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Ellagic acid prevents cognitive and hippocampal long-term potentiation deficits and brain inflammation in rat with traumatic brain injury

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ABSTRACT

Aims: Traumatic brain injury (TBI) remains one of the main clinical problems globally and is a common cause of death among youth. Cognitive defects such as thinking, memory and behavior or mental health disorders are considered as the most frequent effects of severe and moderate TBI. It has been reported that ellagic acid (EA), a natural polyphenol, exhibits protective effects against oxidative damage. This study was performed to examine the EA preventive effects on cognitive impairments, long-term potentiation (LTP) deficits in hippocampus and brain inflammation induced by diffuse TBI in rat.
Main methods: Subchronic oral administration of 100 mg/kg EA, 7 consecutive days before induction of trauma (once daily) was used to elucidate the EA effects on passive avoidance memory and hippocampal LTP following TBI. To illustrate the possible mechanisms related to the preventive effects of EA on brain function following TBI, brain content of IL-1β, IL-6 and blood–brain barrier (BBB) permeability were determined.
Key findings: EA pretreatment significantly ($P < 0.001$) prevented TBI-induced memory and hippocampal LTP impairments in rat. Furthermore TBI induced elevation in brain content of IL-1β, IL-6 and BBB permeability were decreased significantly ($P < 0.001$) due to EA pre-treatment.
Significance: Our findings suggest that EA can prevent cognitive and LTP deficits and also prevent brain inflammation following TBI.

Introduction

Traumatic brain injury (TBI) remains one of the main clinical problems globally and is a common cause of death among youth [17,25]. In the USA, about 1.7 million people survive a TBI annually, among whom 275,000 are hospitalized [17]. TBI occurs in two stages: 1) primary injury, indicated by destruction of the brain tissue and blood vessels, which initiates complex physiological processes involving cellular and molecular events; 2) secondary injury, the processes that occurs hours to days after TBI which lead to further injury on neurons and axons [2,4]. Therapeutic policies have focused on preventing secondary injury [25]. Inflammation has a main role in secondary brain injury [50]. Previous studies have shown many examples of cytokine production after TBI [25].

Cognitive defects such as thinking, memory, and reasoning problems as well as behavior or mental health disorders are among the most frequent sequelae after severe and moderate TBI [3,24,72]. The hippocampal areas especially dentate gyrus (DG) neurons are vulnerable to TBI [27]. It has been shown that neuronal death in the DG following TBI leads to learning and memory impairments in adult rodents [26]. Long-term potentiation (LTP) in hippocampal synapses has been proposed as a model for the cellular changes that underlie learning and memory [64].

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Naturally occurring polyphenols are known as capable alternatives for use as pharmaceuticals ([38]). Previous studies suggested a relation between phenolic food intake and the protection against several diseases [41]. Polyphenols are plant metabolism products and they have antioxidant functions [32,43]. Ellagic acid (EA) (2,3,7,8-tetrahydroxybenzopyrano[5,4,3-cde]benzopyran-5-10-dione) is a polyphenol present in many plant species such as pomegranate plants, grapes, raspberries, blackberries, strawberries and walnuts [14,75]. Several line of studies have shown that EA has different pharmacological effects such as anti-bacterial, anti-inflammatory, immune regulatory and inhibition of tumorigenesis and also it is considered as a potent antioxidant [6,12,18].

The pretreatment approach has long been successfully employed for the neuroprotection against TBI [36,46,79,80]. This recognition has led us to choose the pretreatment strategy in this study. To the best of our knowledge, there is no published scientific report on the effects of EA on brain inflammation, learning and memory deficits induced by TBI. Therefore, the present study intended to examine the preventive effects of EA on avoidance memory and hippocampal LTP deficits induced by closed head injury and determined whether these neuroprotective effects were modulated through anti-inflammatory mechanisms in the brain.

