as a major antioxidant defense system due to their having protective effects against oxidative damage following TBI (13). Likewise, malondialdehyde (MDA) as a product of lipid peroxidation is a highly toxic compound which in part is formed by oxidation of lipids by free radicals (14). An antioxidant is a substance that can prevent or delay oxidative stress-induced damages. Natural antioxidants are divided into four categories: allyl sulfides, carotenoids, flavonoids, polyphenols (15). Recently, the use of medicinal plants and natural substances with a focus on their antioxidant characteristics have been established for health promotion (16,17). Luteolin (LUT, 3′,4′,5,7-tetrahydroxyflavone) belongs to the flavonoid family, constitutes the most common group of polyphenolic compounds and is abundant in plant sources such as celery, carrots, chamomile tea, and green tea. There are several pieces of evidence both from in vitro and in vivo studies that LUT has neuroprotective properties. Additionally, LUT not only acts as an antioxidant (18), and radical-scavenging agent, but also possesses anti-inflammatory properties (19). It shows a potential protective effect against damages induced on neurons and has beneficial effects on memory and learning (20). It was reported that LUT could protect against memory impairment induced by chronic restraint stress in rats, via the modulation of oxidative stress and neuroinflammation level in brain tissues (21).

As LUT has been introduced as a natural source of antioxidant and can reduce the oxidative stress by different mechanisms, the goal of the present study was to investigate the final effects of LUT against memory impairment induced by TBI in male rats through behavioral studies, oxidative stress measurements, and histopathological investigations.

Materials and Methods

Animals

Male Wistar rats, weighing 250±50 g, and aged 2-3 months, were kept in standard cages with a temperature of 24-26° C and a 12/12 h light/dark cycle (light on at 07:00 am). Animal care and handling were performed according to the guidelines set by the Iranian Ministry of Health and Medical Education for lab animals. Animals had access to food and water ad libitum.

Experimental groups

Adult male Wistar rats (250±50 g at the beginning of experiments) were randomly divided into 6 groups (n=8 in each group) and were treated as follows:

- Control group: Animals did not receive any treatment or surgery.
- Sham group: Animals in this group underwent the surgical procedure of TBI without actual induction of TBI and received an equal volume of 1 % DMSO.
- TBI group: Animals underwent the surgical procedure and were injured using a standard TBI device and an equal volume of 1 % DMSO.
- TBI+LUT10: Induction of TBI was performed, and the animals received a dose of 10 mg/kg/intraperitoneal (IP) LUT for one week starting 48 hours after induction of TBI.
- TBI+LUT25 group: Induction of TBI was performed, and the animals received a dose of 25 mg/kg/ intraperitoneal (IP) LUT for one week starting 48 hours after induction of TBI.
- TBI+LUT50 group: Induction of TBI was performed, and the animals received a dose of 50 mg/kg/ intraperitoneal (IP) LUT for one week started 48 hours after Induction of TBI.

Preliminary data from our experiment showed that the middle (25 mg/kg) and high (50 mg/kg) doses of LUT were more effective. Therefore, we focused on the LUT treatment at 50 and 25 mg/kg per day to continue the study.

Induction of TBI

In this study, we intubated all animals before TBI (all animals were intubated with a 16-G angiocatheter using transillumination). The TBI was moderate and was diffused using the Marmarou's weight-drop model (22) . TBI model induced using a standard device according to

the method described as follow:

A 250 g weight was dropped from a two- meter height on the head of an anesthetized rat [ketamine (80 mg/kg) and xylazine (15 mg/kg)] when a metal disc (stainless steel) with 10 mm in diameter and 3 mm thickness is attached to the animal's skull. After the induction of the trauma, we immediately connected rats to the respiratory pump (TSA animal respiratory compact, Germany) and following the restoration of spontaneous breathing the endotracheal tube was removed. The scalp wound of the rats, after the weight drop, was closed with standard suture material and the rats were placed in individual cages (23,24).

