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Protective effects of maternal administration of curcumin and hesperidin in the rat offspring following repeated febrile seizure: Role of inflammation and TLR4

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ABSTRACT

Neuroinflammation has a key role in seizure generation and perpetuation in the neonatal period, and toll-like receptor 4 (TLR4) pathway has a prominent role in neuroinflammatory diseases. Administration of antioxidants and targeting TLR4 in the embryonic period may protect rat offspring against the next incidence of febrile seizure and its harmful effects. Curcumin and hesperidin are natural compounds with anti-inflammatory and antioxidant properties and have an inhibitory action on TLR4 receptors. We evaluated the effect of maternal administration of curcumin and hesperidin on infantile febrile seizure and subsequent memory dysfunction in adulthood.

Hyperthermia febrile seizure was induced on postnatal days 9–11 on male rat pups with 24 h intervals, in a Plexiglas box that was heated to ~45 °C by a heat lamp. We used enzyme-linked immunosorbent assay, Western blotting, malondialdehyde (MDA), and glutathione (GSH) assessment for evaluation of inflammatory cytokine levels, TLR4 protein expression, and oxidative responses in the hippocampal tissues. For assessing working memory and long-term potentiation, the double Y-maze test and Schaffer collateral-CA1 *in vivo* electrophysiological recording were performed, respectively

Our results showed that curcumin and hesperidin decreased TNF-α, IL-10, and TLR4 protein expression and reversed memory dysfunction. However, they did not provoke a significant effect on GSH content or amplitude and slope of recorded fEPSPs in the hippocampus. In addition, curcumin, but not hesperidin, decreased interleukin-1β (IL-1β) and MDA levels.

These findings imply that curcumin and hesperidin induced significant protective effects on febrile seizures, possibly via their anti-inflammatory and antioxidant properties and downregulation of TLR4.

1. Introduction

Epileptic seizures and related disorders are some of the complicated and hard-to-treat neurological disorders among adults and children [1]. According to the International League Against Epilepsy guideline, there are different classifications of seizure types that need clear differential diagnostic procedures [2]. Among them, febrile seizures are associated with a risk of subsequent epilepsy [3] and must be taken seriously because of severity and its predisposing effects in the later periods of a patient's life.

Febrile seizures affect children with a prevalence rate of 2–5% [4]. The American Academy of Pediatrics issued a clinical practice guideline for characterizing febrile seizure as "a seizure accompanied by fever (temperature ≥100.4°F or 38 °C by any method), without central nervous system infection, that occurs in infants and children 6 through 60 months of age." [5]. Brain inflammation (neuroinflammation) not only occurs in different CNS disorders such as autoimmune and epileptic disorders [6], but also this phenomenon has been recognized as a serious predisposing factor for some neurodegenerative disorders such as multiple sclerosis and Alzheimer's and Parkinson's diseases [7].

Abbreviations: FS, febrile seizure; CUR, curcumin; HES, hesperidin; CMC, carboxymethyl cellulose; TLR4, toll-like receptor 4; MDA, malondialdehyde; GSH, glutathione; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-10, interleukin-10; LTP, long-term potentiation; LTD, long-term depression; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation; BBB, blood-brain barrier; ELISA, enzyme-linked immunosorbent assay

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Neuroinflammation is characterized by the activation of astrocytes, microglia, endothelial cells of the blood-brain barrier (BBB), infiltration of monocytes and lymphocytes, and upregulation of a cluster of pro-inflammatory and/or anti-inflammatory mediators in the nervous system [8]. Recent evidence indicates that neuroinflammation leads to seizure generation and perpetuation [9]. Seizures increase levels of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in the brain [10]. These molecules activate several inflammatory signaling pathways, such as toll-like receptor 4 (TLR4) and nuclear factor-κB (NF-κB). Several hours after the beginning of the inflammatory responses, anti-inflammatory cytokines such as interleukin-10 are released, as an immune response, to reduce inflammation. However, pro-inflammatory cytokines induce more release of cytokines via cyclic activation of inflammatory cascades and lead to fever, neuronal injury, and hyperexcitability [8,11].

An increase in core body temperature (hyperthermia) can affect numerous cellular processes, including the functions of several neuronal ion channels and amplitude of major ionic currents and electrical activity of the neurons. These events enhance the rate, magnitude, and/or synchrony of neuronal firing during seizure [12]. Recurrent convulsions may damage some brain structures. Among many brain areas, some are more susceptible to seizure episodes. Some of the consequences of repeated seizures are hippocampal sclerosis and memory dysfunction [13].

Long-term potentiation (LTP) has been recognized as one of the most important mechanisms of synaptic plasticity [14]. Recurrent seizures induce CA1 plastic changes and LTP impairment [15]. LTP induction involves a signal transduction process that includes the depletion of glutamate from synaptic vesicles, activation of N-Methyl-D-aspartate glutamate receptor (NMDAR), Ca^{2+} influx, activations of Ca^{2+}–calmodulin-dependent protein kinases (CaM kinases) II and IV and mitogen-activated protein kinase (MAPK). All of these targets play important roles in spatial memory formation [15].

According to the aforementioned studies, targeting inflammatory processes has significant preventive or treating effects in selected acute and chronic neurological disorders. In recent years, various treatment strategies targeting inflammation using natural products have been reported [8,16]. Two of these natural products, curcumin and hesperidin, were found to have diverse biological effects, including anti-inflammatory properties [17]. Also, it has been reported that these compounds reduced the production of reactive oxygen species (ROS) [18,19]. Production of ROS promotes oxidative stress, which in turn is strictly related to the generation of high-mobility group box 1 (HMGB1), activation of HMGB1-TLR4 axis, and neuroinflammation. This vicious cycle is a potential mechanism for seizure promotion and epileptogenesis [20]

Curcumin is a major component derived from the rhizome of *Curcuma longa* that attenuates inflammation by inhibiting the TLR4/NF-κB signaling pathway [21]. This agent has been reported to regulate numerous transcription factors, adhesion molecules, protein kinases, cytokines, redox status, and enzymes that have been associated with inflammation [22]. Moreover, its pharmacological safety and low cost make it an attractive substance for further studies [22]. It has been documented that curcumin induced an ameliorative effect on seizure severity and memory impairment in the pentylenetetrazol kindling model in mice [23]. Curcumin has been found to cross the BBB [24]. Likewise, one study showed that curcumin, in placental and fetal membranes, significantly decreased gene expression of IL-6 and cyclooxygenase-2 (COX-2) [25].

Hesperidin is a flavanone glycoside extracted from citrus species with diverse biological properties, especially antioxidant and anti-inflammatory effects [26]. Hesperidin acts via blockade of NF-κB and MAPK signaling pathways and consequently reduces pro-inflammatory cytokine production [26]. There is evidence that flavonoids can pass across BBB [27]. Also, both human and animal studies have shown that either natural or synthetic flavonoids can easily cross the placenta [28].

Thus, considering the anti-inflammatory and antioxidative effects of curcumin and hesperidin, their abundance and availability, these natural agents were selected due to their safety in pregnancy in animal studies [28,29].

The goal of the present study was to determine whether administration of curcumin and hesperidin in pregnant rats protects the offspring against repeated febrile seizures. We also aimed to evaluate the role of these natural products on pro-inflammatory cytokines, TLR4 protein, memory function, and electrophysiological properties of LTP recordings in the brains of offspring. Therefore, a comparative study between two different natural anti-inflammatory agents was conducted.

2. Materials and methods

2.1. Materials

The following materials were used in this study: curcumin (Cas. No. 458-37-7, Purity ≥ 90%, Cayman, Michigan, USA), piperine (Cas No. 94-62-2, purity ≥ 95% by HPLC, Golexir, Mashhad, Iran), hesperidin (Cas. No. 520-26-3, purity ≥ 95% by HPLC, Golexir, Mashhad, Iran), TLR4 monoclonal antibody (Cat. No. sc-293072, Santa Cruz, Dallas, USA), protease inhibitor cocktail (Cat. No. P8340, Sigma Aldrich, St Louis, USA), urethane (Cas. No. 51-79-6, Sigma Aldrich, St. Louis, USA), anti-mouse immunoglobulin G-horseradish peroxidase (IgG-HRP) conjugate antibody (Cat. No. 7076, Cell Signaling, Danvers, USA), anti-β-actin antibody (Cat. No. 4967, cell signaling, Danvers, USA), Rat TNF-α (Cat. No. ab100785), IL-1β (Cat. No. ab100768), and IL-10 (Cat. No. ab100765) ELISA kits (all from Abcam, Cambridge, USA).