Materials and methods

Animals and experimental groups

The Ahvaz Jundishapur University of Medical Sciences Institutional Animal Care and Use Committee approved all experiments, and the procedures followed the NIH Guide for the care and use of experimental animals [20]. Adult male Wistar rats (250 ± 20 g) purchased from Ahvaz Jundishapur University of Medical Sciences Animal House (Ahvaz, Iran) were housed in clear cages in temperature (22 ± 2 °C) and humidity (50%) controlled conditions and 12/12 h light/dark cycle. Animals had free access to food and water ad libitum and allowed to adapt to the laboratory conditions for at least 7 days before the study. The rats were randomly assigned to Control, Sham-injury, Veh + TBI and EA + TBI groups (n = 24 for each group). Both Sham-injury and Veh + TBI rats received EA vehicle (10% DMSO in normal saline in a total volume of 10 ml/kg, once daily) orally for 7 consecutive days before induction of trauma (Gavage needle about 11 cm long with a 15° curved blunt ended needle). Animals in the Sham-injury group underwent TBI procedures but were not exposed to TBI while the Veh + TBI animals were exposed. Control and EA + TBI rats orally received a dose of 100 mg/kg EA and 10% DMSO in normal saline as solvent (in a total volume of 10 ml/kg, once daily) for 7 consecutive days before induction of trauma using the Gavage needle (as described above). Animals in the EA + TBI group were exposed to trauma while the rats in the Control group did not undergo any procedure (naive rats). To habituate the animals to oral administration, all rats received normal saline (10 ml/kg, by gavage) daily for three days prior to the experiments. We used 8 rats in each experimental group to perform passive avoidance memory and electrophysiological tests, 8 rats for determination of blood–brain barrier permeability and 8 rats for determination of brain IL-1β and IL-6 content. Every possible effort was made to minimize animal suffering.

Chemicals

We purchased ellagic acid (purity ≥ 95%), Evans blue, dimethyl sulfoxide (DMSO) and Triton X-100 from Sigma-Aldrich Co. (St. Louis, MO, USA), protease inhibitor cocktail from Roche (Basel, Switzerland), T-PER™ Tissue Protein Extraction Reagent from Pierce Biotechnology Inc. (Rockford, IL, USA), and a Bio-Rad protein assay kit from Bio-Rad Laboratories (Hercules, CA, USA). Tris base, sodium phosphate, sodium chloride, potassium phosphate and potassium chloride were of analytical grade and obtained from Merck Co. (Darmstadt, Germany). EA was dissolved in 10% DMSO in normal saline. Drug was freshly prepared so that the necessary dose could be given in a total volume of 10 ml/kg orally route. EA dose and administration schedules were selected based on our pilot studies and previous reports [22,54,66].

Induction of brain trauma

Tracheal intubation was performed while the rats were under an appropriate level of ketamine/xylazine (50/5 mg/kg, IP) anesthesia before TBI [60]. Then animals in the Veh + TBI and EA + TBI groups were exposed to diffuse traumatic brain trauma using an instrument made in the Physiology Research Center of Ahvaz Jundishapur University of Medical Sciences with the Marmarou method [29,39]. As it is instructed in this method, a 200 g weight was dropped from a 2-m height through a free-falling tube onto the head of an anesthetized animal while a steel disk was attached to the animal's skull. After brain trauma induction, the animal was immediately connected to an animal respiratory pump (Ugo Basile, Italy) and as soon as it was spontaneously breathing, it was disconnected from the ventilator and returned to the cage to be cared for [30].

Passive-avoidance test

A step-through latency test in a shuttle-box was performed to evaluate the effects of subchronic EA pretreatment on avoidance memory in rats. The shuttle-box apparatus (Borj sanat, Tehran, Iran) consisted of two equally sized (200 × 250 × 200 mm) compartments, a lighted one and a dark one with two independent grid floors. The compartments were separated by a guillotine door. As an accommodation session, the animal was placed in the lighted chamber while the guillotine door was opened and allowed to explore both compartments for 10 min and then removed. After 10 min the animal was again placed in the lighted compartment facing away from the closed guillotine door and 10 s later the door was elevated and the entering delay of rat into the dark compartment was recorded as initial latency (IL). Immediately after entering the dark chamber, the guillotine door was closed and an unavoidable foot-shock (75 V, 0.2 mA, 50 Hz for 3 s) was delivered using a shock generator. Twenty-four hours after the initial session, the retention test was carried out. In this session the animal was again placed in the lighted compartment and the step-through latency (STL) was measured. The max latency was recorded as 300 s [34,38].

Electrophysiological studies

Surgical procedure

Forty-eight hours after TBI induction, the animals were prepared for electrophysiological recordings. They underwent an appropriate level of ketamine/xylazine (50/5 mg/kg, IP) anesthesia and their heads were mounted on a stereotaxic device for surgery (electrode implantation and EPSP recording) [60]. The animal's body temperature was maintained at 36.5 ± 0.5 °C using a heating pad. The animal's skull was drilled and small holes were made to implant the electrodes. A pair of stimulating metal wire microelectrode (stainless steel, 100 μm in diameter, tip separation 500 μm, CFW, USA) and a pair of recording metal wire microelectrodes (tungsten, 50 μm in diameter, tip separation 1 mm, CFW, USA) were implanted into the perforant pathway (PP) at AP = −7.5 mm from bregma; ML = −4 mm; DV = −3.9 mm from dura and granular cells of DG with stereotaxic coordination of AP = −3.8 mm from bregma; ML = −2.3 mm; DV = −3.5 mm from dura, respectively [49]. In order to decrease brain tissue damage, both electrodes were lowered very slowly (0.1 mm/30 s) [34].