LUT administration

LUT (Shanghai Yuan ye Bio-Technology Co., Ltd, Shanghai, China) with greater than 98% purity was dissolved in saline containing 1% DMSO. LUT or equal volumes of 1% DMSO were injected IP 48 h after the onset of TBI for 1 week, and during this time, behavioral tests were done.

Behavioral study

The cognitive ability was assessed using a step-through passive avoidance test to evaluate the effectiveness of LUT protection in TBI induced rats. We performed behavioral tests in the morning.

Passive avoidance task (PA apparatus and procedures)

The apparatus used for the passive avoidance task was a two-way shuttle box (Borj Sanaat Co.Tehran, Iran), which consisted of two adjacent plexiglass compartments of identical dimensions (27 cm×14.5 cm×14 cm) with grid floors. The floors of the two compartments were covered with stainless steel bars (2 mm diameter) spaced 1 cm apart. The compartment was illuminated by a 5 W lamp mounted on its wall just below a movable transparent Plexiglas ceiling. 48 h after surgery each rat was allowed a 10-minute adaptation period with free access either to the light or dark compartment of the avoidance training box after being placed in a shuttle-box in order to familiarize with instruments.

Two days after this adaptation period, rats were placed into the illuminated compartment and 30 seconds later the sliding door was raised (initial latency was recorded). Upon entering the dark compartment, the door was closed and a 1.5 mA constant-current was applied to the fore and hind paws for 3 seconds. After 20 seconds, the rat was removed from the dark compartment and placed into the home cage. In order to test short-term learning, 24 hours after receiving foot shock, the rats were placed in the illuminated chamber again and 30 seconds later the sliding door was raised and latency of entering the dark compartment was recorded again (as step-through latency). The maximum time considered in this procedure was 300 seconds (25,26).

Biochemical assessment

After learning and memory tests, animals were sacrificed and the hippocampal tissues were dissected and kept at -80° C for biochemical evaluations. Then, we homogenized the hippocampal samples in volumes of 9 g/L ice-cold normal saline (1:9 w/v). Supernatant was collected after centrifugation homogenates at 4000 rpm/min for 10 min at 40° C The supernatants were used for the evaluation of activities of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) using spectrophotometer; in accordance with the protocols provided with the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China).

Histopathological study

At the end of the behavioral tests brains of the rats were perfused using a transcardial injection of normal saline (200 mL) and subsequent amount of 200 mL of paraformaldehyde (4%, PFA, Sigma-Aldrich) in phosphate buffer (0.1 M). The brains carefully and quickly were removed and then, post-fixed in 10% formalin for 72 h at 4° C. After tissue processing and embedding the samples in paraffin, 5μm coronal sections were prepared using microtome (Leica Biosystems, Milan, Italy) and stained with hematoxylin and eosin (H and E) and Cresyl Violet Stainings (Nissl). The images of hippocampus area were prepared using a bright-field microscope (Olympus, CX31, Tokyo, Japan) equipped with an Olympus camera and analyzed by counting intact and dark neurons (DNs) in hippocampal Cornu Ammonis 1 (CA1) area (27). DN is a kind of cell degeneration observed with hyper-electron density and hyperbasophilia characteristics in histopathological investigations (27-29).

Statistical analysis

We expressed the results as mean ± SEM. All data were analyzed using SPSS-22 and compared by one-way ANOVA followed by Tukey's post hoc comparison test. We considered differences statistically significant at $P<0.05$.

Results

Effects of LUT on learning and memory in rats with TBI

We used passive avoidance learning test to evaluate the memory processes and learning performances of all groups. The results of PA test showed that there was a significant reduction in the latency time to enter the dark chamber in the TBI group (Figure 1), when compared to sham and control groups 48 hours after the TBI induction (P<0.05-P<0.001, respectively). Treatment with 25 µg/kg and 50 µg/kg doses of LUT increased the time latency at 48 hours after TBI induction compared to TBI group by P<0.05 and P<0.01, respectively (Figure 1).