2.2. Animals

Virgin female Wistar rats (200–250 g) were mated with males over a period of seven to ten days. For breeding, one male and one female were paired in a wire mesh cage. Day 0 of gestation was confirmed by the presence of the vaginal plug on the paper beneath the mating cages [30]. After conception, the female rats were placed individually in the clear polycarbonate cages and maintained under standard temperature (22 ± 2 °C), humidity (50–55%), and light (12 h light/ 12 h dark cycles). Animals were fed a standard rodent pellet diet and drinking water *ad libitum*. Pregnant females were monitored for the parturition day designated as postnatal day zero (P0). All experiments were conducted according to the international animal care ethics (National Institutes of Health Guide for the Care and Use of Laboratory Animals) and were approved by the Ethics Committee for Human and Animal Care of Ferdowsi University of Mashhad (IR.UM.REC.1398.105).

Pregnant rats were randomly divided into three different treatment groups: those that received curcumin plus piperine (60 mg/kg + 10 mg/kg, respectively) emulsified in 1% carboxymethyl cellulose (CMC), rats that received hesperidin plus piperine (100 mg/kg + 10 mg/kg, respectively) in 1% CMC, and rats that received 1% CMC plus piperine (10 mg/kg) as vehicle (4 ml/kg). Piperine, a bioenhancer (an agent that enhances the bioavailability of the drugs when combined), was used as a concomitant treatment for improving the intestinal absorption of natural products or slowing down their metabolism [31]. The drug solutions and vehicle were gavaged from day 0 of gestation until parturition (the presence of vaginal plug after mating was designated as the first day of gestation: day 0). After parturition, the male offspring, in each group, were divided into two subgroups: those that were exposed to hyperthermia-induced febrile seizure as seizure subgroups and those that were not exposed to hyperthermia as control subgroups. So, the study arrangement was as follows: vehicle, vehicle + febrile seizure (V + FS), curcumin (CUR), curcumin + febrile seizure (CUR + FS), hesperidin (HES), and hesperidin + febrile seizure (HES + FS) (Table 1).

Tab. 1. The final sample size was adjusted for the expected death of animals. Total n was divided as follows: Double Y-maze test (n = 7),

Table 1
Design of study groups.

Offspring groups	Maternal administration (4 ml/kg, oral) from gestation to parturition	Febrile seizure induction (P9-P11)	Total number of offspring
Vehicle	1% CMC + 10 mg/kg of piperine	No	n = 36
V + FS	1% CMC + 10 mg/kg of piperine	Yes	n = 40
CUR	(60 mg/kg of curcumin + 10 mg/kg of piperine) in 1% CMC	No	n = 36
CUR + FS	(60 mg/kg of curcumin + 10 mg/kg of piperine) in 1% CMC	Yes	n = 36
HES	(100 mg/kg of hesperidin + 10 mg/kg of piperine) in 1% CMC	No	n = 36
HES + FS	(100 mg/kg of hesperidin + 10 mg/kg of piperine) in 1% CMC	Yes	n = 36

electrophysiological study (n = 6–7), Western blotting (n = 4), enzyme-linked immunosorbent assay (n = 6), malondialdehyde assessment (n = 6), glutathione assessment (n = 6). V: vehicle, CMC: carboxymethyl cellulose, CUR: curcumin, HES: hesperidin, FS: febrile seizure.

2.3. Febrile seizure induction

Febrile seizures were induced on days 9–11 after parturition (P9–P11) on male rat pups [32] with 24 h intervals between sessions. Experiments were conducted in a Plexiglas box (14.5 × 14.5 × 15 cm). The chamber was heated to ~45 °C [32] by a heat lamp that was held 16.5 cm above the chamber floor by placing a cap on the Plexiglas chamber. Hyperthermia was induced for 20–25 min and core body temperature was measured before experiments, during seizure episodes, and at the end of the experiments by using a custom-made sensitive digital rectal thermometer. Normothermia controls were submitted to the same treatment, except that their core body temperature was maintained at the baseline during their stay in the Plexiglas chamber. The behaviors of the rat pups were monitored by the experimenter and videotaped for a more detailed analysis. Behavioral characteristics of hyperthermic seizures of the rat pups have a broad spectrum as described previously [33,34]; they include jumping, rearing, sudden stop of the movement followed by facial automatisms (chewing), jerky gait, running in tight circles, clonic movements of the forelimb, tonic flexion of the body, and one or more tonic-clonic seizures often associated with a loss of postural control. Seizure characteristics were measured based on behavioral manifestation, which includes baseline temperature, threshold temperature (an index for indicating the seizure onset), maximal temperature (an index for indicating the final stage of seizure), threshold time (an index for indicating the time of seizure onset), and hyperthermia duration (an index for indicating hyperthermia induction time to reach the final stage of seizure). Following hyperthermia, animals were placed on a cool surface to regain normal core body temperature and then were returned to their mothers. For biochemical studies, 6 h after hyperthermia-induced febrile seizure on P11, pups' brains were dissected and kept frozen at −80 °C. Other animals were returned to their cages and were maintained until puberty for electrophysiological and behavioral studies.

2.4. Behavioral study

2.4.1. Double Y-maze apparatus

The double Y-maze was made of black Plexiglas, according to Bett et al. (2012) [35] (Fig. 1). Briefly, it consisted of a start box, two choice boxes, and four goal boxes, connected with alleyways. Each of the boxes was octagonal, with 25 cm between parallel walls. The octagons had 10.35 cm wide and 30 cm high walls. The connecting alleyways were 25 cm long and 8 cm wide with 10 cm high walls. Each goal box contained a round plastic food bowl that was stuck firmly to the floor of the box. We equipped each goal box with a different shape and color to be distinguished from others by animals. The figure was attached to the wall opposite the alleyway. The maze was elevated 60 cm from the floor on bar stools, and it was located in an experimental room with well-controlled conditions.

2.4.2. Behavioral training in the maze

Behavioral assessment was performed with some modifications, according to Bett et al. (2012) [35] by a blind experimenter. Forty-two male Wistar rats weighing 250–300 g (n = 7 in each group) were used for this experiment. The goal of the present study was for the rat to recognize a piece of food reward (Kellogg's Froot Loops cereal) in one of the four goal boxes. Rats were subjected to the food deprivation protocol until they reached 85 to 90% of their initial body weight before the start of training. In the familiarization trial, each animal was introduced to the double Y-maze apparatus for 10 min/day for two consecutive days before initial training without any food reward. Then, training days were started and continued for four days according to the following protocol. At the beginning of each trial, rats were placed in the arena facing the south wall of the start box and their heads oriented towards the wall. Rats were trained to perform the task from the start box on the maze and find one reward location among four possible locations. One of the four goal boxes was baited in each trial, and the other boxes contained cereal dust to remove olfactory cues. On other training days, the other goal boxes were baited so that all of them were baited during the procedure. To encourage the animal to return to the rewarded goal box, each Froot Loop cereal was divided into four pieces, and the pieces were put into the food bowl so that the animal could not consume all the food prior to the end of the trials. Each daily trial was performed for 20 min or until 20 trials had been completed, whichever came earlier [36]. On a trial, if the rat found the rewarded goal box, it was allowed to eat for 10 s, and if the rat went to an unrewarded box, a Plexiglas barrier was placed at the intersection of the goal box and alleyway for 10 s to confine the animal to this area before the beginning of the next trial. The maze was wiped with a mild soapy solution after each trial, and the interval between two consecutive trials was 10 s. Animals were allowed to move freely in the maze, and once they found the food reward location, the first correct entry was considered. Rats were trained until they reached 70% accuracy (entries to rewarded goal box) over 20 trials/day for 4 days. After 6 days of training (2 days of introductory training + 4 days of initial training), the final test was performed on the seventh day. All experiments were performed between 10:00 and 16:00 hr.

