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Original article

Impact of levetiracetam on cognitive impairment, neuroinflammation, oxidative stress, and neuronal apoptosis caused by lipopolysaccharides in rats

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ABSTRACT

Introduction: Neuroinflammation is associated with the elevation of toxic proinflammatory mediators that promote neurodegeneration and subsequently affect cognition. Causes of inflammation in the neuronal cells are believed to initiate various neurodegenerative disorders, mainly Alzheimer's disease. Levetiracetam is a second-generation antiepileptic drug. There is evidence supporting the memory-enhancing effect of levetiracetam from numerous experimental and clinical studies. Therefore, this research focused on finding its protective effects against lipopolysaccharides prompted cognitive impairment and exploring possible mechanisms underlining their neuroprotection.

Methodology: Two doses (100 or 200 mg/kg) of levetiracetam were administrated orally for 30 days. Additionally, four doses (250 μg/kg) of lipopolysaccharide were injected peripherally to induce neurotoxicity. Behavioral tests were carried out using various maze models. At the end of the tests, brain tissues were collected for biochemical evaluations. Cholinergic, neuroinflammatory, apoptosis, and oxidative-related parameters were analyzed in the brain homogenate to explore the possible mechanisms of action of levetiracetam.

Results: In lipopolysaccharide-induced rats, levetiracetam indicated a reduction ($p < 0.01$) in transfer latency using the elevated plus-maze. An improvement ($p < 0.01$) in novel and familiar objects exploration time using novel object recognition test. A rise ($p < 0.05$) in novel arm entries and extended time spent in the novel arm using the Y-maze test. In extension, the levels of acetylcholine ($p < 0.001$), anti-inflammatory factors (transforming growth factor-β1; $p < 0.01$ and interleukin-10; $p < 0.05$), and an antioxidant (catalase; $p < 0.01$) were elevated in lipopolysaccharide-induced rats after the administration of levetiracetam. In contrast, inflammatory factors (cyclooxygenase-2; $p < 0.05$, nuclear factor kappa B; $p < 0.05$, tumor necrosis factor-α; $p < 0.01$, and interleukin-6 ($p < 0.01$), apoptosis inducers (BCL2-asso ciated X protein; $p < 0.05$ and Caspase-3 ($p < 0.001$), and oxidative stress (malondialdehyde; $p < 0.05$) were considerably reduced with levetiracetam in lipopolysaccharide-induced rats.

Conclusion: The collective results suggested that levetiracetam may be able to treat neuroinflammatory-related memory loss by enhancing cholinergic activity while reducing neuroinflammation, cellular apoptosis, and oxidative stress.

1. Introduction

Neurodegenerative diseases are notified as a diverse collection of complications marked by the gradual deterioration of both central or peripheral nervous systems' structures and functions. Hence, dementia is a neurodegenerative condition, according to WHO almost 55 million people have dementia globally and the forecast is around 10 million new cases per year (Chowdhary et al., 2022). Therefore, the high prevalence of dementia will be a major burden on the world's healthcare systems in the future (Wong, 2020). Pathologically, the exact cause of dementia is still unclear. However,

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some principles including neuroinflammation, cholinergic deficits, oxidative stress, and cellular apoptosis are suggested to have vital roles in memory impairment (Mani et al., 2021). Hence, inflammatory vulnerable to neuronal cells results in the decline of cognitive functions. Microglia found in CNS act as the main components of the brain's immunological defense system, and have the ability to recognize harmful or external infections. The stimulation of microglia results in neuroinflammation by triggering a number of inflammatory mediators like proinflammatory cytokines, transcription factors, chemokines, reactive oxygen/ nitrogen species (ROS/RNS), and peptides (Ahmad et al., 2022; Iqubal et al., 2018). Additionally, the cholinergic neurons have been offered as internal regulators of immunological responses or neuro-immune interactions. The effect of cholinergic neurons on the anti-inflammatory effect has also been demonstrated preclinically by using acetylcholinesterase (AChE) inhibitors. Hence AChE inhibitors prevent inflammatory cytokines synthesis in the brain (Tyagi et al., 2007). Also, acetylcholine (ACh) has been shown to control the discharge of cytokines including IL-6, IL-1β, and IL-18 in the brain (Borovikova et al., 2000).

Brain tissue is principally vulnerable to oxidative harm due to increased oxygen utilization and the presence of more oxidizable polyunsaturated fatty acids. Generally, neuronal cells present in brain areas are highly vulnerable to the imbalanced liberation of ROS from cellular metabolism and various enzymatic process. Moreover, the excessive production of ROS leads to either cellular apoptosis or a necrotic pathway and results in neuronal cell damage (Jelinek et al., 2021). Intracellularly from mitochondria and extracellularly from inflammation are considered as potential sources of free radicals. When anti-oxidant processes are out of balance with these reactive molecules it can damage DNA, proteins, and lipids, which causes cell toxicity and tissue damage (Ozkul et al., 2007). Besides, the overproduction of ROS impairs mitochondrial Ca^{+2} homeostasis and affects its membrane potential, which ultimately triggers the apoptosis pathway. Additionally, inappropriate apoptosis is strongly linked with increased neuronal damage in various neurodegenerative disorders, including AD (Radi et al., 2014).

Inducing neuroinflammation can be done in a variety of methods, with lipopolysaccharides (LPS) being one of them. A powerful endotoxin, LPS triggers the production of a large number of pro-inflammatory cytokines in a variety of cell types, which may lead to an acute inflammatory response (Batista et al., 2019). Toll-like receptor 4 (TLR4) is activated when LPS binds to it, leading to the release of pro-inflammatory cytokines including tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-1α, and IL-1β (Keymoradzadeh et al., 2020; Lee et al., 2002; Bluthé et al., 1992). The LPS induction also elevated nitric oxide (NO) and prostaglandin E2 (PGE2) levels in the brain via an inflammatory response mediated by the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases (MAPK) signaling pathways (Tang et al., 2023). The antioxidant system is weakened and oxidative stress increases as a result of this process (Mirahmadi et al., 2018). Memory and learning impairment can be caused in part by neuroinflammation and oxidative stress (Zhao et al., 2019). In addition, enhanced AChE activity with LPS-induced neuroinflammation indicates an inverse link between cholinergic functions and neuroinflammation (Bison et al., 2009; Tyagi et al., 2008).

Levetiracetam (LEVE) is an antiepileptic medication that has a broad spectrum of activity. It is used as an alone or adjuvant medication to treat generalized seizures and focal onset (Itoh et al., 2019). The broad anticonvulsant effects of LEVE are proposed by various mechanisms including anti-inflammatory, anti-apoptotic, and antioxidative effects that have recently received much attention (Srivastava et al., 2016). In order to prevent synaptic transmission, the neuromodulator LEVE blocks N-type calcium channels by acting on synaptic vesicle protein 2A (SV2A) (Deshpande and Delorenzo, 2014). LEVE is well-recognized to reverse cognitive decline, behavioral abnormalities, hippocampal remodeling, and synaptic dysfunction (Sanchez et al., 2012). The evidences have presented that cognitive performance is improved by low doses of LEVE in both preclinical and clinical settings. The regulation of hippocampal hyperactivity is assumed to be the basis for LEVE's therapeutic actions (Haberman et al., 2017). In addition, LEVE attenuated the age- and AD-associated memory loss and learning functions in the aging-induced mice experimental model (Devi and Ohno, 2013). It also enhanced the acetylation of histone H4, which decreases histone deacetylase (HDAC) functions and facilitates the establishment of long-term memory (Eyal et al., 2004; Stefanko et al., 2009). Therefore, LEVE may have the ability to manage AD effectively in inpatient care.