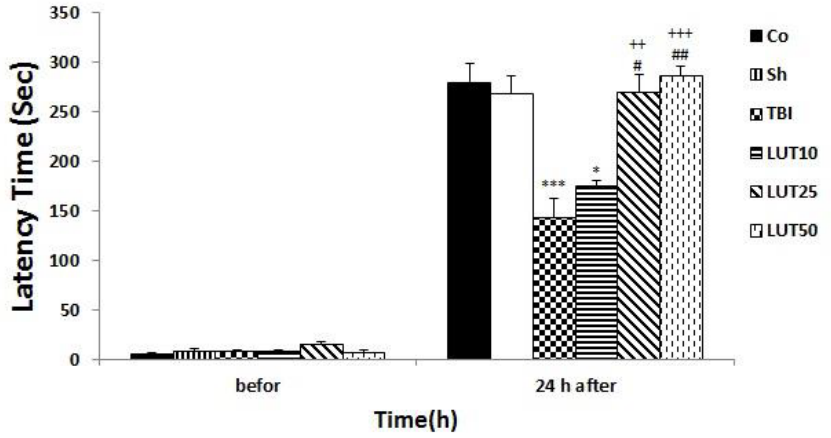

Figure 1. Effects of LUT on learning and memory in rats with TBI, Comparison the time spent in the dark (before shock) and light (after shock) room in PA test. $^{*}P$<0.05 and $^{***}P$<0.001 in comparison with Control group, ^{++}P<0.01 and ^{+++}P<0.001 in comparison with TBI group, $^{\#}P$ <0.05 and $^{\#\#}P$<0.01 in comparison with luteolin (10mg/kg) group. Co: normal group without any treatment or surgery, TBI: standard TBI device induction group with ICV injection of solvent, TBI+LU10: standard TBI device induction group with ICV injection of luteolin

Effects of LUT on anti-oxidant activity in rats with TBI

To evaluate the effect of LUT on TBI outcomes, the levels of MDA, SOD, and CAT in the brain tissues were measured. Biochemical analyses of brain tissues showed that MDA concentration in the hippocampal tissues was higher in TBI and LUT (10 µg/kg) groups than both control and sham groups (P<0.001), (Figure 2). Treatment with 25 µg/kg and 50 µg/kg doses of LUT significantly decreased the level of MDA in the hippocampus (P<0.001) compared to the TBI group. SOD (P<0.001) and CAT activities (P<0.01) were lower in the hippocampal tissues of TBI and LUT (10 µg/kg) groups than those of the control and sham groups. Treatment of animals with doses of 25 µg/kg and 50 µg/kg of LUT increased the levels of SOD (P<0.001) and CAT activity (P<0.05) in hippocampal tissues as compared to the TBI group (Figure 2).

Effects of LUT on the number of dark neurons of CA1 region in rats with TBI

The number of DNs (dark neurons) were counted in CA1 region of hippocampus in different groups in Nissl staining. The mean numbers of dark neurons in TBI, LUT10, LUT 25 and LUT50 groups were significantly higher than those in Control and Sham groups (P< 0.05, Figure 3) And a significant decrease was recorded in the mean number of DNs in LUT10, LUT 25 and LUT50 groups (P<0.001) when compared with TBI group (Figure 3).