2.5. Electrophysiological study

Electrophysiological study was performed based on previous studies [37,38]. For electrophysiological studies, adult rats weighing 250–300 g (n = 6–7 in each group), were anesthetized deeply using urethane (1.6 g/kg, intraperitoneally). Then, the head of the animal was placed in a stereotaxic apparatus, and 0.2 ml lidocaine 1% was injected subcutaneously at the incision site for local anesthesia. After skin incision, the skull was exposed, and the proper locations of the CA1 region of the hippocampus and the Schaffer collateral pathway were determined using Paxinos and Watson's atlas [39]. Then, two tiny holes were made with a dental drill at these sites. For recording field excitatory postsynaptic potential (fEPSP), a bipolar stainless steel stimulating electrode (125 μm diameter, A-M Systems, Carlsborg, USA) was placed in the Schaffer collateral pathway of the right hippocampus

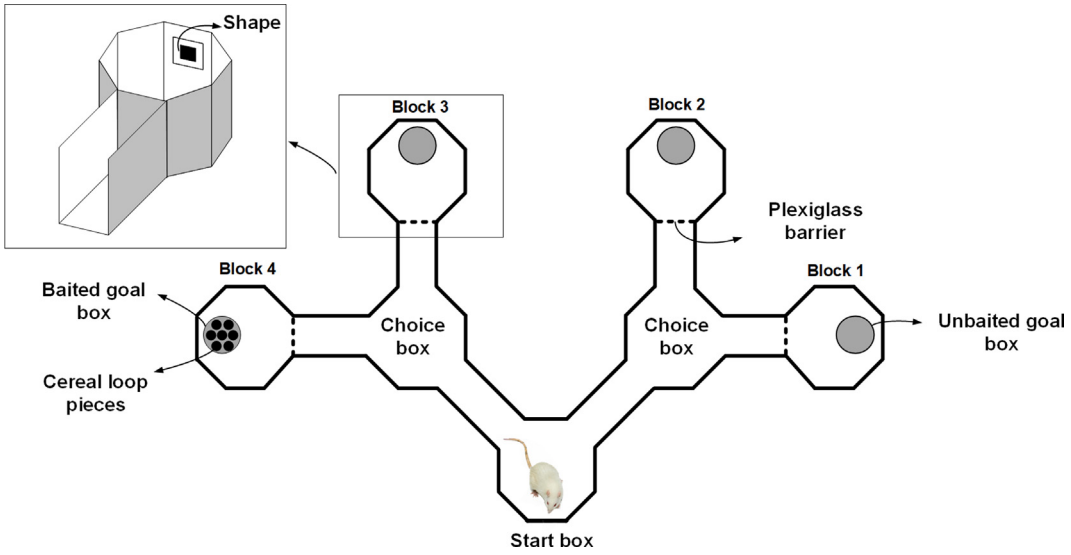

Fig. 1. Schematic drawing of the double Y-maze apparatus. The inside of the apparatus was shown in white instead of black for better understanding. The dashed line presents the open state of the Plexiglas barrier.

(AP = -4.2 mm; ML = 3.8 mm; DV = -2.7⁻-3.8 mm) and a unipolar recording electrode with the above-mentioned characteristics was implanted into the CA1 region of the ipsilateral hippocampus (AP = -3.4 mm; ML = 1.5 mm; DV = -4.4⁻-5.1 mm; at an angle of 52.5˚) [40]. Implantation of electrodes in the correct position was determined using physiological and stereotaxic indicators. For this purpose, coordinates of each region were defined by the stereotaxic atlas. Then, paired-pulse facilitation (PPF) was used as one of the physiological indicators. To elicit PPF, paired stimuli were applied to the Schaffer collateral pathway with a 50 ms interstimulus interval [41]. Furthermore, the depth profile of electrode implantations into the Schaffer collateral-CA1 region of the hippocampus and properties of elicited field potentials were checked at the beginning of each experiment [42].

After surgery, the rats were allowed to rest for 30 min to stabilize before taking baseline measurements. The stimulating and recording electrodes were connected to a stimulator and an amplifier, respectively. fEPSPs were recorded from the CA1 region of the hippocampus following high-frequency stimulation of the ipsilateral Schaffer collateral pathway. Extracellular field potentials were amplified (100×) and filtered (1 Hz to 3 kHz bandpass). Signals were recorded through an analog-to-digital interface (Data Acquisition D3111, Science Beam Institute, Tehran, Iran). The stimulation and recording protocol was performed according to the paired-pulse 50 (PP50) procedure. This protocol of stimulation was adjusted to evoke 50% of the maximal response for baseline recording before and after LTP induction. After obtaining a steady-state baseline response, an input-output (I/O) protocol was performed by gradually increasing the stimulus intensity in a range from 100 to 1000 μA to evaluate synaptic potency before long-term potentiation (LTP) induction. Then fEPSP was recorded as an output. After achieving a maximum response, baseline recording was performed for 30 min after I/O protocol. Then, LTP was induced by high-frequency stimulation (HFS) at 100 Hz, and recording was continued for 90 min post HFS protocol. Therefore, the duration of the recording period was 120 min. The slope and amplitude of fEPSP were calculated as reference values of LTP. 720 trials at 10 s intervals were recorded for each animal, and for reporting each time point in the graphs, 30 consecutive trials were averaged and compared within and between groups. For preliminary data extraction and analysis, the eProbe software package (Science Beam Institute, Tehran, Iran) was used.

2.6. Biochemical studies

For biochemical studies, after decapitation, the brains were removed and dissected on ice. The brain was cut in half along the midline, and the left and right sides of the hippocampi were collected. Tissues were placed in cryotubes and immediately frozen in liquid nitrogen. Then, samples were stored at −80 ˚C until subsequent use.

2.6.1. Western blot

2.6.1.1. Tissue preparation. To prepare samples for Western blot analysis, hippocampi were homogenized in a lysis buffer containing 50 mM Tris-HCl pH: 7.4, 2 mM EDTA, 2 mM EGTA, 10 mM NaF, 1 mM sodium orthovanadate (Na_3VO_4), 10 mM β-glycerophosphate, 0.2% (w/v) sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride (PMSF), and complete protease inhibitor cocktail on ice using a Polytron homogenizer (Polytron PT 10–35 GT, Kinematica AG, Switzerland) [43]. The homogenates were centrifuged at 10000*g* for 10 min at 4 ˚C. Supernatants were collected on ice, and the total protein content in supernatants was measured using a Bradford protein assay dye reagent (Bio-Rad, Hercules, USA). Equal portions of supernatant were mixed with 2x SDS sample buffer containing 100 mM Tris-base, 4% w/v SDS, 20% v/v glycerol, 0.2% w/v bromophenol blue, and 10% v/v 2-mercaptoethanol [43]. Samples were incubated in boiling water for 5 min, allowed to cool, and then were stored at −80 ˚C until use.

2.6.1.2. Western blotting analysis for TLR4. Western blot analysis was carried out according to Vahdati Hassani et al. (2014) [43]. Equal amounts of proteins (20 μg) were separated by 10% SDS-polyacrylamide gels and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, USA) by electrophoresis. The membranes were blocked with 5% skim milk in Tris-buffered saline Tween 20 (TBS-T20) for 3 h at room temperature. Then they were incubated with the following primary antibodies: anti-mouse TLR4 (1:1000 in TBS-T20) and anti-β-actin antibody (1:1000 in TBS-T20) for 2 h at room temperature. The membranes were then washed 3 times for 5 min with TBS-T20 buffer and incubated with anti-mouse IgG-HRP conjugated antibody (1:3000 in TBS-T20) as a secondary antibody for 1.5 h at room temperature. The membranes were washed three times as before. Finally, the PVDF membranes were exposed to an enhanced chemiluminescence reagent (Pierce ECL; Thermo Fisher Scientific, Waltham, USA) and H_2O_2 for 3 min and the intensity of protein bands was measured using an Alliance gel doc system (Alliance 4.7

Gel doc, UVtec, UK) and its software (UVtec software). Western blot densities were normalized against β-actin as internal control.

2.6.2. The enzyme-linked immunosorbent assay (ELISA)

Frozen hippocampal tissues were homogenized with ice-cold phosphate-buffered saline (PBS) containing complete protease inhibitor cocktail and centrifuged at 10000*g* for 10 min at 4 °C. The supernatants were removed, aliquoted to three tubes, and stored at −80 °C for later use. The total protein concentration in the supernatant was determined using a Bradford protein assay dye reagent (Bio-Rad). Equal amounts of proteins were loaded for the enzyme-linked immunosorbent assay. The levels of TNF-α, IL-1β, and IL-10 were measured using Abcam ELISA Kits (Abcam, Cambridge, USA) following the manufacturer's instructions. All samples were run in duplicate, and results were expressed as pg/mg.

2.6.3. Measurement of malondialdehyde (MDA)

For measurement of MDA as a biomarker for lipid peroxidation, the thiobarbituric acid (TBA) reaction method was employed. MDA measurement was done according to the protocol described by Mihara and Uchiyama (1978) [44]. Hippocampi of rat pups were weighed and homogenized with cold 1.15% KCl to make a 10% homogenate. Briefly, 1.5 ml of 1% H_3PO_4 and 0.5 ml of 0.6% thiobarbituric acid aqueous solutions were added to 250 μl of this homogenate, and the mixture was heated in boiling water for 45 min [45]. After cooling, 2 ml of n-butanol was added, and the mixture was then vortexed vigorously for 1 min followed by centrifugation at 3000*g* for 20 min. The butanol phase (supernatant) was transferred to a glass tube, and absorbance of the supernatant was measured spectrophotometrically at 532 nm [45]. The MDA level in the rat hippocampus was calibrated with the MDA standard curve and expressed as nmol/g tissue.