Administration of LEVE displayed an anti-inflammatory effect by decreasing the IL-1β systems immunoreactivity of the astrocytic and glial cells in the rat piriform cortex and hippocampus (Kim et al., 2010). Current research reported that elevated expression of pro-inflammatory cytokine levels during epileptogenesis after status epilepticus (SE) was suppressed by LEVE. In continuation, the treatment of LEVE declined mononuclear phagocyte phagocytosis and cytokine expression within two days later SE (Matsuo et al., 2022; Itoh et al., 2016). Also, LEVE inhibited BV-2 microglial stimulation *in vitro*, which was evidenced by phagocytic activity, cytokine expressions, and morphological changes (Itoh et al., 2019). Moreover, LEVE is said to have an anti-inflammatory impact that restores the *in vitro* electrophysiological characteristics of astrocytic cells. (Haghikia et al., 2008). More studies also addressed that LEVE affected IL-1β and TNF-α expressions in epileptic rat models (Himmerich et al., 2013; Kim et al., 2010). In pentylenetetrazole-induced kindling mice, LEVE results in altering the hippocampal antioxidants and brain-derived neurotrophic factor (BDNF) expression (de Souza et al., 2019). Early, LEVE was shown to decrease neuronal cell cytotoxicity and apoptosis and enhance angiogenesis in the ischemic experimental stroke model in rats (Yao et al., 2021). Therefore, the purpose of the current study was directed to examine how LEVE protects against LPS-induced memory deficiency, cholinergic dysfunction, neuroinflammation, cellular apoptosis, and oxidative stress in rat models.

2. Materials and method

2.1. Animals

For the present experiment, 24 male, 3-month-old, Sprague Dawley rats (body weight 160–240 g) were collected from Qassim University's College of Pharmacy. During the entire experiment, rats were kept in typical lab settings with a 12 h light–dark cycle. Rats were accommodated in three per cage. Retain normal temperature [22 °C (±1)] and humidity [45%–55%]. Standard animal pellets and water were allowed freely accessible. Ethical approval of the experimental protocols was granted by the Committee of Research Ethics, Deanship of Scientific Research of Qassim University (Approval ID 21–21-14; 13–6-2022).

2.2. Vehicle

LEVE was procured from Toronto Research Chemicals, Canada (TRC: L331500), and dissolved in normal saline (NS; 0.09% w/v) for oral administration (p.o.) to the rats. The LPS (SKU: L2630) were acquired from Sigma-Aldrich, USA, and also dissolved in NS for intraperitoneal (i.p.) administration.

2.3. Experimental design and drug treatments

The rats were allocated to four groups (n = 6) for the experimental procedure. The schedules of the prophylactic drug (LEVE) treat-

ment and induction of neurotoxicity (LPS) were followed according to previous reports with minor modifications (Kamdi et al.,2021; Mani et al., 2022a). The control rats were administered 30 days with the vehicle (NS; 10 mL/kg, p.o.) and were injected (days 22, 23, 24, and 25; four doses) with NS (10 mL/ kg, i.p.). The LPS-induced group was administered 30 days with the NS (10 mL/kg, p.o.), and neuronal deficits were induced with LPS (250 μg/kg, i. p.) on days 22, 23, 24, and 25 of the experimental schedule. For drug-treated groups, the animals were administered 30 days with LEVE (100 or 200 mg/kg, p.o.), and neuronal deficits were induced with LPS, similar to the LPS-induced group. The doses of LPS (Rahim et al., 2021) and LEVE (Mani et al., 2022a) used in this experiment are based on our previous reports. For the assessment of cognitive performance, EPM procedures were carried out on days 26 (training) and 27 (retention). On days 28 (training session) and 29 (test session), the NOR test was implemented. Both of the Y-maze test sessions (training and test sessions) were conducted on day 30. At the end, the brain tissues were utilized for enzyme-linked immunosorbent assay (ELISA) tests (Fig. 1).

2.4. Elevated plus-maze (EPM) test

The EPM test is a commonly performed behavioral test to assess memory-related behavior. It has four arms, two enclosed and two open, with 50 cm length and 10 cm width, and is 50 cm above the ground. For the first day of training (day 26), each rat was placed at a fixed open-arm end and away from the midpoint. Transfer latency (TL) of day 1 was referred to as the learning capacity of animals. TL is defined as "the time taken for a rat (in seconds) to move from the open arm to either one closed arm" (Mani et al., 2022b). After 24 h (day 27), the TL was targeted as retention of the learned task, which is referred to as the memory.

2.5. Novel object recognition (NOR) test

The test is commonly utilized to assess the ability of laboratory animals' memory to recognize novel objects. The NOR comprises an open wooden box apparatus with dimensions of 80 × 60 × 40 cm. The test technique was modified based on a similar study by Mani et al. (2022b). Two types of objects (familiar objects: two rectangular boxes, and a novel object: a cylindrical box) were used to assess discrimination ability. The objects have the same height and firmness, which were not moved by animals during the test. This experiment followed three phases: habituation, training, and test. During the habitual phase (day 28), without using any objects, each rat was permitted to explore for 5 min. After 24 h (day 29), the training phase was implemented by allowing each animal to explore two uniform rectangular boxes (familiar objects: FO1 and FO2) for 5 min and recording the exploration times of familiar objects (ETFOs). After three hours, each animal was given five minutes to explore two objects: a novel object (NO) and a familiar object (FO1). The exploration times of a novel object (ETNO) and an ETFO were documented for each animal. They were calculated as "the time spent by each animal when directing its nose to an object with a distance ≤ 2 cm and touching it using its nose". Also, the discrimination index (DI) was determined by following a previous report (Mani et al., 2022b).

2.6. Y-maze test

It is an extensive model for evaluating behavioral tasks for spatial memory among rodents by evaluating animals' ability to explore novel places. A wooden Y-maze consists of three arms (A, B, and C) at 120° angles with a measurement of 50 × 10 × 18 cm. At each arm's end, there was an image with various patterns, and the device was set down on the floor. The test protocol was modified from a similar study by Mani et al, (2022a; 2022b). The test comprised two sessions: training (first trial) and test (second trial) sessions on day 30 of the treatment schedule. During the training, a novel arm (A) was blocked, and each animal was free to explore the other two arms (B and C; known arms) for 5 min. In the test, rats were allowed to explore A, B, and C) freely including the novel one for 5 min. The number of entries in the novel arm (NENA) and both known arms (NEKA) were counted. An animal entering an arm with 85% of its body was considered an entry. The time spent by rats in a novel arm was also assessed and each animal's percentage of time spent in novel arms [TSNA (%)] was calculated (Mani et al, 2022a; 2022b).