Electrophysiological recordings and LTP induction

Following the stimulation of PP, the field potential recordings were obtained in DG granular cells. The PP was stimulated every 30 s. The

electrodes were placed in a way such that the max field excitatory post-synaptic potential (fEPSP) can be evoked. The voltage difference between the peaks of the first positive wave and the first negative deflection was determined as the post-tetanic stimulation population spike (PS) amplitude. The max slope between the starting point of fEPSP and the first positive peak was considered as the fEPSP slope. The Biochart software (Biochart software version 1.53, Science Beam Co., Iran) was used to amplify (1000×), filter (0.1 Hz to 3 kHz), digitize, record, and analyze the extracellular field potentials. A high-frequency stimulation (HFS) protocol consists of 10 bursts of 20 stimuli (0.2 ms) at 400 Hz, and a 10 s interburst interval [37,69] was used to induce LTP. The stimulus intensity that was able to elicit a PS amplitude and fEPSP slope of about 80% of max response was used as the HFS stimulus intensity. Different intensities were used to obtain the input/output (I/O) curve and a stimulus intensity which produced 40% of max response was defined as the baseline intensity (before and after HFS) for LTP recording [19,69]. LTP was recorded for periods of −1, 0, 0.25, 0.5, 1, and 3 h after the HFS [34,59,69].

Brain sample collection and ELISA assays

Animals were anesthetized with ketamine/xylazine (as described above) 48 h after trauma, and were perfused intracardially with phosphate buffered saline (pH = 7.4) for 1 min. Animal brains were immediately removed and frozen in a freezer (−80 °C) until assay. Each brain was weighed and homogenized in the T-PER™ Tissue Protein Extraction Reagent (Pierce) with 0.5% Triton X-100, 150 mM NaCl, 50 mM Tris, and Roche protease inhibitor cocktail (500 mg tissue per 2 ml of the reagent). Samples were then shaken for 90 min using a shaker and the suspensions were centrifuged (4000 ×*g* for 15 min at 4 °C) and the supernatants were collected. To confirm that an equal amount of protein from each sample was used for ELISA assays, the Bio-Rad protein assay kit (based on the Bradford dye-binding method) was used to estimate the protein content of the supernatants [8,61,68].

ELISA kits for IL-1β and IL-6 were purchased from eBioscience (San Diego, USA) and the assay was performed according to the manufacturer's guidelines. The concentrations of IL-1β and IL-6 were quantified as picograms of antigen per milliliter of the supernatant.

Determination of blood–brain barrier permeability

Blood–brain barrier (BBB) permeability was determined through measuring extravascular Evans blue dye and using a spectrophotometer device. 48 h after trauma, brain vascular permeability was measured by the injection of Evans blue dye via the femoral vein [28,44]; at 47 h after trauma, animals were anesthetized with ketamine/xylazine (as described above), next, 20 mg/kg Evans blue dye 2% (1 ml/kg) was injected through the femoral vein. One hour after injection (at 48 h after trauma), thorax was opened and descending aorta was clipped. Then, 200–300 ml isotonic saline solution was infused into the left ventricle for 20 min to remove intravascular Evans blue dye. For this purpose, jugular vein was cut bilaterally and infusion was continued until complete removal of Evans blue [28,44]. The rats were decapitated and each brain was immediately removed and homogenized using phosphate buffered saline. In order to precipitate the protein, trichloroacetic acid was added. Then each sample was cooled and centrifuged. The supernatant was taken and the spectrophotometer was used to measure the absorbance of Evans blue at 620 nm. The amount of color based on μg/mg brain tissue was calculated by the following formula:

Evans blue dye (μg) in brain tissue (g) = (13.24 × 20 × absorbance) / tissue weight. A higher amount of dye in brain tissue represents more vascular permeability and more severe blood–brain barrier disruption [28,44].

Evaluation of neurological outcomes

Neurological outcome was assessed based on veterinary coma scale (VCS) [28,31]. In this scale a total score (3–15) is obtained by adding the scores of motor response (1–8), visual response (1–4) and respiratory response (1–4) (Table 1) [31]. A higher score represents better neurological outcome. In the present study, neurological outcomes were also assessed at −1, 1, 4, 24, and 48 h post trauma injury. Based on the VCS score, the severity of head injury can be categorized; mild (13–15), moderate (9 –12), and severe (8 or less) [31,52,53,61,65] (Table 1).