2.6.4. Measurement of glutathione (GSH)

GSH content was measured according to Moron et al. (1979) [46]. Glutathione content was measured using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), the reduction of which resulted in the production of yellow-colored 5-thio-2-nitrobenzoic acid (TNB). Tissues were prepared as explained in the previous section. Hippocampi were homogenized in cold PBS (pH 7.4) to make a 10% homogenate. The homogenate and 10% trichloroacetic acid (TCA) were mixed together at a ratio of 1:1, vortexed, and centrifuged at 5000*g* for 10 min at 4 °C. Then, 250 μl of 0.04% DTNB reagent was added to 250 μl of supernatant plus 1 ml PBS (0.1 M, pH 8). Finally, the absorbance of the liberated TNB was measured at 412 nm. Tissue GSH content was expressed as nmol/g tissue.

2.7. Statistical analysis

In this study, continuous variables were expressed as mean ± standard error of mean (SEM). A one-way analysis of variance (one-way ANOVA), two-way ANOVA, repeated measures ANOVA, and Tukey's post-hoc test were used to compare between groups as appropriate. Statistical analysis was undertaken using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, USA). All statistical tests were two-sided, and a $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of maternal administration of curcumin and hesperidin on seizure characteristics

Baseline temperature ($F(2,109) = 0.5741$, $p = 0.5649$; Fig. 2A), threshold temperature ($F(2,109) = 0.2940$, $p = 0.7458$; Fig. 2B), maximal temperature ($F(2,109) = 0.2597$, $p = 0.7717$; Fig. 2C), and hyperthermia duration ($F(2,109) = 1.839$, $p = 0.1639$; Fig. 2D) were evaluated for each seizure group. Statistical analysis revealed no significant differences between groups in the above-mentioned characteristics. In the threshold time index, results showed a significant difference between curcumin-treated and the related control group on postnatal days 9 and 10 ($F(2,109) = 3.820$, $p = 0.0365$ for postnatal day 9, $p = 0.0149$ for postnatal day 10; Fig. 2E).

3.2. Effects of maternal administration of curcumin and hesperidin on working memory in the offspring in adulthood

The double Y-maze results revealed that hyperthermia-induced febrile seizure significantly decreased correct entries to the goal box in the V + FS group compared with the vehicle group ($F(5,36) = 13.17$, $p < 0.0001$). However, maternal administration of curcumin and hesperidin enhanced working memory and correct entries to the goal box in both curcumin- and hesperidin-treated groups following repeated febrile seizure ($F(5,36) = 13.17$, $p = 0.0001$, $p = 0.0009$; respectively, compared with V + FS group). No significant difference was observed in the normothermia groups ($F(5,36) = 13.17$; $p = 0.9996$ for vehicle vs CUR, $p = 0.9694$ for vehicle vs HES) (Fig. 3).

3.3. Effects of maternal administration of curcumin and hesperidin on TLR4 protein expression

Western blotting analysis was carried out in order to quantify TLR4 protein expression in the hippocampi of rat pups. The results showed that TLR4 protein expression was significantly increased 6 h after hyperthermia-induced febrile seizure on P11 in the V + FS group compared with the vehicle group ($F(5,18) = 6.774$, $p = 0.0112$). Also, there was a significant decrease in TLR4 protein expression in CUR + FS and HES + FS groups compared with the V + FS group ($F(5,18) = 6.774$, $p = 0.0101$, $p = 0.0148$; respectively). No significant differences in TLR4 protein expression were found between vehicle, CUR, and HES groups ($F(5,18) = 6.774$, $p = 0.8292$ for vehicle vs CUR, $p = 0.9437$ for vehicle vs HES) (Fig. 4 A, B).

3.4. Effects of maternal administration of curcumin and hesperidin on cytokine concentration

The data analyses of pro-inflammatory/anti-inflammatory cytokines are presented as follows:

Pro-inflammatory cytokines: The results revealed that TNF-α and IL-1β concentration were significantly increased in the hippocampal tissue following repeated febrile seizures in the V + FS group compared with the vehicle group (TNF-α: $F(5,30) = 7.874$, $p = 0.0140$, IL-1β: $F(5,30) = 12.59$, $p = 0.0012$). CUR + FS and HES + FS groups showed significant differences with the V + FS group (TNF-α: $F(5,30) = 7.874$, $p = 0.0004$ and $p = 0.0471$; IL-1β: $F(5,30) = 12.59$, $p = 0.0051$ and $p = 0.9712$; respectively) (Fig. 5A, B).

Anti-inflammatory cytokine: Investigation of IL-10 protein levels showed that there was a significant difference between V + FS/vehicle, CUR + FS/V + FS, and HES + FS/V + FS groups ($F(5,30) = 14.51$, $p = 0.0002$, $p = 0.0191$, and $p < 0.0001$; respectively) (Fig. 5C). Also, no significant differences between normothermia groups were found at any of the above-mentioned cytokines ($p > 0.05$).

3.5. Effects of maternal administration of curcumin and hesperidin on oxidative stress

In MDA analysis, a significant increase was observed between V + FS and vehicle groups ($F(5,30) = 25.09$, $p < 0.0001$). Also, the CUR + FS group showed a significant decrease in MDA levels compared with the V + FS group ($F(5,30) = 25.09$, $p = 0.0144$). But there was not a significant difference between HES + FS and V + FS groups ($F(5,30) = 25.09$, $p = 0.8029$). Normothermia groups showed no significant differences in MDA levels ($F(5,30) = 25.09$, $p = 0.5648$ for vehicle vs CUR, $p = 0.2307$ for vehicle vs HES) (Fig. 6A).