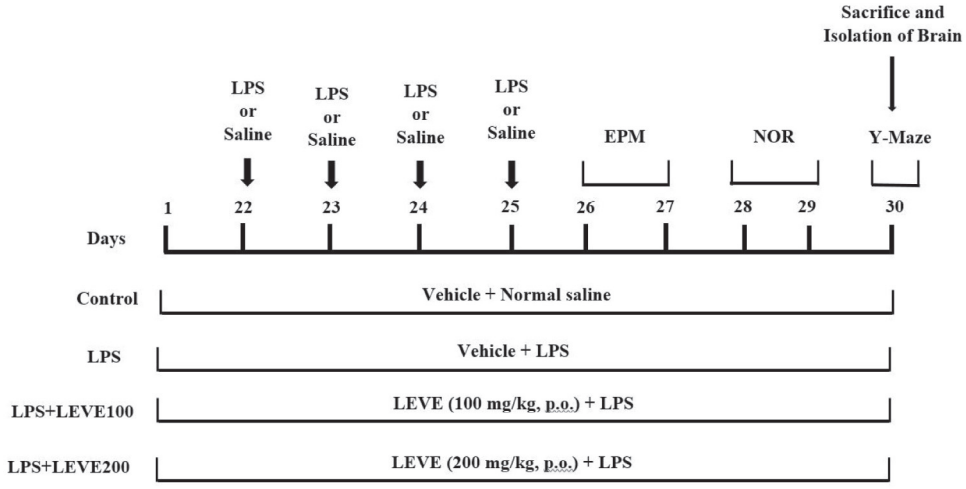

Fig. 1. Timeline of the experiments using rats. Groups of animals were administered with vehicle or levetiracetam (LEVE; 100 or 200 mg/kg, p.o.) for 30 days. To induce neuronal toxicity, four injections of lipopolysaccharide (LPS; 250 μg/kg, i.p; days 22, 23, 24, and 25) were given to groups other than the control group. The elevated plus-maze (EPM) assessments were conducted on day 26 (training) and day 27 (retention). On day 28 (habituation) and day 29 (training and test sessions), the novel object recognition (NOR) test was conducted. Whereas, both sessions (training and test sessions) of the Y-Maze test were conducted on day 30. After the Y-maze test, all the animals were sacrificed and brain tissues were collected for ELISA tests.

2.7. Isolation of brain tissues

After maze experiments (day 30), each rat was sacrificed using cervical dislocation under mild diethyl ether anesthesia by inhalation using a desiccator chamber. After sacrifice, all rats' brains were carefully collected. The brain tissue was homogenized using phosphate-buffered saline (PBS; pH 7.4) at ice-cold (4 °C). Then centrifuged for 10 min at 4000 rpm to collect the aliquot and stored at −80 °C. The biuret colorimetric method was followed to calculate the content of total protein in each brain tissue homogenate sample.

2.8. Determination of cholinergic, neuroinflammatory, apoptosis, and oxidative markers in the brain homogenate

Specific sandwich ELISA kits from MyBioSource (MyBioSource, Inc., USA) were used to estimate each targeted marker. The estimation is based on the intensity of colour that happens when the biotinylated detection antibody reacts with the particular antigen in the presence of SABC. A microplate reader (ELx800, BioTek Instruments, USA) was utilized to quantify the absorbance spectrophotometrically at 450 nm, and a standard curve was plotted to estimate the specific quantity of each indicator. For a cholinergic parameter, the level of ACh (catalog number (CN): MBS262132) was measured. Neuroinflammation-related parameters such as cyclooxygenase (COX)-2 (CN: MBS725633), NF-κB (CN: MBS764450), TNF-α (CN: MBS2507393), IL-6 (CN: MBS27010823), IL-10 (CN: MBS702776) and transforming growth factor (TGF)-β1 (CN: MBS824788) were estimated. The levels of B-cell lymphoma (Bcl)-2 (CN: MBS452319), BCL2-associated X prot ein (Bax; CN: MBS2703209), and Caspase-3 (CN: MBS261814) levels were measured as apoptotic parameters. Furthermore, malondialdehyde (MDA; CN: MBS268427) for oxidative stress and catalase (CN: MBS2704433), as well as glutathione (GSH; CN: MBS265966) levels for antioxidant parameters, were analyzed in the brain homogenate. Assay concepts, techniques, and calculations were carried out following the manufacturer's catalog.

2.9. Statistical analysis

Results were represented as mean ± standard deviation (SEM). The one-way ANOVA was employed to evaluate all the data, and the Tukey-Kramer post hoc analysis was used to determine the degree of variance. In particular, to compare between the exploration time of two different objects (FO1 vs FO2 and FO1 vs NO) in the NOR test, the Student's unpaired *t*-test was applied. GraphPad 9.5.0 version (GraphPad Software Inc., United States) was utilized for the statistical analysis. A *p*-value of ≤ 0.05 was referred statistical significance.

3. Results

3.1. Pre-treatment with LEVE decreased the TL of LPS-induced animals in the EPM test

Fig. 2 indicates the effect of LEVE on the retention of TL. Using one-way ANOVA analysis [$F(3,20) = 12.79$, $p < 0.001$] showed a significant difference in TL time changes between experimental groups. Further *post hoc* analysis indicated that treatment with LPS considerably ($p < 0.001$) raised the TL time (46.83 ± 3.53 S) in comparison to the control group (24.17 ± 1.30 S). Compared with LPS-induced rats, LEVE (100 mg/kg, p.o.) lowered the TL time (32.33 ± 2.86 S; $p < 0.01$). Moreover, LEVE (200 mg/kg, p.o.) significantly reduced the TL time further (26.83 ± 3.11 S; $p < 0.001$).

3.2. Pre-treatment with LEVE enhanced the memory parameters of LPS-induced animals in the NOR test

To assess the impact of LEVE on cognitive targets as recognition memory in LPS-induced rats, the NOR test was used (Fig. 3). The targeted parameters were extracted in both training (objects: FO1 and FO2) and test (objects: FO1 and NO) session when the animals explored to pointing objects from each session.

Fig. 3**A** shows the consequences of LEVE on the ETFOs in LPS-induced rats during a training session. Applying one-way ANOVA analysis revealed considerable modifications [FO1: $F(3,20) = 13.62$, $p < 0.001$ and FO2: $F(3,20) = 10.02$, $p < 0.001$] in the ETFOs between the groups. It was found that there was an extensive reduction ($p < 0.001$) in the ETFO of LPS-induced rats (FO1: 17.67 ± 0.76 S and FO2: 16.17 ± 1.76 S) as associated with the corresponding control (FO1: 31.83 ± 1.92 S and FO2: 29.67 ± 2.43 S). Interestingly, LEVE at 100 mg/kg (FO1: 25.33 ± 1.36 S, $p < 0.05$ and FO2: 25.67 ± 1.43 S, $p < 0.01$) and 200 mg/kg (FO1: 28.17 ± 2.12 S, $p < 0.01$ and FO2: 26.17 ± 1.52 S, $p < 0.01$) showed an elevation in LPS-induced rats during the ETFOs. Moreover, analyzing of unpaired *t*-test between the parallel groups of FO1 and FO2 revealed that there were no significant changes in ETFO among the objects.