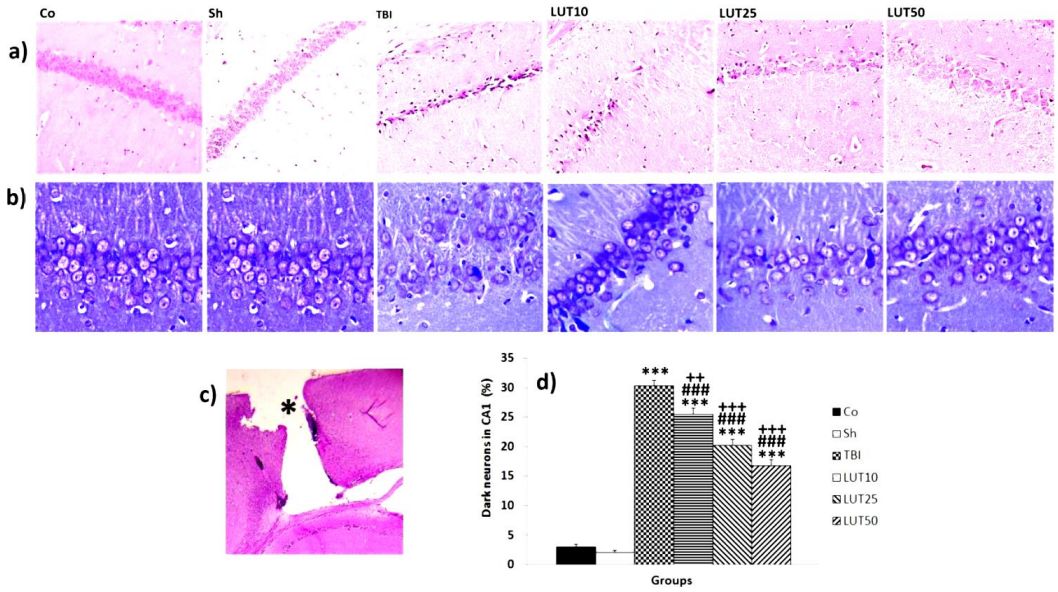

Figure 2. Photomicrograph of CA1 area of hippocampus in different groups: a H&E staining (x100), b, Cresyl Violet Stainings (Nissl) (×400) **c** (*) TBI site, **d** Comparing the dark neuron number in different groups. $^{***}P$<0.001 in comparison with Control group; ^{++}P<0.01 and ^{+++}P<0.001 in comparison with TBI group, ###P<0.001 compared to luteolin group; Co: normal group without any treatment or surgery, TBI: standard TBI device induction group with ICV injection of solvent, TBI+LU10: standard TBI device induction group with ICV injection of luteolin.