Table 1
Veterinary coma scale.
Adapted from [31].

	Variable	Score
Motor function	Normal movement	8
	Mildly drowsy with spontaneous, purposeful movements	7
	Lethargic, unable to stand, but maintains sternal recumbency	6
	Lethargic, withdraws to pinch, and lifts head with attention to visual stimuli; no sternal recumbency	5
	Withdraws or pedals to pinch	4
	Spontaneous pedaling	3
	Extensor posturing (spontaneous or to stimuli)	2
	Flaccid to stimuli	1
Eye function	Open	4
	Open on stimulation	3
	Normal eyelid reflexes	2
	No eyelid response to stimuli	1
Respiration	Normal	3
	Ataxic	2
	Apneic	1

Statistical analysis

The results are presented as mean ± SEM and the data were analyzed by one-way ANOVA followed by Tukey's post-hoc test. Neurological scores were analyzed using repeated measures one-way ANOVA followed by Tukey's post hoc test. LTP data were analyzed using repeated measures two-way ANOVA followed by Tukey's post hoc test. P-values less than 0.05 were considered to be statistically significant. Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, USA).

Results

According to data analysis, there were no differences among the Control and Sham-injury groups in all experimental parameters (data not shown). Therefore, to verify the results, comparisons between the Control group and the other groups were conducted.

Passive avoidance memory

Fig. 1 shows the preventive effects of subchronic pretreatment of EA on initial latency (IL) and step-through latency (STL) in TBI rats. One-way ANOVA analysis followed by Tukey's post-hoc test revealed that after oral administration of EA (100 mg/kg) for 7 consecutive days before induction of trauma (once daily), there were no differences in IL between groups [$F(2,21) = 1.142$, $P < 0.38$]. STL significantly increased in TBI rats pretreated with EA [$F(2,21) = 117.1$, $P < 0.001$, for Control (257.48 ± 16.10) vs. Veh + TBI (32.65 ± 3.28) and $F(2,21) = 86.61$, $P < 0.001$, for EA + TBI (209.90 ± 12.09) vs. Veh + TBI)].

Electrophysiology

PS amplitude

Fig. 2A shows the PS amplitude in all tested groups. Repeated measures two-way ANOVA followed by Tukey's post-hoc test indicated that PS amplitude during 0.25, 0.5, 1, and 3 h after HFS in Veh + TBI has decreased significantly [$(F(10,105) = 17.37$, $P < 0.001)$] vs. Control group. Oral administration of EA (100 mg/kg) for 7 consecutive days before TBI induction (once daily) reversed PS amplitude significantly at all recording times [$F(2,21) = 20.85$, $P < 0.001$] compared to Veh + TBI.

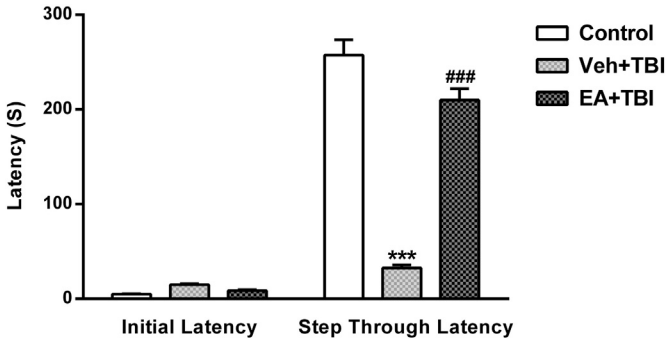

Fig. 1. Initial latency and step-through latency (memory retention) 48 h after TBI. Memory retention was impaired in the Veh + TBI group (***$p < 0.001$ vs. Control). As seen in Fig. 1, oral administration of EA (100 mg/kg) for 7 consecutive days before TBI induction (once daily) reversed memory 48 h after TBI (###$p < 0.001$ vs. Veh + TBI). Values are represented as mean ± SEM, one-way ANOVA followed by post-hoc Tukey's test, n = 8.