Fig. 3**B** displays the effect of LEVE on the ETFO and ETNO of LPS-induced rats while performing test sessions. The statistical analysis revealed alterations [FO1: $F(3,20) = 10.15$, $p < 0.001$ and NO: $F(3,20) = 17.82$, $p < 0.001$] in the ETFO and ETNO between the groups. Both the ETFO and ETNO of rats (FO1: 11.00 ± 1.00 S and NO:16.67 ± 1.99 S) were lowered ($p < 0.001$) in LPS-induced groups when related to the control (FO1: 21.67 ± 1.71 S and NO: 44.50 ± 3.16 S). Considered ETFO, LEVE in both doses (100 mg/kg: 19.17 ± 1.68 S, and 200 mg/kg: 19.00 ± 1.32 S) showed an elevation ($p < 0.01$) in LPS-induced rats. Further, the ETNO with LEVE 200 mg/kg treatment increased ($p < 0.001$) to 38.83 ± 2.81S in LPS induction. Besides, the lower dose of LEVE 100 mg/kg showed an elevation ($p < 0.01$) of ETNO to 34.17 ± 3.25 S. Interestingly, the comparison of exploration time between the objects (FO1 and NO) explained that each group of rats was spending higher time (control: $p < 0.001$, LPS: $p < 0.05$, LPS + LEVE100: $p < 0.01$, and LPS + LEVE200: $p < 0.001$) with NO as compared to FO1.

The discrimination ability of rats to two dissimilar objects in NOR was evaluated as the DI of different groups (Fig. 3**C**). The obtained results highlighted a significant variance [$F(3,20) = 9.013$, $p < 0.001$] in DI values among the treated groups. Administration of LPS significantly ($p < 0.01$) declined the DI values (19.79 ± 1.67%) from the control animals (34.27 ± 3.89%). Among two doses of LEVE, the higher dose of 200 mg/kg considerably ($p < 0.01$) increased the DI value (34.22 ± 1.25%) as allied with the LPS-induced group.

3.3. Pre-treatment with LEVE enhanced the memory parameters of LPS-induced animals using the Y-maze test

Fig. 4 illustrates how LEVE affected the NEKA and NENA, and TSNA (%) during a test session using a Y-maze in LPS-induced rats. In the test session, the animals were permitted to explore the entire maze including all the arms.

Fig. 4**A** shows that a significant variation [$F(3,20) = 4.674$, $p < 0.05$] in the NEKA was noted between the treatment groups. The further *post-hoc* comparison revealed a decrease ($p < 0.05$) in NEKA with LPS induction (3.67 ± 0.33) showing a cognitive deficit in LPS treatment when related to the control group (5.83 ± 0.40). On the other hand, the administration of 200 mg/kg, p.o. LEVE displayed a considerable ($p < 0.05$) increase in NEKA (6.00 ± 0.63), while with 100 mg/kg, p.o.; 5.17 ± 0.54 of LEVE, no changes were established in comparison to LPS-induced.

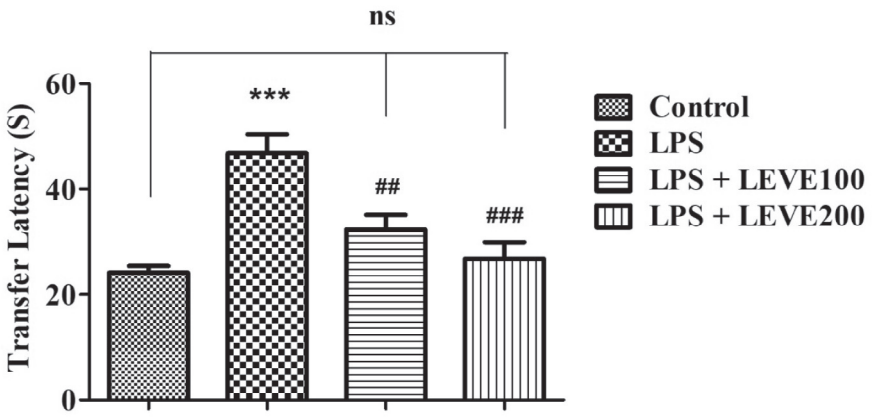

Fig. 2. Effect of levetiracetam on retention of transfer latency (s) of LPS-induced rats using elevated plus-maze. The results are expressed by mean ± SEM (n = 6). One-way ANOVA followed by Tukey-Kramer multiple comparisons test. $^{***}p < 0.001$ as compared to the control group; ns- not significant as compared to the control group; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to the LPS-induced group.

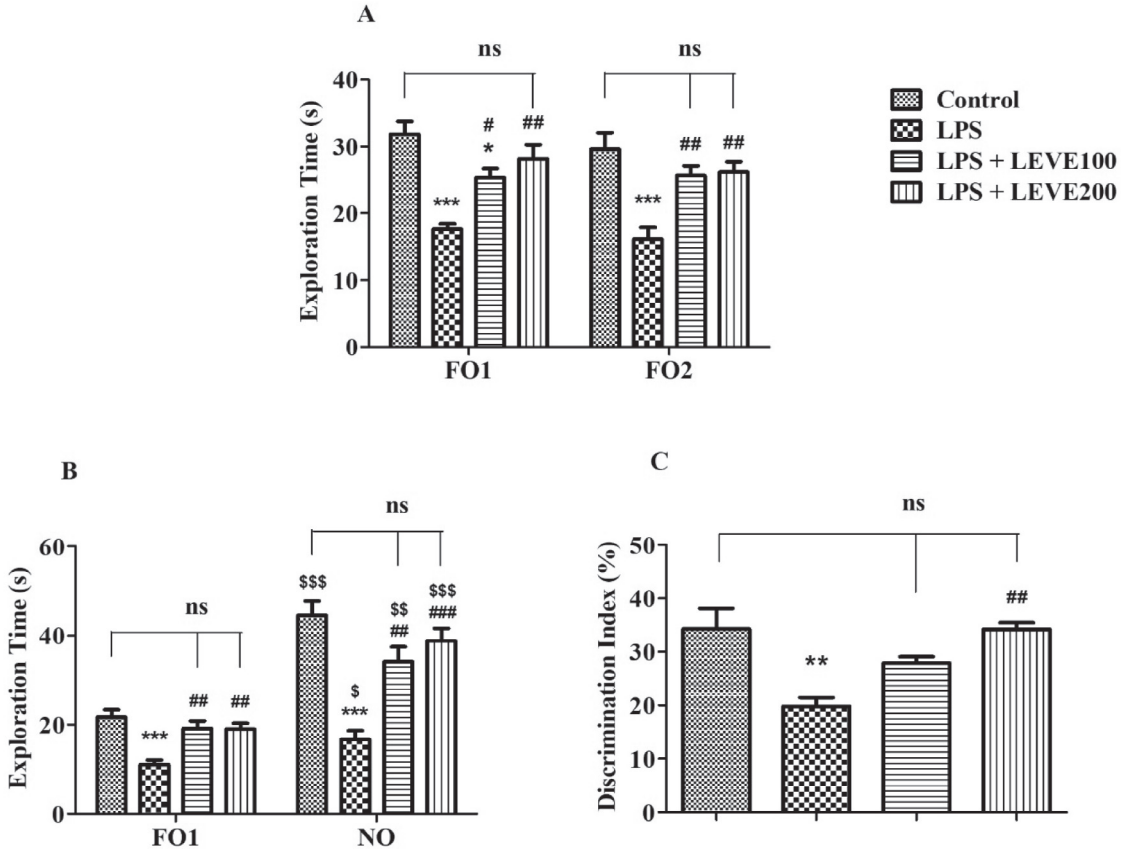

Fig. 3. Effect of levetiracetam on (A) exploration time of familiar objects (FO1 and FO2) during the training session, (B) exploration time of familiar (FO1) and novel (NO) objects during the test session, and (C) discrimination index (DI) of LPS-induced rat model during the test session using novel object recognition test. The results are expressed by mean ± SEM (n = 6). One-way ANOVA followed by Tukey-Kramer multiple comparisons test for comparisons within the groups. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ as compared to the control group; ns- not significant as compared to the control group; $\#p < 0.05$, $\#\#p < 0.01$ and $\#\#\#p < 0.001$ as compared to the LPS-induced group. Further, the Student's unpaired *t*-test was used to compare between exploration time of two different objects (Fig. 3A; FO1 vs FO2 and Fig. 3B; FO1 vs NO). There were no significant variations between the corresponding groups of FO1 and FO2; $\$p < 0.05$, $p < 0.01$, and $\$p < 0.001$ as compared to the corresponding groups of FO1 and NO.