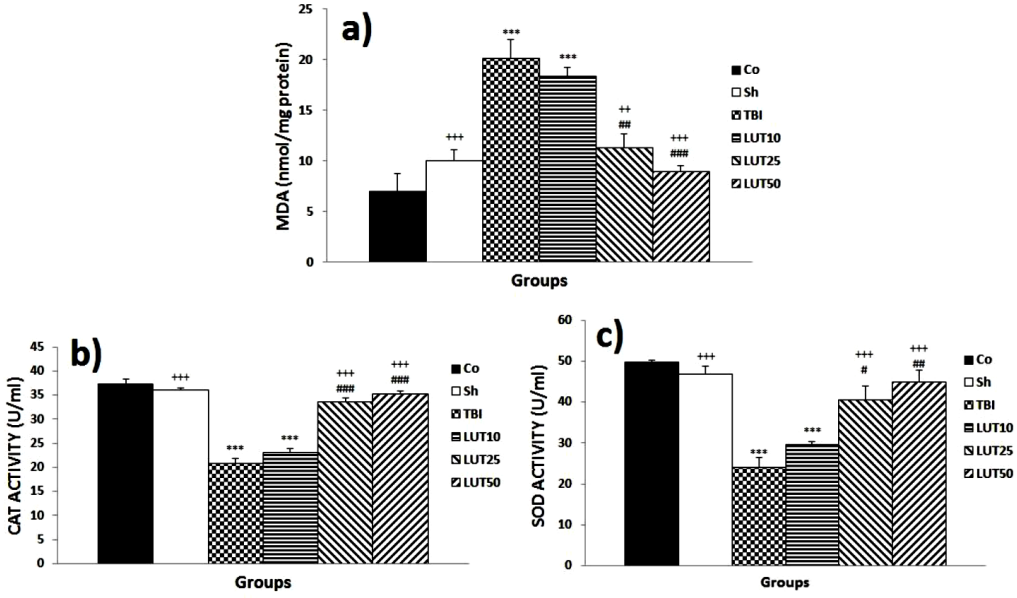

Figure 3. The antioxidant activity of LUT on hippocampal area of rats with TBI. **a** The MDA concentrations in hippocampal tissues, **b** The CAT activities in hippocampal tissues, **c** The SOD activities in hippocampal tissues. $^{***}P$<0.001 in comparison with Control group; ^{++}P<0.01 and ^{+++}P<0.001 in comparison with TBI group, ###P<0.001 in comparison to luteolin group. Co: normal group without any treatment or surgery, TBI: standard TBI device induction group with ICV injection of solvent, TBI+LU10: standard TBI device induction group with ICV injection of luteolin

Discussion

In this study, we used LUT to reduce TBI induced memory impairments in a rat model. Marmarou's weight-drop model was carried out to perform the experiment as a standard model of TBI (22). Different animal models have been introduced to induce TBI impairment in animal studies (30). Among them, a weight-drop model of TBI for rodents has been demonstrated to characterize the pathophysiology of this acquired brain injury. This kind

of cognitive impairment is similar to that observed in sporadic Alzheimer's dementia. It has been proven that induction of TBI in rats significantly induce memory impairment, Aβ plaques aggregation, τ protein hyperphosphorylation, neuroinflammation and apoptosis(31).

The results of Behavioral tests in the current study showed that the latency time to enter the dark chamber decreased in TBI group, which was due to a cognitive disability in this group and indicated the success of TBI model establishment. In addition, to confirm the model, the number of DNs and oxidative stress level were also examined in the TBI group, and both showed increasing trends. In the present study, increased MDA level and decreased CAT and SOD activities were observed which indicated the oxidative stress condition after induction of TBI. According to the literature, high-energy oxidants and oxidative stress are associated with TBI as mediators of secondary defect. The high amount of ROS production, as a result of the endogenous antioxidant system exhaustion and excitotoxicity, induces peroxidation of cellular structures, cleavage of DNA, oxidation of protein and suppression of the mitochondrial electron transport chain (32). Oxidative stress is the major agent involved in the pathophysiology of TBI, which leads to increased apoptosis and cell damage, progressive memory loss and cognition through increased free oxygen radicals (33). Furthermore, Ezaki et al. showed the degeneration of hippocampal neurons of both (CA1) and (CA3) areas following TBI in mouse induced by scalp incision (34). All these experimental findings confirmed the results of the current study.

There are numerous studies aimed at finding or developing pharmacotherapeutic agents targeted at ameliorating outcome of traumatic brain injury patients (35). According to the literature, the lack of success of neuroprotective agents clinically against TBI has been proven. However, using herbal and natural products with antioxidant properties against neurological impairments has been suggested in different studies (16). LUT is a member of the flavonoids family. The pharmacological function of LUT is thought to be partly associated with its antioxidant properties (36). Since the LUT as a small molecule can freely penetrate the blood-brain barrier (BBB) and enters the brain by peripheral administration, it is a reliable candidate in brain tissue impairments such as TBI or other cognitive disorders (37).

In this study, LUT with different doses of 10, 25 and 50 mg/kg was investigated for possible improvement of memory impairments by reducing DNs in the hippocampal region following TBI induction. The results of the present study indicate that the administration of LUT in doses of 25 and 50 mg/kg improved learning and memory by reducing the number of dark neurons and oxidative stress level after TBI in rats. Different studies demonstrated the neuroprotective effects of LUT against TBI. In a similar study, Sawmiller *et al*., (2014) reported that the administration of LUT improved memory and learning and reduced oxidative stress factors in animals with induced TBI and AD (38). Liu *et al*., (2013), demonstrated that LUT as a neuroprotective agent-induced memory-enhancing effect via modifying oxidative stress (39).