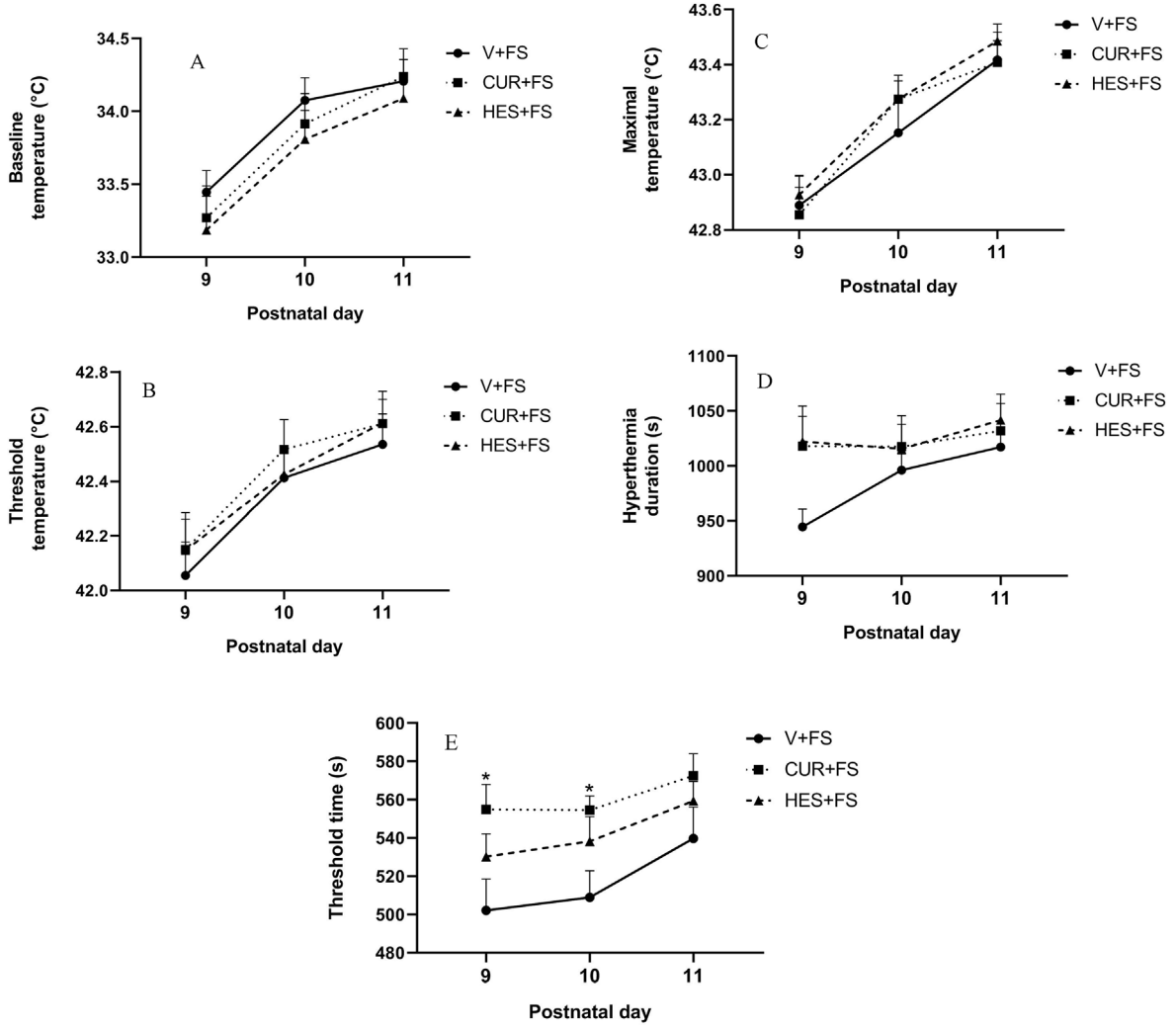

Fig. 2. Seizure time and temperature changes during hyperthermia-induced febrile seizures. Relationship between baseline temperature changes and postnatal days 9–11 in three seizure groups (A). Relationship between threshold temperature for seizure onset and postnatal days (B). Relationship between the maximal temperature in the final stage of seizure and postnatal days (C). Hyperthermia induction time to reach the final stage of seizures on different postnatal days (D). Relationship between threshold time of seizure onset and postnatal days (E). (n = 36–40, *p < 0.05 compared with the V + FS group, two-way ANOVA mixed design followed by Tukey's post-hoc test, data were expressed as mean ± SEM). V: vehicle, CUR: curcumin, HES: hesperidin, FS: febrile seizure.

Analysis of GSH content revealed that there were no significant differences between any groups (F (5,30) = 0.1904, p = 0.9639; Fig. 6B).

3.6. Effects of maternal administration of curcumin and hesperidin on electrophysiological changes (LTP) in offspring adulthood

Hyperthermia-induced febrile seizures decreased amplitude and slope of fEPSPs in the V + FS group, but it was not statistically significant at different time points between groups. In other words, the V + FS group had the lowest value of amplitude and slope. Also, there was no significant change in amplitude or slope of fEPSPs following maternal treatment with curcumin and hesperidin (amplitude: F (5,31) = 0.5930, p = 0.7053, slope: F (5,31) = 0.7939, p = 0.5623; Fig. 7A, B).

4. Discussion

Adverse effects of recurrent febrile seizure, in the neonatal period, on the brain is potentially related to inflammatory and oxidative processes [47]. We aimed to evaluate the effect of maternal treatment with two natural anti-inflammatory and antioxidative agents on the harmful effects of febrile seizures in rat pups.

In the present study, we investigated whether maternal administration of curcumin and hesperidin protects rat pups against adverse effects of repeated febrile seizures. Also, we evaluated the effects of these compounds on seizure incidence, pro- and anti-inflammatory cytokine expressions, TLR4 protein expression, oxidative stress of the hippocampus (in the neonatal period), working memory, and long-term potentiation (in the adulthood period) following repeated febrile seizures in the offspring.

The findings revealed that maternal treatment with curcumin increased seizure latency and reversed impaired working memory following febrile seizures. It also decreased hippocampal TNF-α, IL-1β, IL-10, MDA, and TLR4 protein. Maternal administration of hesperidin decreased TNF-α, IL-10, and TLR4 protein and reversed impaired working memory. Hesperidin did not change IL-1β, MDA, or seizure-related parameters. Also, curcumin and hesperidin did not induce any significant effect on GSH content or fEPSP properties. Comparison of the results of curcumin and hesperidin administration has been discussed separately in each section.

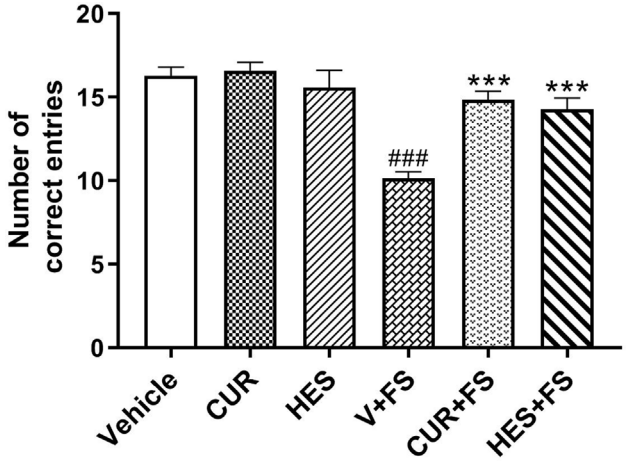

Fig. 3. Rat correct entries to the goal box in the double Y-maze task in adult rats. Maternal administration with curcumin and hesperidin reversed working memory impairment in adult rats with a history of febrile seizure. (n = 7, ###p < 0.001 compared with the vehicle group; ***p < 0.001 compared with the V + FS group, one-way ANOVA followed by Tukey's post-hoc test, data were expressed as mean ± SEM). V: vehicle, CUR: curcumin, HES: hesperidin, FS: febrile seizure.

4.1. Maternal administration, febrile seizure, and seizure-related parameters

Our findings showed that all seizure groups had an incremental trend of response to time-temperature parameters of FS during postnatal days 9–11. In other words, all animals, including the control group, showed age-dependent enhancement in response to hyperthermia. Curcumin and hesperidin did not change the main seizure-related parameters, including baseline temperature, threshold temperature, maximal temperature, and hyperthermia duration. However, curcumin elevated threshold time for seizure onset.

4.2. Maternal administration, febrile seizure, and inflammation

There are reports showing that inflammation has a crucial role during FS [48,49]. The balance between pro-inflammatory (TNF-α, IL-1β, and IL-6) and anti-inflammatory cytokines (IL-1RA and IL-10) affects the severity of fever [50]. Experimental studies confirm that inflammation and inflammatory mediators are major contributors to febrile and epileptic seizure development [50]. Accordingly, we evaluated the effect of curcumin and hesperidin on selected pro- and anti-inflammatory cytokines. The results of the present study showed that maternal administration of curcumin decreased TNF-α and IL-1β and diminished IL-10 in the hippocampi of the rat pups. In agreement, a study by Zhou et al. (2017) indicated that curcumin administration, via upregulation of phosphorylated Akt, suppressed enhanced placental levels of TNF-α, IL-1β, and IL-6 in lipopolysaccharide (LPS)-treated animals [51]. Similar results were reported by Chen et al. (2018). They showed that administration of curcumin significantly decreased IL-6 in the amniotic fluid and fetal brain following maternal infection with LPS [52]. Also, anti-inflammatory activity of curcumin and downregulation of NF-κB expression in the placental and fetal tissues in the preterm birth model has been documented previously in animals [53]. Kaur et al. (2015) noted that curcumin treatment decreased hippocampal TNF-α, IL-1β, IL-6, and MCP-1 levels in an experimental model of chronic epilepsy [54]. In agreement, two other studies reported that administration of curcumin attenuated hippocampal TNF-α following experimental models of epilepsy [55,56]. There are conflicting reports regarding the effect of curcumin on IL-10. Hoppe et al. (2013) found that curcumin increased IL-10 release in beta-amyloid-induced toxicity in rat organotypic hippocampal slice culture [57]. However, Shirley et al. (2008) reported that stimulated curcumin-treated dendritic cells produced lower levels of IL-10 [58]. In our study, the levels of IL-10 were decreased in the CUR + FS group compared with the V + FS group. We have no explanation for this finding. However, this effect

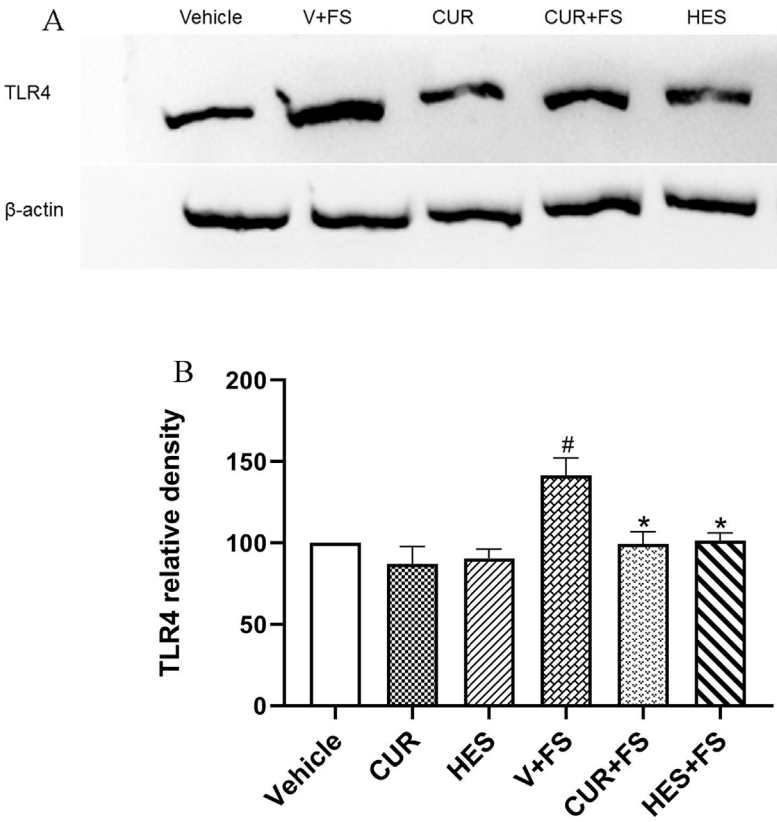

Fig. 4. Representative Western blot bands of TLR4 in the hippocampi of rat pups in the normothermia and seizure groups (A), and graphic representation of its protein expression levels (B). Maternal administration of curcumin and hesperidin resulted in significantly reduced protein expression of TLR4 induced by hyperthermia febrile seizure. β-actin was considered as internal control. (n = 4, #p < 0.05 compared with the vehicle group; *p < 0.05 compared with the V + FS group, one-way ANOVA followed by Tukey's post-hoc test, data were expressed as mean ± SEM). V: vehicle, CUR: curcumin, HES: hesperidin, FS: febrile seizure.