Fig. 4**B** presents the outcome of LEVE on the NENA of LPS induction. A one-way ANOVA analysis represented the significant differences [$F(3,20) = 10.63$, $p < 0.001$] in the NENA. Then, in comparison with the control group (10.00 ± 0.52), it was seen that LPS-induced rats indicated an extensive ($p < 0.001$) decrease in NENA (5.83 ± 0. 48), which showed a cognitive deficit with LPS induction. However,

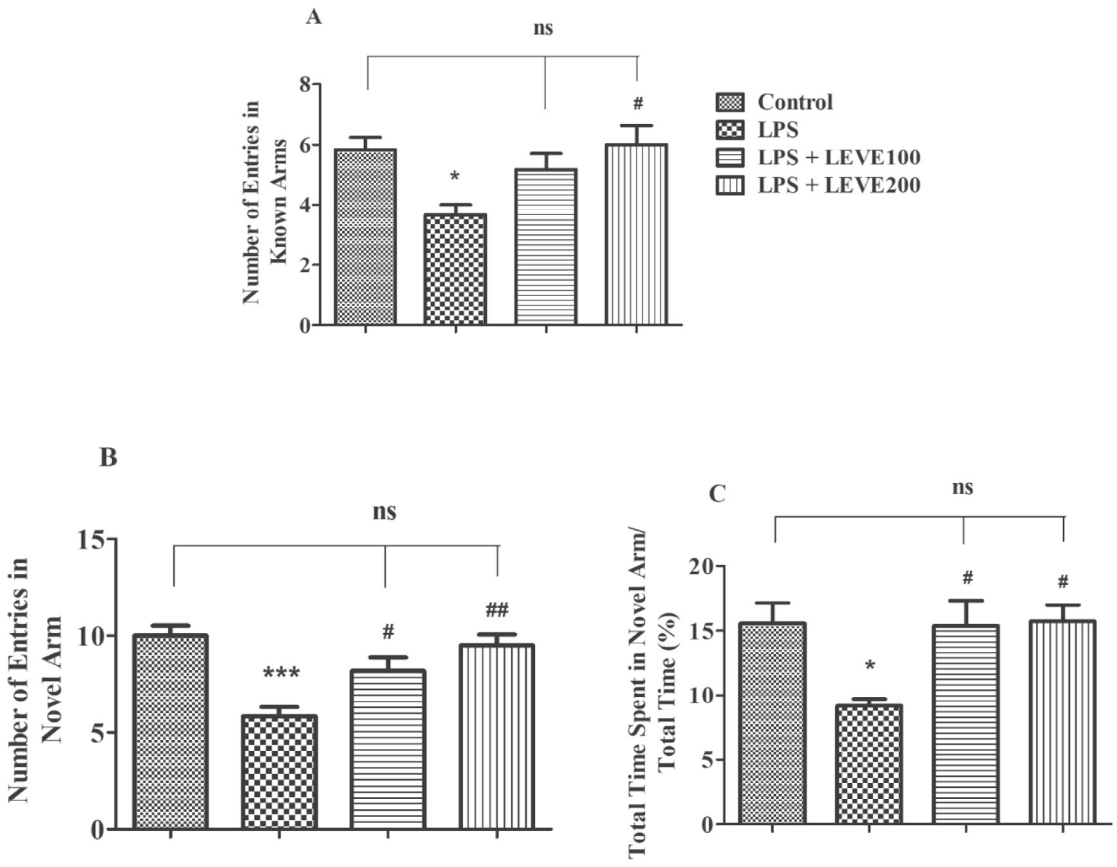

Fig. 4. Effect of levetiracetam on (A) the number of entries in known arms (NEKA), (B) the number of entries in the novel arm (NENA), and (C) the percentage of time spent in the novel arm [TSNA(%)] in the test session of LPS-induced rat model using Y-maze. The results are expressed by mean ± SEM (n = 6). One-way ANOVA followed by Tukey-Kramer multiple comparisons test. $^{*}p < 0.05$ and $^{***}p < 0.001$ as compared to the control group; ns- not significant as compared to the control group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ as compared to the LPS-induced group.

the administration of LEVE (100 and 200 mg/kg, p.o.) in comparison to LPS-induced rats, had significantly higher numbers of entries (8.17 ± 0.70; $p < 0.05$ and 9.50 ± 0.56; $p < 0.01$, correspondingly) which explained the improvement in memory functions.

The effect of LEVE on the TSNA (%) during the test session is presented in Fig. 4**C**. Statistical analysis between all the treated groups revealed a substantial difference ($F(3,20) = 4.933$, $p < 0.05$) in TSNA (%). Only the LPS treatment declined ($p < 0.05$) the value of the TSNA (%) (9.17 ± 0.55%) as considered to the control group (15.56 ± 1.61%). In comparison to LPS-induced rats, the treatment of LEVE in both doses (100 and 200 mg/kg, p.o.) upgraded the TSNA (%) significantly (15.39 ± 1.94% and 15.72 ± 1.28%, respectively; $p < 0.05$). Interestingly, there were no significant changes among the control group and LEVE groups.

3.4. Pre-treatment with LEVE upregulated brain ACh levels in LPS-induced rats

The impacts of LEVE on ACh levels in LPS-induced rat brains are presented in Fig. 5. A considerable variation was seen in brain ACh levels [$F(3,20) = 13.45$, $p < 0.001$] after treatment. It was shown that LPS induction reduced ($p < 0.001$) the ACh level (13.07 ± 1.6 5 pg/mg protein) in rats as related to the control rats (27.86 ± 2. 12 pg/mg protein). LEVE 200 mg/kg administration increased ACh levels (28.80 ± 2.59 pg/mg protein; $p < 0.001$) when compared to LPS-induced. In contrast, no considerable alterations in ACh levels (19.77 ± 1.54 pg/mg protein) with LEVE at 100 mg/kg compared to LPS-induced rats' brain homogenate.

3.5. Pre-treatment of LEVE-altered neuroinflammatory mediators in LPS-induced rats

The results of neuroinflammatory parameters such as COX-2, NF-κB, TNF-α, IL-6, IL-10, and TGF-β1 altered with the treatments with LEVE and LPS are listed in Fig. 6.