In the current study, injection of LUT with 25 and 50 mg/kg doses decreased the level of MDA and increased the CAT and SOD levels. These antioxidant properties of LUT could protect the neurons against oxidative stress. In agreement with these results, it has been reported that LUT injection reverses the oxidative stress state induced by TBI, which appears to be due to decreasing the level of MDA and increasing CAT and SOD activities (40). Xu *et al*., (2014) reported that LUT (with greater than 98% purity) improved learning and memory impairments following TBI in a Marmarou′s weight-drop model of mice by improving neurological deficits. Their results showed that LUT treatment reduced oxidative stress by increasing SOD and CAT activities and decreasing the level of MDA. They also showed that LUT protected the mice from TBI for 15 days post-TBI by decreasing mRNA and protein expressions of pro-inflammatory factors IL-1b and TNF-a and increasing the autophagy via expressions of autophagy markers. Moreover, LUT reduced BBB disruption, neuronal degeneration and alleviated brain edema in Wistar rat (9). Qiao et al. (2012) indicated that the LUT neuroprotection against cerebral ischemia was strongly related to its effect of increasing the endogenous antioxidant capability and thus mitigating the oxidative stress during ischemic stroke. LUT has direct antioxidant property by modulating or scavenging free radicals in ischemia-induced cellular injury(41). In favor of our findings, Zhao *et al*. reported that LUT can suppress the chronic cerebral TBI-induced cognitive dysfunction. They concluded that this effect might be attributed to the protective role of LUT against oxidative stress and inflammatory damages (42).

The findings of the present study show that LUT could subside the memory impairment resulted from TBI. The neuroprotective effect of LUT against TBI can be due to the antioxidative characteristic of this agent and inhibition of free radical production as well as the reduction of neuronal death in the hippocampus. Therefore, it may be suggested that LUT is a potential

therapeutic candidate for neural disorders such as TBI and that further studies including clinical trials are necessary to confirm this hypothesis.

Acknowledgments

This study was part of a Ph.D. thesis 931590 approved in mashhad University of Medical Sciences.