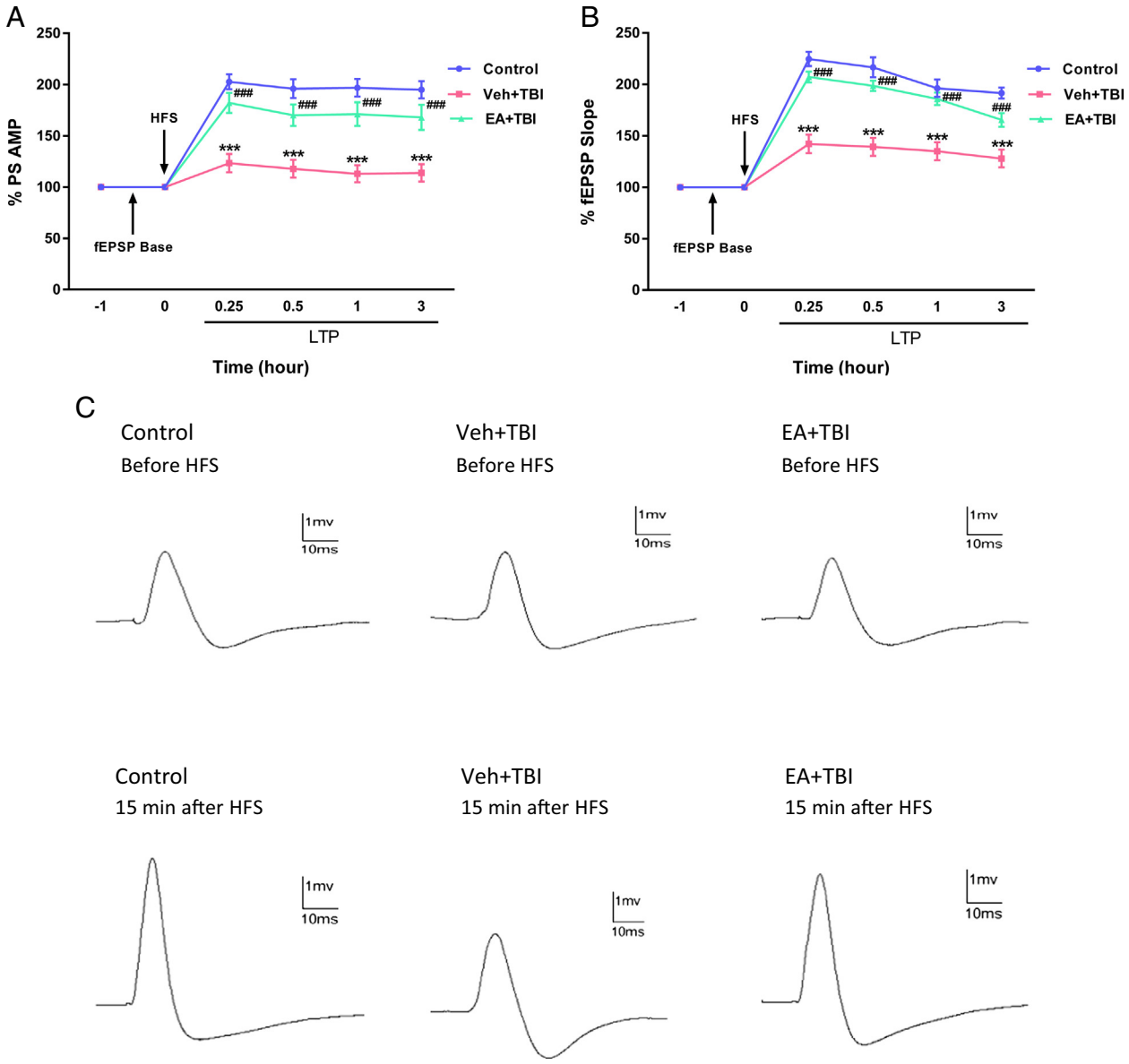

Fig. 2. Population spike amplitude (A), fEPSP slope (B) and sample traces recorded (C) from the hippocampal dentate gyrus area before and after high frequency stimulation. PS amplitude and fEPSP slope were impaired in the Veh + TBI group (***$p < 0.001$ vs. Control). Fig. 2 shows that oral administration of EA (100 mg/kg) for 7 consecutive days before TBI induction (once daily) reversed amplitude and slope 48 h after TBI (###$p < 0.001$ vs. Veh + TBI). Values are represented as mean ± SEM, RM-ANOVA followed by post-hoc Tukey's test, n = 8.

PS slope

Fig. 2B indicates the effects of oral administration of EA (100 mg/kg) for 7 consecutive days before TBI induction (once daily) on PS slope in TBI rats. Repeated measures two-way ANOVA analysis followed by Tukey's post-hoc test revealed that in all LTP recording times PS slope significantly decreased in the Veh + TBI group [($F(10,105) = 19.43$, $P < 0.001$)] vs. Control, while in the EA + TBI group it was increased significantly [($F(2,21) = 24.79$, $P < 0.001$)] vs. Veh + TBI.