Treatments with LPS and LEVE exhibited a modification [$F(3,20) = 5.961$, $p < 0.01$] in the brain COX-2 levels (Fig. 6**A**). The control animals resulted in COX-2 levels of 0.136 ± 0.014 ng/mg protein. Four doses of LPS caused an increase ($p < 0.05$) in COX-2 levels in the brain concerning control animals, and the levels of the COX-2 were 0.187 ± 0.012 ng/mg protein in LPS-induced rats. LEVE treatments offered significant protections, and the COX-2 levels were decreased to 0.129 ± 0.008 ng/mg protein ($p < 0.01$) and 0.134 ± 0.010 ng/mg protein ($p < 0.05$) in the brain at 100 and 200 mg/kg, respectively, as related to the LPS-induced animals. These values were comparable to the control values.

The effect of a transcription factor NF-κB after the treatment with LPS and LEVE is explained in Fig. 6**B**. A notable variation [$F(3,20) = 10.23$, $p < 0.001$] in brain NF-κB levels were found between the groups. The NF-κB levels (14.71 ± 1.116 ng/mg protein; $p < 0.01$) were elevated after the LPS injections as matched to the control (9.587 ± 0.399 ng/mg protein). However, the oral adminis-

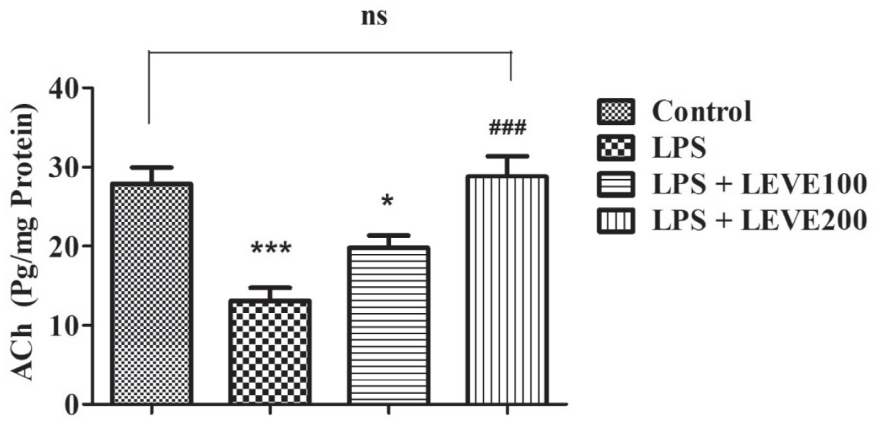

Fig. 5. Effect of levetiracetam on acetylcholine (ACh) levels in brain homogenates of the LPS-induced rat model. The results are expressed by mean ± SEM (n = 6). One-way ANOVA followed by Tukey-Kramer multiple comparisons test. $^{*}p < 0.05$ and $^{***}p < 0.001$ as compared to the control group; ns- not significant as compared to the control group; $^{\#\#\#}p < 0.001$ as compared to the LPS-induced group.

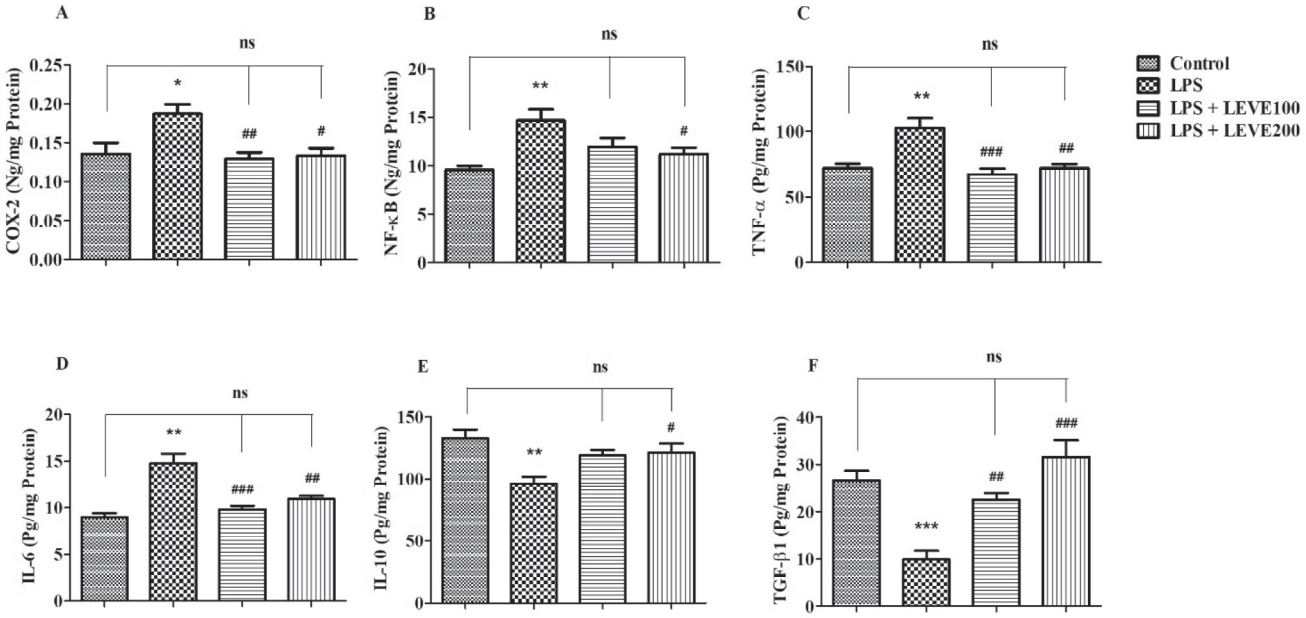

Fig. 6. Effect of levetiracetam on neuroinflammatory parameters like (A) COX-2, (B) NF-κB, (C) TNF-α, (D) IL-6, (E) IL-10, and (F) TGF-β1 in LPS-induced rat model. The results are expressed by mean ± SEM (n = 6). One-way ANOVA followed by Tukey-Kramer multiple comparisons test for comparisons of within the groups. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ as compared to the control group; ns- not significant as compared to the control group; $\#p < 0.05$, $\#\#p < 0.01$, and $\#\#\#p < 0.001$ as compared to LPS-induced group.

tration of LEVE 20 0 mg/kg showed a considerable decline (11.21 ± 0.645 ng/mg protein; $p < 0.05$) in NF-κB levels as matching with LPS-induced rats.

Fig. 6**C** and Fig. 6**D** highlight the influence of LEVE on pro-inflammatory cytokines like TNF-α and IL-6 in the brain of LPS toxicity. There were inter-group alterations in the levels of TNF-α [$F(3,20) = 10.23$, $p < 0.001$] and IL-6 [$F(3,20) = 18.32$, $p < 0.001$] in the brain. Tukey-Kramer *post hoc* analysis exposed that the brain's TNF-α level (102.60 ± 7.89 pg/mg protein) of LPS induction displayed an elevation ($p < 0.01$) when corresponding to the control group (72.24 ± 3.32 pg/mg protein) as shown in Fig. 6**C**. However, the treatment of LEVE at 100 mg/kg significantly (67.36 ± 4.40 pg/mg protein; $p < 0.001$) reduced TNF-α levels, and also LEVE at 200 mg/kg significantly (72.24 ± 3.08 pg/mg protein; $p < 0.01$) declined TNF-α levels in comparison to LPS-induced group.