References

1. Williams AL. Traumatic brain injury. Physical Management for Neurological Conditions E-Book. 2018:p153.
2. Zohar O, Lavy R, Zi X, Nelson TJ, Hongpaisan J, Pick CG, et al. PKC activator therapeutic for mild traumatic brain injury in mice. Neurobiol Dis 2011;41:329-37.
3. Comper P, Bisschop S, Carnide N, Tricco A. A systematic review of treatments for mild traumatic brain injury. Brain Inj 2005;19:863-80.
4. Girgis F, Pace J, Sweet J, Miller JP. Hippocampal neurophysiologic changes after mild traumatic brain injury and potential neuromodulation treatment approaches. Front Syst Neurosci 2016;10:8.
5. Aligholi H, Rezayat SM, Azari H, Mehr SE, Akbari M, Mousavi SMM, et al. Preparing neural stem/progenitor cells in PuraMatrix hydrogel for transplantation after brain injury in rats: A comparative methodological study. Brain Res 2016;1642:197-208.
6. Lowenstein DH, Thomas MJ, Smith DH, McIntosh TK. Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. J Neurosci 1992;12:4846-53.
7. Guo Z, Cupples LA, Kurz A, Auerbach SH, Volicer L, Chui H, et al. Head injury and the risk of AD in the MIRAGE study. Neurology 2000;54:1316-23.
8. Bains M, Hall ED. Antioxidant therapies in traumatic brain and spinal cord injury. Biochim Biophys Acta 2012;1822:675-84.
9. 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.
10. Liu Y, Fu X, Lan N, Li S, Zhang J, Wang S, et al. Luteolin protects against high fat diet-induced cognitive deficits in obesity mice. Behav Brain Res 2014;267:178-88.
11. Mahakizadeh S, Akbari M, Sharifzadeh M, Rastegar T, Abolhassani F, Hassanzadeh G. Wharton'jelly mesenchymal stem cells and insulin effect on BDNF expression in CA1 and CA3 regions of rats' hippocampus after chronic hypoxia. J Contem Med Sci 2018;4:63-9.
12. Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 2004;3:205-14.
13. Loh KP, Qi J, Tan BKH, Liu XH, Wei BG, Zhu YZ. Leonurine protects middle cerebral artery occluded rats through antioxidant effect and regulation of mitochondrial function. Stroke 2010;41:2661-8.
14. Bazrafkan M, Nikmehr B, Shahverdi A, Hosseini SR, Hassani F, Poorhassan M, et al. Lipid peroxidation and its role in the expression of NLRP1a and NLRP3 genes in testicular tissue of male rats: A model of spinal cord injury. Iran Biomed J 2018;22:151.
15. Guhr G, Lachance PA. Role of phytochemicals in chronic disease prevention. Nutraceuticals: Designer foods III garlic, soy, and licorice. 1997:311-64.
16. 16. Bagheri A, Talei S, Hassanzadeh N, Mokhtari T, Akbari M, Malek F, et al. The neuroprotective effects of flaxseed oil supplementation on functional motor recovery in a model of ischemic brain stroke: Upregulation of BDNF and GDNF. Acta Med Iran 2018;55:785-92.
17. Mokhtari T, Faghir Ghanesefat H, Hassanzadeh G, Moayeri A, Jafar Haeri SM, Rezaee Kanavee A, et al. Effects of Flaxseed oil supplementation on renal dysfunction due to ischemia/reperfusion in rat. JBRMS 2017;4:22-9.
18. Zeng Lh, Zhang Hd, XU Cj, Bian YJ, Xu XJ, Xie QM, et al. Neuroprotective effects of flavonoids extracted from licorice on kainate-induced seizure in mice through their antioxidant properties. J Zhejiang Univ Sci B 2013;14:1004-12.
19. Thilakarathna SH, Rupasinghe H. Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients 2013;5:3367-87.
20. Beking K, Vieira A. Flavonoid intake and disability-adjusted life years due to Alzheimer's and related dementias: Public Health Nutr 2010;13:1403-9.
21. Tian X, Sun L, Gou L, Ling X, Feng Y, Wang L, et al. Protective effect of l-theanine on chronic restraint stress-induced cognitive impairments in mice. Brain Res 2013;1503:24-32.
22. Marmarou A, Foda MAA-E, Brink Wvd, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats: Part I: Pathophysiology and biomechanics. J Neurosurg 1994;80:291-300.
23. Khaksari M, Razmi Z, Hekmat AS, Naderi V, Rostami S. The effects of cyclooxygenase inhibitors on the brain inflammatory response following traumatic brain injury in rats. Iran J Basic Med Sci 2012;15:1102-5.
24. Keshavarzi Z, Khaksari M, Gholamreza A. The Effects of Female Sexual Hormones and Estrogen Receptor Agonists