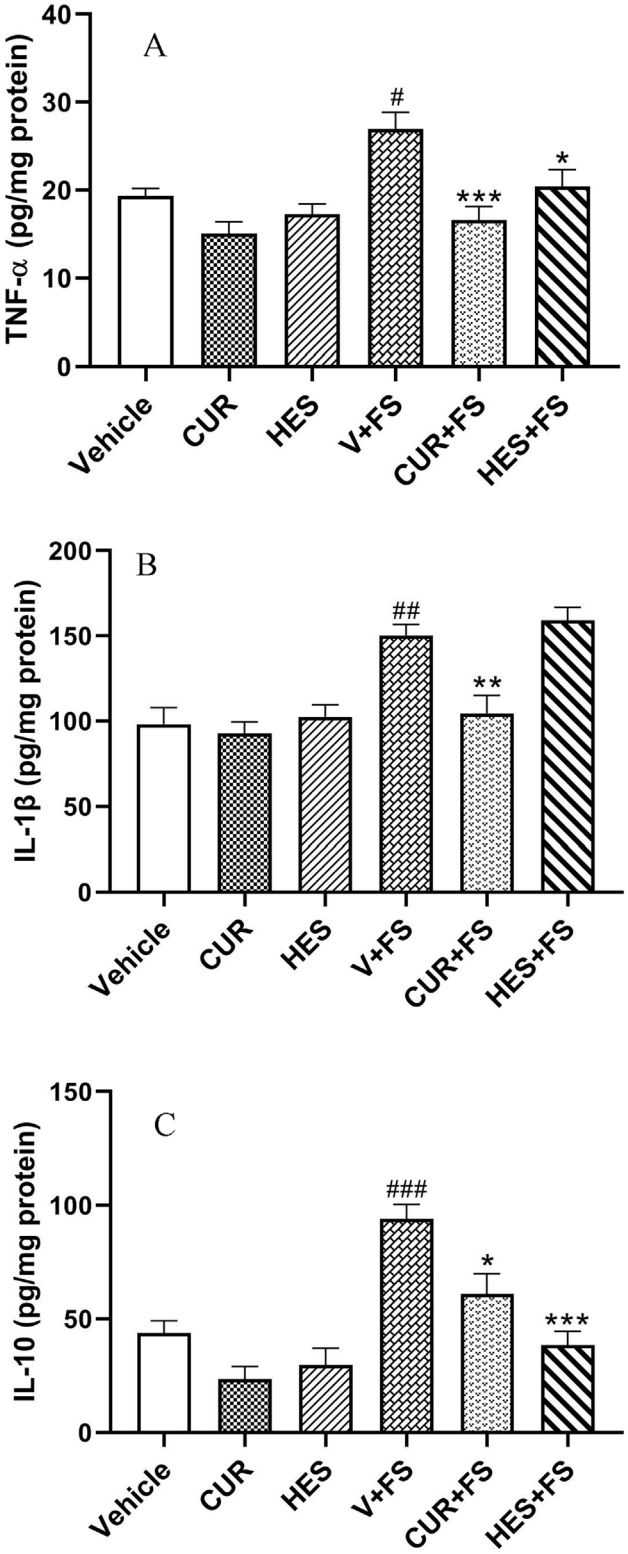

Fig. 5. Enzyme-linked immunosorbent assay of hippocampal TNF-α (A), IL-1β (B), and IL-10 (C) levels in rat pups. Maternal administration of curcumin was more effective than hesperidin and significantly reduced pro- and anti-inflammatory cytokines compared with the V + FS group after hyperthermia-induced febrile seizure. (n = 6, #p < 0.05, ##p < 0.01, ###p < 0.001 compared with the vehicle group; *p < 0.05, **p < 0.01, ***p < 0.001, compared with the V + FS group, one-way ANOVA followed by Tukey's post-hoc test, data were expressed as mean ± SEM). V: vehicle, CUR: curcumin, HES: hesperidin, FS: febrile seizure.

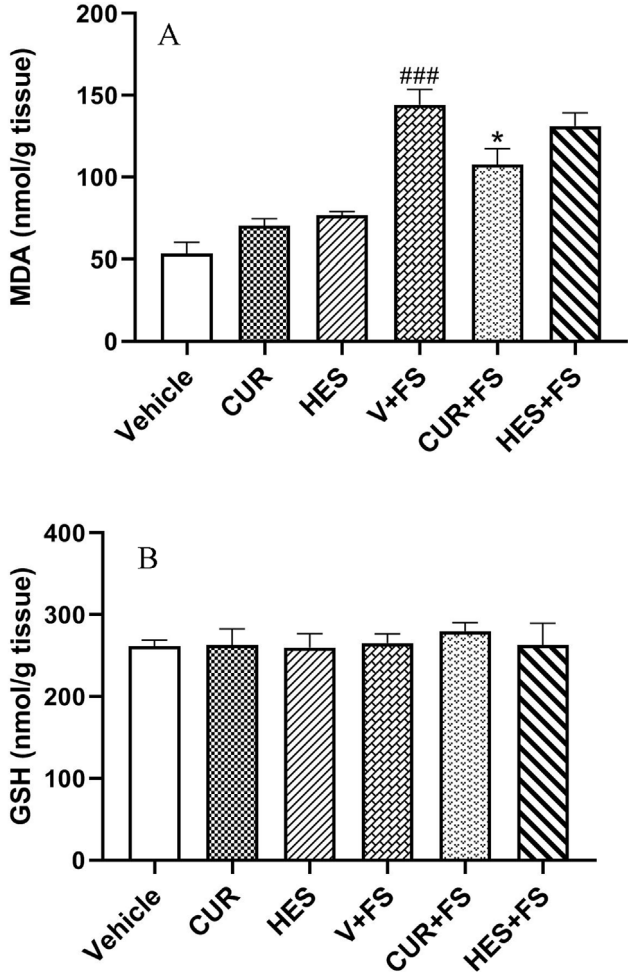

Fig. 6. Measurement of hippocampal MDA (A) and GSH (B) in rat pups. Maternal administration of curcumin significantly reduced hippocampal MDA levels following repeated febrile seizures. GSH levels were not statistically significant in any experimental group. (n = 6 for MDA and n = 6 for GSH assessment, ###p < 0.001 compared with the vehicle group; *p < 0.05 compared with the V + FS group, one-way ANOVA followed by Tukey's post-hoc test, data were expressed as mean ± SEM). V: vehicle, CUR: curcumin, HES: hesperidin, FS: febrile seizure.

may be justified by the modulatory effect of curcumin on the inflammation.

The results of the present study also indicated that maternal administration of hesperidin decreased TNF-α and IL-10 with no significant effect on IL-1β. It has been reported that administration of hesperidin induced a prophylactic effect against diabetic embryopathies [28]. It also induced such preventive effects on formaldehyde-induced toxicity in pregnant rats [59]. Hesperetin, as hesperidin aglycone, reduced plasma levels of inflammatory cytokines such as TNF-α and IL-6 in a prenatal valproate-induced autism model [60]. In addition, hesperidin attenuated TNF-α and IL-1β in the hippocampus and other brain regions following neuroinflammation, traumatic brain injury, and stress [61–63]. The effect of hesperidin on IL-10 has been reported with controversies; it increased IL-10 in a mouse model of multiple sclerosis [64] and decreased IL-10 in macrophages [65]. As aforementioned, in the present study, hesperidin decreased IL-10 concentration significantly. This may imply that hesperidin induces modulatory actions on IL-10 depending on site, dose, and duration of exposure.