Correspondingly, for IL-6 levels, as likened to the control group (9.00 ± 0.40 pg/mg protein), there was a substantial ($p < 0.01$) increase followed by LPS toxicity (14.78 ± 1.01 pg/mg protein). LEVE (100 and 200 mg/kg, p.o.) protected the elevated LPS-induced IL-6 levels (9.84 ± 0.39 pg/mg protein; $p < 0.001$ and 10. 97 ± 0.29 pg/mg protein; $p < 0.01$, respectively) in the brain (Fig. 6**D**). In comparison, the doses of LEVE (100 and 200 mg/kg, p.o), no alterations in brain TNF-α and IL-6 levels as associated to the control group.

Further, the administration of LEVE affected brain anti-inflammatory cytokines like IL-10 and TGF-β1 in LPS-induced animals (Fig. 6**E** and Fig. 6**F**). In the analysis of IL-10 and TGF-β1 levels, there were significant differences [$F(3,20) = 5.888$, $p < 0.001$ and $F(3,20) = 16.67$, $p < 0.001$] in inter-group comparison. Fig. 6**E** shows a substantial decrease in IL-10 levels in the brain ($p < 0.01$) in LPS-

induced rats (96.32 ± 5.56 pg/mg protein) matched to the control animals (132.40 ± 7.57 pg/mg protein). When LEVE was tested at 100 (118.90 ± 4.25 pg/mg protein) and 200 mg/kg levels, only at a higher dose significantly (121.40 ± 7.04 pg/mg protein; $p < 0.01$) decreased the elevated IL-10 levels by LPS induction.

In addition, using one-way ANOVA analysis of TGF-β1 levels [$F(3,20) = 16.67$, $p < 0.001$] exposed an alteration among the treatments, as shown in Fig. 6**F**. As referred to a control group (26.69 ± 1.10 pg/mg protein), there was a decrease ($p < 0.001$) in TGF-β1 level in LPS-induced rats (9.90 ± 1.78 pg/mg protein). Nevertheless, LEVE in both dose levels (100 and 200 mg/kg, p.o.) increased (22.52 ± 1.40 pg/mg protein; $p < 0.01$ and 31.60 ± 3.40 pg/mg protein; $p < 0.001$, respectively) the TGF-β1 level in LPS-induced rats. Also, there were no significant changes in TGF-β1 levels at treated doses of LEVE, when matched with the control group.

3.6. Pre-treatment of LEVE-affected apoptosis proteins in LPS-induced animals

To examine the effect of LPS and LEVE on the process of cellular apoptosis, Bcl-2, Bax, and Caspase-3 levels were detected in the brain (Fig. 7). A substantial alteration ($F(3,20) = 5.550$, $p < 0.01$) in Bcl-2 levels among the treatments. Referred to control rats (1696 ± 144.4 pg/mg protein), LPS induction resulted in a significant drop (1077 ± 24.11 pg/mg protein; $p < 0.01$) in the brain Bcl-2 levels. Besides, LEVE (100 and 200 mg/kg, p.o.) did not alter BCL-2 levels as allied to the LPS-induced group (Fig. 7**A**).

Considering pro-apoptosis proteins, Bax ($F(3,20) = 4.533$, $p < 0.05$) as well as Caspase-3 ($F(3,20) = 10.77$, $p < 0.001$) levels were altered between groups. Four doses of LPS injections caused significant elevations in both Bax (0.376 ± 0.0275 ng/mg protein; $p < 0.05$) and Caspase-3 (13.46 ± 1.163 ng/mg protein; $p < 0.001$) levels as a match to respective controls of Bax (0.2618 ± 0.0241 ng/mg protein) and Caspase-3 (9.2 ± 0.9768 ng/mg protein). For Bax levels, only LEVE (100 mg/kg, p.o.) exhibited a restoration in brain Bax levels (0.2697 ± 0.0296 ng/mg protein) as associated with the LPS-induced group (Fig. 7**B**). However, both LEVE doses returned ($p < 0.001$) the Caspase-3 levels (6.91 ± 0.7716 ng/mg protein for 100 mg/kg and 7.592 ± 0.5616 ng/mg protein for 200 mg/kg) to normal (Fig. 7**C**).

3.7. Pre-treatment of LEVE ameliorated oxidative stress in LPS-treated animals

Fig. 8 highlights the results of LPS and LEVE on an oxidative marker MDA and antioxidants like catalase and GSH levels in brain tissues. A significant deviation in MDA levels ($F(3,20) = 9.630$, $p < 0.001$) was recorded considering all the groups (Fig. 8**A**). Moreover, LPS induced a significant elevation of MDA levels (2.796 ± 0.1459 nmol/mg protein; $p < 0.001$) when matched to the control group (1.655 ± 0.1324 nmol/mg protein). Both doses of LEVE reduced this elevation of the brain MDA levels (2.084 ± 0.2090 nmol/mg protein for 100 mg/kg and 2.088 ± 0.1017 nmol/mg protein for 200 mg/kg; $p < 0.05$).

On the other hand, both antioxidant markers like catalase ($F(3,20) = 9.400$, $p < 0.001$) and GSH ($F(3,20) = 5.836$, $p < 0.01$) established significant alterations between the groups. After LPS induction, the levels of catalase (9.051 ± 0.5600 ng/mg protein; $p < 0.05$) and GSH (7.880 ± 0.6739 μg/mg protein; $p < 0.01$) declined considerably as associated with corresponding control values of catalase

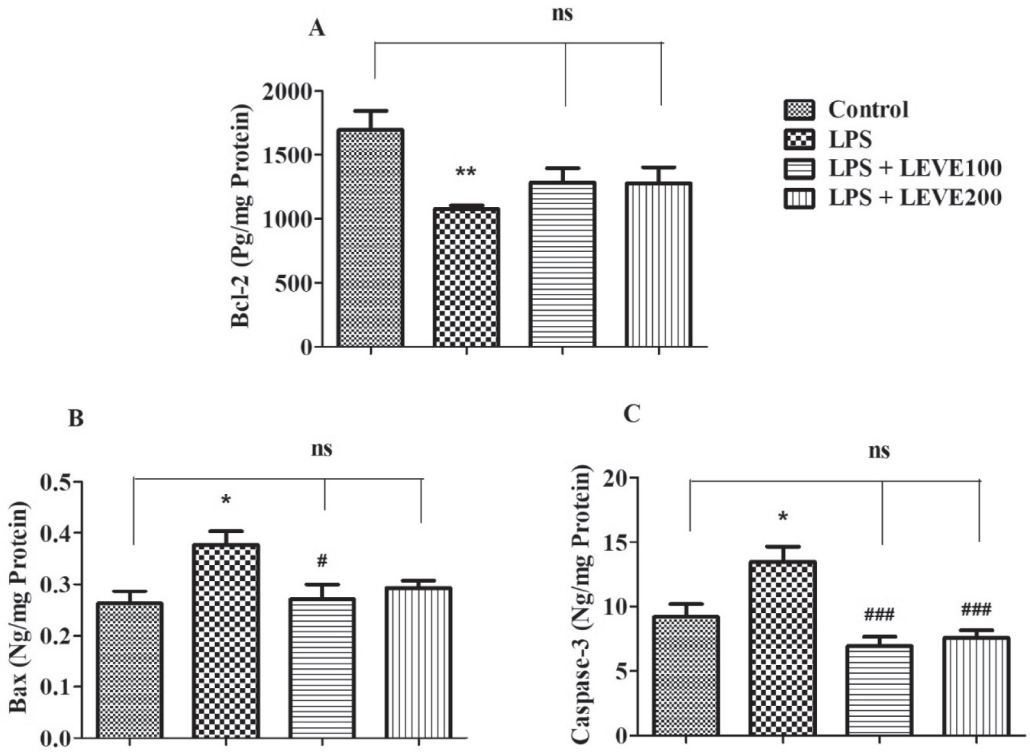

Fig. 7. Effect of levetiracetam on apoptosis parameters (A) Bcl-2, (B) Bax, and (C) Caspase-3 in LPS-induced rat model. The results are expressed by mean ± SEM (n = 6). One-way ANOVA followed by Tukey-Kramer multiple comparisons test for comparisons of within the groups. $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared to the control group; ns- not significant as compared to the control group; $\#p < 0.05$ and $\#\#\#p < 0.001$ as compared to LPS-induced group.