Sample recorded traces

Sample recorded traces from hippocampal DG area before and after the 400 Hz tetanic stimulation are represented in Fig. 2C.

Brain IL-1β content

Fig. 3 shows the effects of oral administration of EA (100 mg/kg) for 7 consecutive days before TBI induction (once daily) on whole brain tissue IL-1β content. One-way ANOVA analysis followed by Tukey's post hoc test revealed a significant increase of IL-1β in the Veh + TBI group [($F(2,21) = 86.79$, $P < 0.001$)] vs. Control, while in the EA + TBI group it was decreased significantly [($F(2,21) = 86.79$, $P < 0.001$)] vs. Veh + TBI.

Brain IL-6 content

Fig. 4 shows the effects of oral administration of EA (100 mg/kg) for 7 consecutive days before TBI induction (once daily) on whole brain tissue IL-6 content. One-way ANOVA analysis followed by Tukey's post hoc test revealed a significant increase of IL-6 in the Veh + TBI group [($F(2,21) = 65.87$, $P < 0.001$)] vs. Control, while in the EA + TBI group it was decreased significantly [($F(2,21) = 65.87$, $P < 0.001$)] vs. Veh + TBI.

Brain Evans blue dye content

Fig. 5 shows the amount of brain Evans blue dye content in the studied groups. A higher amount of Evans blue dye in brain tissue represents more vascular permeability and more severe blood–brain barrier disruption. One-way ANOVA analysis of data and Tukey's post-hoc test indicated that the amount of Evans blue dye in the Veh + TBI group increased significantly [($F(2,21) = 63.49$, $P < 0.001$)] compared to the Control group, while there was a significant decrease in the EA + TBI [($F(2,21) = 63.49$, $P < 0.001$)] vs. the Veh + TBI group.

Evaluation of neurological outcomes

As seen in Fig. 6, according to repeated measures one-way ANOVA analysis followed by Tukey's post-hoc test, the score of neurological outcomes in the Veh + TBI group was significantly decreased [($F(10,105) = 49.73$, $P < 0.001$)] vs. Control in all recording times after TBI. The score of neurological outcomes in the EA + TBI group at 1, 4, 24 and 48 h after TBI showed a significant increase [($F(2,21) = 275.5$, $P < 0.001$)] compared to Veh + TBI.

Discussion

The present study intended to evaluate the preventive effects of subchronic pretreatment of EA, a natural polyphenolic compound, on brain function such as cognitive and neurological outcome, induction and persistence of LTP at the DG area of hippocampus and brain tissue inflammation after TBI in rats. TBI produced a marked impairment in passive avoidance memory, which was associated with a significant decrease in hippocampal LTP, as well as impaired BBB permeability and elevated levels of IL-1β and IL-6 in brain tissue. The main findings of this study indicate that pretreatment with EA: 1) significantly improved passive avoidance memory; 2) significantly reversed the PS amplitude and slope toward Control; 3) significantly restored BBB permeability; 4) significantly recovered brain content of IL-1β and IL-6 to normal levels: and 5) significantly restored neurological outcome to normal level.

The hippocampus, a critical region for memory formation, is one of the most susceptible parts of the brain during TBI. LTP as a cellular mechanism of memory is a long lasting enhancement in synaptic plasticity, and much focus on LTP has been in the hippocampus neural circuits [13,47,63]. Following TBI in rat, a stable incapability to induce LTP has been shown in several models of TBI [51,58]. Weight-drop injury (closed head injury), fluid percussion injury (FPI), cortical contusion injury (CCI), and in vitro TBI models such as biaxial stretch are the most common models to induce TBI in rodents [10,40]. Closed head injury was used in the present study because this model leads to a diffuse axonal injury in rodents, the same happens in humans following TBI [1].

Our data indicated that EA prevented the increase of brain contents of IL-1β and IL-6 which may lead to maintenance of hippocampal LTP, restoration of neurological behaviors and also repair of the BBB. These findings are in consistent with Chao et al.'s findings about the effect of EA, which efficiently decreases the expression of inflammatory cytokines including IL-1β and IL-6 in rats and shows its anti-inflammatory activity [11].