4.3. Maternal administration, febrile seizure, and TLR4 protein expression

In the present study, induction of FS enhanced the protein

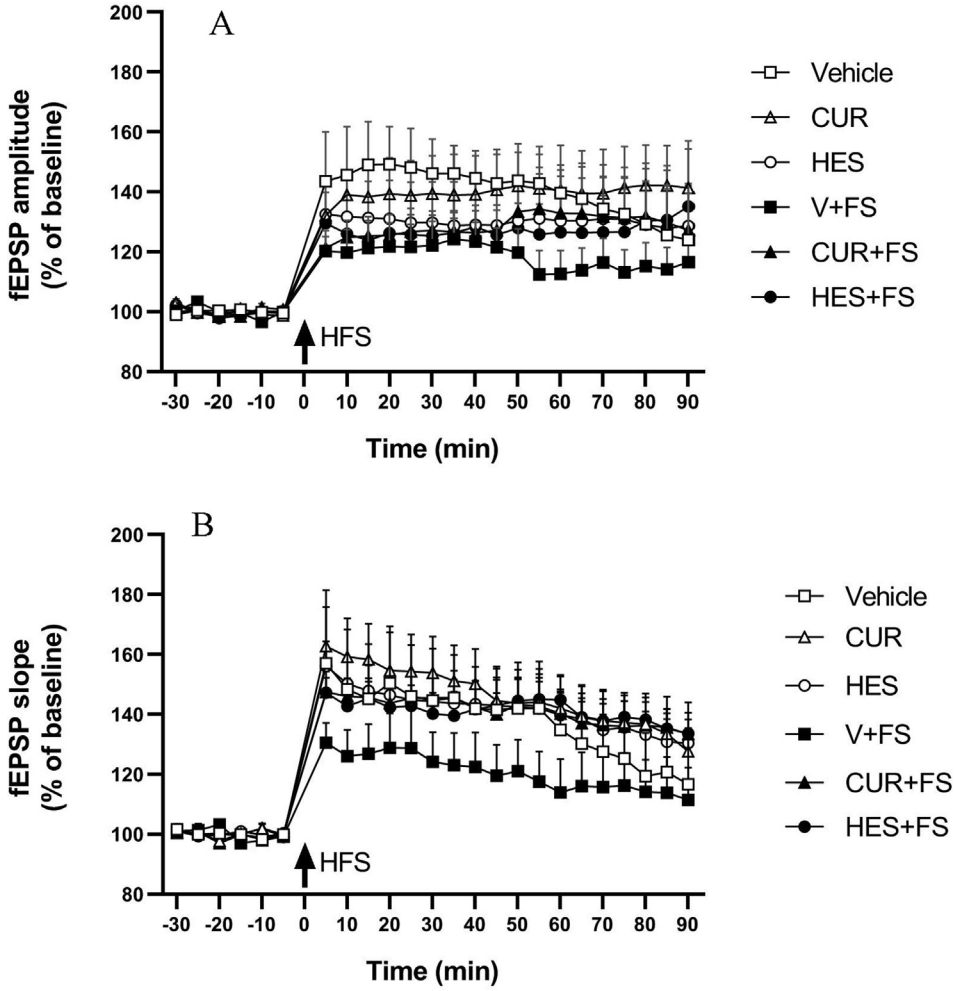

Fig. 7. Comparison of fEPSP amplitude (A) and slope (B) after LTP induction in the CA1 area of the hippocampus using 100 Hz tetanic stimulation in adult rats. (n = 6–7, repeated measures ANOVA followed by Tukey's post-hoc test, data were expressed as mean ± SEM). LTP: long-term potentiation, V: vehicle, CUR: curcumin, HES: hesperidin, FS: febrile seizure.

expression of TLR4 in the hippocampi of rat pups. In accordance, it was reported that seizure increases HMGB1 release from neurons and glia [66]. HMGB1 acts as a TLR4 ligand. This signaling pathway (HMGB1-TLR4) contributes to precipitation and recurrence of both acute and chronic seizures [66]. This pathway is also involved in mesial temporal lobe epilepsy in immature rats and children [67].

We showed that maternal administration of curcumin reduced hippocampal protein of TLR4. In line, it was reported that curcumin reduced TLR4 expression in the hippocampus and other brain regions in traumatic brain injury [21], cerebral ischemia [68,69], fructose-induced neuroinflammation [70], and ethanol-induced neurodegeneration [71].

Our results also showed that maternal administration of hesperidin, similar to curcumin, reduced TLR4 protein expression in the hippocampi of rat pups. We did not find evidence regarding the effect of hesperidin on TLR4 protein expression in the CNS. However, hesperetin reduced TLR4 expression in the cortices and hippocampi in two different experimental models of neuroinflammation [72,73].

In addition to previous findings, the effect of curcumin and hesperetin on the downstream signaling pathways of TLR4 was studied. The results showed that these natural compounds affected the following pathways: TLR4/MyD88/NF-κB, TLR4/p38/MAPK, Nrf2/TLR4/RAGE, Nrf2/TLR4/NF-κB, and TLR4/NF-κB [21,69,71–73]. All these reports show that TLR4 is an important target for curcumin and hesperidin, and TLR4 is involved, at least in part, in the anti-inflammatory effects of these molecules in various experimental models.

4.4. Maternal administration, febrile seizure, and oxidative stress

To evaluate the effect of FS on oxidative stress, we measured hippocampal MDA and GSH. The results showed that, following hyperthermia-induced febrile seizure, MDA was increased. However, it did not change GSH content significantly. The effect of seizure on MDA and GSH contents has been reported with controversies. In agreement with our results, a significant increase in the hippocampal MDA level was reported in adult rats subjected to hyperthermia during immaturity [74]. Also, it was reported that plasma, serum, and erythrocyte MDA levels were increased in patients with febrile seizures [75–77]. In an experimental model of seizure induced by maximal electroshock, GSH levels showed no difference between electroshock and control groups [78,79]. Also, in other types of seizure induced by kainic acid, pentylenetetrazol, or pilocarpine, the levels of GSH did not change significantly [80–82]. However, it was reported that hyperthermia increased GSH level of the hippocampal region [74].

Maternal administration of curcumin decreased elevated MDA content in the hippocampus. However, it did not change GSH content significantly. In accordance, there are considerable reports showing that curcumin is able to decrease and increase MDA and GSH in the brain, respectively [83–89]. The effect of curcumin on GSH has been documented with controversies. For example, it was reported that

curcumin did not have a significant effect on brain GSH level despite decreasing MDA level in the ischemia/reperfusion and irradiation insult [90,91]. On the other hand, the effect of curcumin on GSH may be dependent on its dose. Gupta et al. (2009) showed that pretreatment with curcumin at doses of 100 and 200 mg/kg, but not 50 mg/kg, significantly reversed the oxidative stress markers [92]. Accordingly, it is a possibility that the dose of curcumin we employed (60 mg/kg) was not able to induce a significant effect on GSH.

A limited number of studies have been devoted to examining the effects of hesperidin and hesperetin on oxidative stress in the brain. In these studies, treatment with hesperidin and hesperetin reduced MDA levels [93–95]. However, similar to curcumin, hesperidin induced dose-dependent effects on GSH content, and it did not change GSH at the dose of 100 mg/kg significantly [95]. This effect is in agreement with the present results showing that hesperidin at the dose of 100 mg/kg did not change GSH. However, it should be noted that in our study FS did not change GSH content in the hippocampus. So, it is a possibility that in the absence of FS action on GSH, curcumin and hesperidin did not induce their modulatory effects on GSH.

4.5. Maternal administration, febrile seizure, and memory function

The double Y-maze task has been used as a valid model to evaluate working and reference memories [96], spatial memory, and spatial navigation in rodents [35,97]. In this task, we found that rats with a history of FS had decreased correct entries to the baited box, implying that they had impaired working memory.

Our results show that curcumin and hesperidin reverse memory impairment and reduce the adverse effects of seizures on working memory.

There are reports showing that FS may induce memory disturbance. As examples, in a recent study, Dai et al. (2019) reported that prolonged febrile seizures induced by hyperthermia in rat pups resulted in memory deficits in adulthood and induced inheritable deficits in the next generation through DNA methylation [98]. Also, Yang et al. (2009) noted that early-life frequently repetitive febrile seizures (FRFS) exhibited long-term astrocyte activation and induced memory deficits [99]. Another study showed that developmental febrile seizures, by neuronal restrictive silencing factor enhancement, resulted in spatial memory impairment [100]. Altogether, febrile seizures cause memory impairment through different mechanisms. In addition, inflammation has been reported as an important factor in memory impairment [101]. TLR4 and oxidative stress have key roles in the initiation and propagation of inflammation. The present study showed that, in addition to elevation in MDA content, FS increased TLR4 protein in the hippocampus. So, it is a possibility that memory impairment of the rats with a history of FS was mediated by enhancement of these factors. In accordance, it was reported that TLR4 deficient mice ($TLR4^{-/-}$) had spatial reference memory acquisition, memory retention, and cognitive function better than their wild-type counterparts [102,103]. Wang et al. (2009) demonstrated that lipid peroxidation had a key role in memory impairment induced by carbon monoxide in a step-down-type passive avoidance test [104]. Guerrero et al. (1999) showed that treatment with vitamin E, as a free radical scavenger, reduced the lipid peroxidation level in the hippocampus and attenuated memory impairment following oxidative stress [105].