on The NF-κB p50 and p65 Levels Following TBI in Rat Intestine. J Neurol Sci (Turkish) 2012;29.
25. Sarkaki A, Rezaiei M. Improving active and passive avoidance memories deficits due to permanent cerebral ischemia by pomegranate seed extract in female rats. Malays J Med Sci 2013;20:25-34.
26. Salmani H, Hosseini M, Beheshti F, Baghcheghi Y, Sadeghnia HR, Soukhtanloo M, et al. Angiotensin receptor blocker, losartan ameliorates neuroinflammation and behavioral consequences of lipopolysaccharide injection. Life Sci 2018;203:161-70.
27. Mokhtari T, Akbari M, Malek F, Kashani IR, Rastegar T, Noorbakhsh F, et al. Improvement of memory and learning by intracerebroventricular microinjection of T3 in rat model of ischemic brain stroke mediated by upregulation of BDNF and GDNF in CA1 hippocampal region. Daru 2017;25:4.
28. Amiri S, Yousefi-Ahmadipour A, Hosseini M-J, Haj-Mirzaian A, Momeny M, Hosseini-Chegeni H, et al. Maternal exposure to silver nanoparticles are associated with behavioral abnormalities in adulthood: Role of mitochondria and innate immunity in developmental toxicity. Neurotoxicology 2018;66:66-77.
29. Bideskan ARE, Ataei ML, Mansouri S, Hosseini M. The effects of tamoxifen and soy on dark neuron production in hippocampal formation after pentylenetetrazole-induced repeated seizures in rats. Pathophysiology 2015;22:125-35.
30. Ooigawa H, Nawashiro H, Fukui S, Otani N, Osumi A, Toyooka T, et al. The fate of Nissl-stained dark neurons following traumatic brain injury in rats: difference between neocortex and hippocampus regarding survival rate. Acta Neuropathol 2006;112:471-81.
31. Salkovic-Petrisic M, Hoyer S. Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach. J Neural Transm Suppl 2007;72:217-33.
32. Cornelius C, Crupi R, Calabrese V, Graziano A, Milone P, Pennisi G, et al. Traumatic brain injury: oxidative stress and neuroprotection. Antioxid Redox Signal 2013;19:836-53.
33. Varadarajan S, Yatin S, Kanski J, Jahanshahi F, Butterfield DA. Methionine residue 35 is important in amyloid β-peptide-associated free radical oxidative stress. Brain Res Bull 1999;50:133-41.
34. Ezaki J, Shimada R, Shibuya M, Kibayashi K. Hippocampal neuronal degeneration in the traumatic brain injury mouse: non-trivial effect of scalp incision. Neurol Res 2016;38:994-1002.
35. Eghwrudjakpor P, Allison A. Oxidative stress following traumatic brain injury: enhancement of endogenous antioxidant defence systems and the promise of improved outcome. Niger J Med 2010;19:14-21.
36. Ashaari Z, Hassanzadeh G, Alizamir T, Yousefi B, Keshavarzi Z, Mokhtari T. The Flavone Luteolin Improves Central Nervous System Disorders by Different Mechanisms: A Review. J Mol Neurosci 2018;65:491-506.
37. Xia F, Wang C, Jin Y, Liu Q, Meng Q, Liu K, et al. Luteolin protects HUVECs from TNF-α-induced oxidative stress and inflammation via its effects on the Nox4/ROS-NF-κB and MAPK pathways. J Atheroscler Thromb 2014;21:768-83.
38. Sawmiller D, Li S, Shahaduzzaman M, Smith AJ, Obregon D, Giunta B, et al. Luteolin Reduces Alzheimer's Disease Pathologies Induced by Traumatic Brain Injury. Int J Mol Sci 2014;15:895-904.
39. Liu Y, Tian X, Gou L, Sun L, Ling X, Yin X. Luteolin attenuates diabetes-associated cognitive decline in rats. Brain Res Bull 2013;94:23-9.
40. Choi SM, Kim BC, Cho YH, Choi KH, Chang J, Park MS, et al. Effects of flavonoid compounds on β-amyloid-peptide-induced neuronal death in cultured mouse cortical neurons. Chonnam Med J 2014;50:45-51.
41. Qiao H, Dong L, Zhang X, Zhu C, Zhang X, Wang L, et al. Protective effect of luteolin in experimental ischemic stroke: upregulated SOD1, CAT, Bcl-2 and claudin-5, down-regulated MDA and Bax expression. Neurochem Res 2012;37:2014-24.
42. Zhao G, Qin GW, Wang J, Chu WJ, Guo LH. Functional activation of monoamine transporters by luteolin and apigenin isolated from the fruit of Perilla frutescens (L.) Britt. Neurochem Int 2010;56:168-76.