Cytokines are found at low concentrations in the nervous system but rapidly increase in pathological conditions such as brain trauma [5]. As shown in our results, the brain contents of IL-1β and IL-6 were increased after TBI (Fig. 3, Fig. 4). Recent studies indicate that IL-1β and IL-6 are involved in the molecular and cellular mechanisms of learning and memory [5,77]. Both neurogenesis and memory in the hippocampus depends on cytokines [5]. IL-6 leads to a significant reduction in LTP expression which affects hippocampal synaptic plasticity [67]. IL-6 inhibitory effects are related to the activation of the interleukin 6 signal transducer (IL6ST). The phosphorylation of IL6ST leads to its association with Janus kinase (JAK) tyrosine-protein kinases and signal transducer and activator of transcription 3 (STAT-3) which are together with an

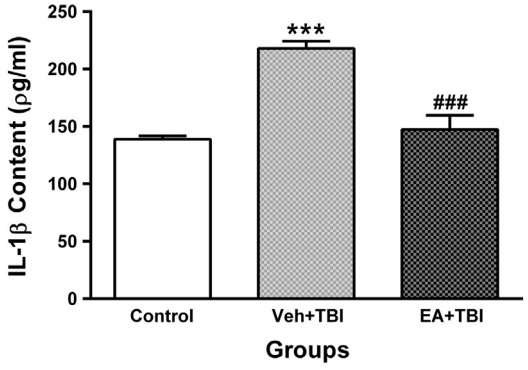

Fig. 3. This figure indicates the brain tissue content of IL-1β 48 h after TBI. IL-1β was elevated significantly after TBI (***$p < 0.001$ vs. Control). Oral administration of EA (100 mg/kg) for 7 consecutive days before induction of TBI (once daily) reversed IL-1β 48 h after TBI (###$p < 0.001$ vs. Veh + TBI). Values are represented as mean ± SEM, one-way ANOVA followed by post-hoc Tukey's test, n = 8.

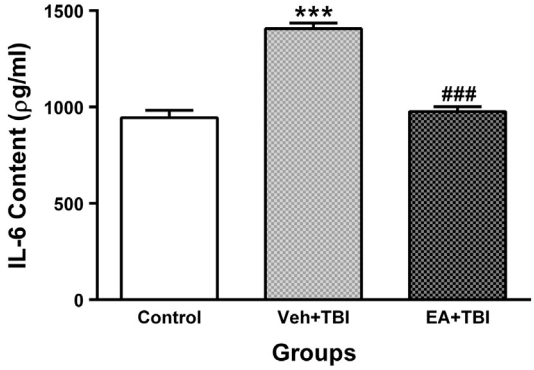

Fig. 4. This figure shows the brain tissue content of IL-6 48 h after TBI. IL-6 was elevated significantly after TBI (***$p < 0.001$ vs. Control). Oral administration of EA (100 mg/kg) for 7 consecutive days before induction of TBI (once daily) reversed IL-6 48 h after TBI (###$p < 0.001$ vs. Veh + TBI). Values are represented as mean ± SEM, one-way ANOVA followed by post-hoc Tukey's test, n = 8.

inhibition of the mitogen activated protein kinase (MAPK)/extra cellular signal-regulated kinase (ERK) pathway and has no significant effects on the stress-activated protein kinases (SAPK)/jun amino-terminal kinases (JUK) pathway [67]. As seen in our results the decreased fEPSP slope and PS amplitude observed in rats with TBI were associated with elevated levels of IL-6 in brain tissue (Fig. 4). The mechanisms that regulate IL-6 expression in the hippocampus have yet to be elucidated [76]. Many activities of IL-1β on glial cells are mediated by IL-6 [70].

It is clear that IL-1β has a role as a neuromodulator in the hippocampus [62] and the maintenance of LTP in hippocampus depends on it [15,55,73]. Although IL-1β receptors are found in all parts of the brain, the highest densities of these receptors are in the hippocampus and they are localized in the granular neurons of DG [7,48,56]. It has been shown that synaptic transmission enhancement which happens during LTP is closely linked to AMPA-type glutamate receptor (AMPAR) activities [33]. Furthermore previous studies have shown the role of IL-1β as an N-methyl-D-aspartate receptor (NMDAR) modulator [74]. On the other hand it has been suggested that AMPAR behavior is modulated via activation of NMDARs and its associated Ca^{2+} influx [9,21,35]. IL-1β raises the Ca^{2+} influx through NMDA receptors by activating Src kinases which leads to NR2A/B subunit phosphorylation [74]. Elevated levels of IL-1β cause memory impairment [23]. It has been suggested that three events affecting the magnitude of LTP, involving glutamate release modulation, the function of NMDARs and calcium channel influx can be influenced by IL-1β [45]. Our observations illustrated that inhibition of hippocampal LTP was associated with a significant increase in brain content of IL-1β (Fig. 3). The results of our study have shown that subchronic administration of EA before TBI induction maintained the hippocampal LTP due to a significant decrease in brain content of IL-1β and IL-6. As shown in Fig. 3 and Fig. 4, EA pretreatment reduces brain content of IL-1β and IL-6 to normal levels. Together with other parts of our results, the effect of EA on LTP recovery may directly depend on its anti-inflammatory effects through decreasing the elevated levels of IL-1β and IL-6.