We evaluated the effects of curcumin and hesperidin on memory deficits of rats with a history of FS. The results showed that maternal administration of either curcumin or hesperidin reversed the memory impairment in these rats. To our knowledge, the consequences of maternal administration of hesperidin or curcumin on memory performance of the offspring in adulthood have been studied very little so far.

Abu-Taweel (2019) reported that maternal administration of curcumin from day one of pregnancy until postnatal day 15, concomitant with mercuric chloride, resulted in shorter escape latencies to reach the platform in the Morris water-maze test compared with the mercuric chloride-treated group and decreased unsuccessful trials to reach the platform. Also, times spent in the open and closed arms of the elevated plus-maze test had significant differences compared with the mercuric chloride-exposed group [106]. In addition, co-treatment with curcumin significantly attenuated the endothelin-1-mediated cell death in primary hippocampal cultured neurons by blocking c-Jun expression [107]. In vitro study of the effect of hesperidin on embryonic neuronal cultures showed that hesperidin reduced cell death and increased synaptogenesis and pre-synaptic activity [108].

On the other hand, numerous studies are showing that these natural compounds are able to enhance memory performance in various animal models [23,71,73,86,94,109] and clinical practice [110–112].

4.6. Maternal administration, febrile seizure, and long-term potentiation

It has been reported that seizure susceptibility is increased via the induction of neuroinflammation and oxidative stress in the hippocampus of the adult male rats following LPS-induced inflammation [113]. Neuroinflammation is an inflammatory response to the brain injury that results in the activation of microglia, increased production of pro-inflammatory cytokines, and generation of reactive species such as ROS. An imbalance between production and degradation of ROS leads to oxidative stress [113]. Chronic neuroinflammation affects LTP via NMDAR or voltage-dependent calcium channel (VDCC) [114]. Both anti- and pro-inflammatory mediators are important for normal brain function, and deviations in inflammatory levels outside of the physiological range may impair neural plasticity [115]. del Rey et al. (2013) reported that activation of a cytokine network in the brain involving IL-1β, IL-1ra, IL-18, IL-6, and TNFα is a physiologically relevant process during LTP and learning [116]. There have been some contradictory reports about the role of IL-1β and TNFα in long-term potentiation or inhibition [117–119]. Moreover, the formation of LTP in the Schaffer collateral-CA1 synapses following neuroinflammation may be age-dependent. Liu et al. (2012) indicated that chronic systemic inflammation induced LTP impairment in middle-aged rats but not in young rats [120]. On the other hand, reactive oxygen species (ROS) are involved in both normal LTP and age-related impairment of LTP. The physiological range of superoxide production is necessary for LTP. In contrast, excessive production of superoxide leads to the formation of other ROS, such as hydrogen peroxide (H2O2) and hydroxyl radical (•OH), that interfere with LTP. Thus, the amount of the particular ROS concentration or its identity dictates a different response [121]. In our study, hyperthermia febrile seizure was induced in the neonatal period, and electrophysiological recording was performed in adulthood. In a recent study, in line with our findings, Kudryashova et al. (2020) found that neonatal pro-inflammatory stress on postnatal days 3–5 did not have a significant effect on the amplitude of LTP in the juvenile male rats. The authors suggested that higher resistance to LPS-induced stress may explain these findings in male rat pups [122]. Shin et al. (2016) showed that maternal separation for 4 h per day from P2 to P20 exhibited no significant effect on LTP in the CA3-CA1 synapses during adulthood [123]. Collectively, neuroinflammation and oxidative stress may affect LTP in an age-dependent, sex-dependent, or concentration-dependent manner.

Electrophysiological recording of the Schaffer collateral-CA1 showed that febrile seizure decreased amplitude and slope of fEPSPs of the rats with a history of febrile seizures. However, it was not statistically significant. There are conflicting data about the synaptic plasticity after febrile seizures. Zhang et al. (2011) reported that febrile seizure did not affect synaptic plasticity in the lateral perforant path of the rat hippocampal dentate gyrus, and fEPSP slope was not significant between control and FS animals after 100 Hz tetanus stimulation [124]. However, Notenboom et al. (2010) found enhanced long-term potentiation and reduced long-term depression (LTD) in adult rats with a prior history of experimental febrile seizures at P10. In this mentioned study, the slope of fEPSP was significantly higher in hyperthermia rats

compared with the controls [125]. Contrary to the previous study, Chang et al. (2005) showed that early life repetitive febrile seizures led to impaired long-term potentiation and facilitated long-term depression at the Schaffer collateral-CA1 synapses [126]. Also, another study indicated that 12 weeks after FS, the amplitude and slope of evoked fEPSP following LTP were reduced in the FS group [127].

Our results showed that curcumin- and hesperidin-treated rats had LTP indices almost similar to their normal control rats. There is a report showing that curcumin treatment failed to rescue aging-induced impairment of basal synaptic transmission at Schaffer collateral-CA1 synapses [128]. In addition, hesperidin did not affect basal synaptic transmission and theta-burst LTP [129]. However, there are studies reporting a modulatory effect for curcumin on LTP impairment. For example, the HIV-1 gp120 V3 peptide suppressed LTP in the rat hippocampal CA1 region that was reversed by curcumin [130,131]. Similarly, curcumin modulated abnormal increase in LTP in the $Tsc2^{+/-}$ mouse model of tuberous sclerosis [132].

4.7. Febrile seizure, working or spatial memory, and LTP (linkage or not)

There are controversial reports about LTP and memory. It was reported that activation of the NMDA receptor and stimulation of CaM kinase II play important roles in the hippocampal LTP and hippocampal-dependent memory, including the spatial memory [133,134]. However, Kikusui et al. (2000) indicated that a selective NMDA receptor antagonist did not impair spatial working memory but completely blocked hippocampal CA1 LTP [135]. Also, rab3A null-mutant mice that lack both mossy fiber (MF)-LTP and MF-LTD revealed no impairment in spatial, contextual, or working memory [136]. Also, in another study, it was found that spatial memory was disassociated from LTP changes [137].

Febrile seizures may affect gene expression and structural remodeling in the hippocampus [138]. cAMP-responsive element-binding protein (CREB) is a multifaceted regulator of synaptic plasticity [139]. Phosphorylation of CREB significantly was decreased in the hippocampi of adult rats with a history of early-life FRFS after learning tasks and correlated with memory deficits [140]. CREB mutant mice exhibited deficits in memory tasks, but LTP recording in the hippocampal CA1 and dentate gyrus was reported as normal [141]. Early growth response gene 3 (EGR3), is one of the genes that regulate an array of target genes to mediate physiological processes such as synaptic plasticity, memory, and cognition [142]. It was reported that repeated electroconvulsive seizure reduced Egr1, Egr2, and Egr3 protein levels in the rat frontal cortex [143]. On the other hand, $Egr3^{-/-}$ mice showed impaired LTD and Y-maze performance with normal hippocampal LTP. The authors indicated that fewer sequential arm entries in the Y-maze were associated with LTD impairment [144].

Consistent with these studies, we observed a significant memory dysfunction that was not concomitant with significant changes in electrophysiological indices. So, we suggest that febrile seizures may affect memory function via changes in the expression of genes involved in the synaptic plasticity and hippocampal remodeling. We may also suggest that working memory impairment was associated with LTD but not LTP changes. This hypothesis needs to be justified by more studies.

5. Conclusion

Our study revealed that curcumin had better anticonvulsive, anti-inflammatory, and antioxidative effects than hesperidin. It is possible that curcumin, via a reduction in brain inflammation, TLR4 protein expression, and lipid peroxidation, induced its anticonvulsive effect in rat pups exposed to febrile seizures.

CRediT authorship contribution statement

Rabi Atabaki: Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Ali Roohbakhsh:** Conceptualization, Methodology, Validation, Resources, Data curation, Writing - review & editing, Supervision, Funding acquisition. **Ali Moghimi:** Conceptualization, Methodology, Validation, Resources, Data curation, Writing - review & editing, Supervision, Funding acquisition. **Soghra Mehri:** Methodology, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2020.106720.

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