To our knowledge, this is the first study using EA to prevent synaptic plasticity impairment following TBI. The results of the present study suggest that EA may act as a potent neuroprotective component against TBI. Our results indicate that hippocampal LTP was recovered to control levels. The decreased fEPSP slope and PS amplitude observed in rats with TBI were significantly prevented in EA pretreated animals. According to our behavioral data and neurological outcome scores, EA pretreatment improves the neurological behaviors. Furthermore, in EA pretreated rats the BBB function was normal and the brain content of IL-1β and IL-6 had no significant changes when compared with the Control group. Our data indicated that EA decreases brain content of IL-1β and IL-6 (compared with TBI induced rats) which may lead to restoring hippocampal LTP, restoring neurological behaviors and also maintenance of BBB, which may show the effects of EA on astrocyte recovery due to its anti-inflammatory actions. Although several reports have indicated different effects of EA such as antimutagenic, anti-inflammatory, antiviral, anticarcinogenic, antioxidant and free-radical scavenging activities [16,42,57,71,78], the exact mechanisms by which EA induces these effects have not been fully revealed.

Conclusion

Our findings suggest that EA is able to prevent cognitive, learning and memory deficits induced by TBI and prevent brain inflammation

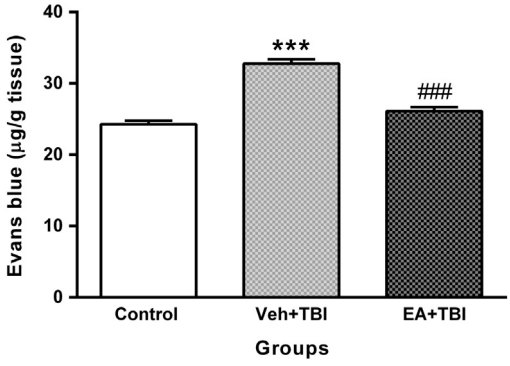

Fig. 5. Brain tissue Evans blue dye content 48 h after TBI. Evans blue dye content was increased significantly after TBI (***$p < 0.001$ vs. Control). Oral administration of EA (100 mg/kg) for 7 consecutive days before induction of TBI (once daily) reversed it 48 h after TBI (###$p < 0.001$ vs. Veh + TBI). Values are represented as mean ± SEM, one-way ANOVA followed by post-hoc Tukey's test, n = 8.

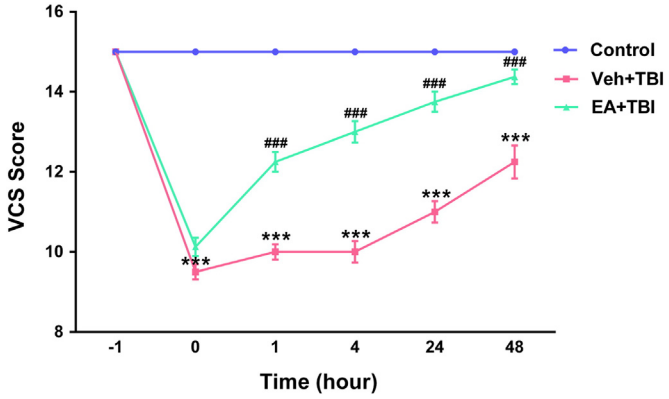

Fig. 6. Veterinary coma scale (VCS) 48 h after TBI. VCS has decreased significantly after TBI (***$p < 0.001$ vs. Control). Oral administration of EA (100 mg/kg) for 7 consecutive days before induction of TBI (once daily) reversed it 48 h after TBI (###$p < 0.001$ vs. Veh + TBI). Values are represented as mean ± SEM, RM-ANOVA followed by post-hoc Tukey's test, n = 8.

following traumatic brain injury, thereby subsequently diminishing the inflammatory factors such as IL-1β and IL-6. Inflammation-related factors are among the primary causes of impairment induced by TBI. The preventive effects of EA can be explained via modulation of these factors which refers to its antioxidative and anti-inflammatory nature. Our results support the role of EA in the prevention of impairment induced by TBI.

Conflict of interest statement

The authors have no conflict of interest to declare.

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