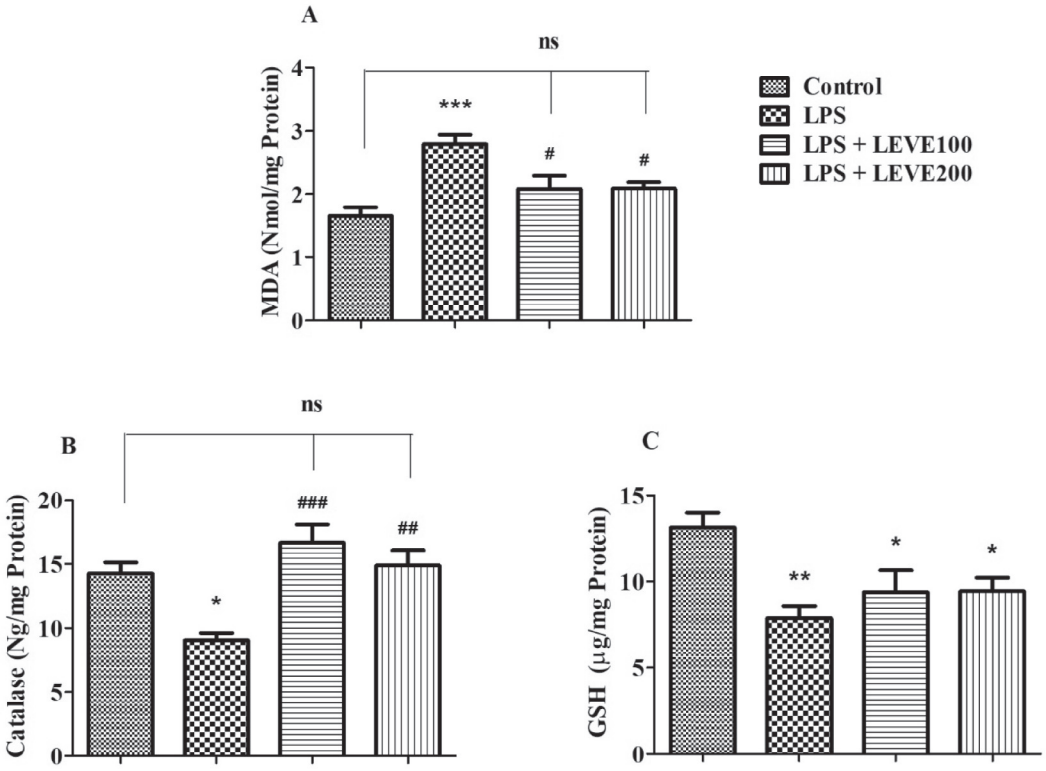

Fig. 8. Effect of levetiracetam on oxidative parameters (A) MDA, (B) Catalase, and (C) GSH in LPS-induced rat model. The results are expressed by mean ± SEM (n = 6). One-way ANOVA followed by Tukey-Kramer multiple comparisons test for comparisons of within the groups. *p < 0.05, **p < 0.01, and ***p < 0.001 as compared to the control group; ns- not significant as compared to the control group; #p < 0.05, ##p < 0.01, and ###p < 0.001 as compared to LPS-induced group.

(14.26 ± 0.8498 ng/mg protein) and GSH (13.12 ± 0.8653 µg/mg protein). LEVE at 100 mg/kg (16.68 ± 1.414 ng/mg protein; p < 0.001) and 200 mg/kg (14.85 ± 1.235 ng/mg protein; p < 0.01) prominently restored catalase levels in the brain (Fig. 8**B**). In contrast, there were no significant alterations in GSH levels by both doses of LEVE as compared to the LPS-induced animals (Fig. 8**C**).

4. Discussion

Recent investigations provide some scientific evidence on LEVE improves cognitive functions, elevates cholinergic functions, and prevents inflammation formation in neurons, neuronal apoptosis process, and oxidative vulnerability. Memory loss, changed mental processes, and behavioral changes are some of the signs of dementia, which is a collection of conditions affecting the brain that can cause morbidity and reliance in the elderly. The central cholinergic neurons regulate signals across the cerebral cortex and maintain normal cognitive functions. Degeneration of cholinergic neurons by inflammatory process and oxidative vulnerability have been concomitant to a number of dysfunctions including impaired learning, memory, and attention (Gamage et al., 2020). The neuronal toxicity was induced with four doses of systemic LPS injection. From the present results, the administration of LEVE ameliorates LPS-induced cognitive deficiencies by preventing neuronal inflammation and improving cholinergic functions.

Using the Y-maze, NOR, and EPM tests, this research confirmed previous findings that LPS-induced rats exhibit memory impairment (Mani et al., 2021; 2022c). In this study, LEVE was also evaluated for its impact on memory function, with two dosages (100 and 200 mg/kg, p.o.). Our findings indicated that LEVE reversed memory impairment in a dose-dependent manner, with outcomes progressively improving at larger doses. Some of our results showed that memory impairment could not be reversed with a modest dosage of 100 mg/kg, but memory functions were enhanced with the administration of large doses of 200 mg/kg.

The NOR test was utilized in the current study to assess how LEVE and LPS affected rats' ability to distinguish between FO and NO, which is directly related to their working memory (Mani et al., 2022b). Initially, animals were involved in a training session with two identical objects (FO1 and FO2) to preserve their working memory (Mani et al., 2022b; Silvers et al., 2007). There was a reduction in ETFOs of LPS-induced rats as matched to corresponding controls evidenced the induction of LPS affected the ability of rat's acquisition. After the LEVE treatment, an increase in ETFOs might support the neuroprotective effects of LEVE. Moreover, a comparison of the exploration time of each group between the similar objects (FO1 and FO2) highlighted there was no significant variation in exploration time during a test session.

Considering the test session of NOR, when the rats were allowed to explore the two different objects (FO1 and LO), LPS-induced rats also recorded less ETFO and ETNO values as compared to the corresponding control group. The treatment with both doses of LEVE exhibited improvement in the exploration time from both sessions, showing neuroinflammation causes memory impairment, and LEVE remedies this deficit. Interestingly, the extended ETNO as compared to ETFO has suggested the preference of rats to spend higher time with NO and the discrimination ability of both objects (Mani et al., 2022b; Silvers et al., 2007). These findings proposed

that LEVE might assist as a treatment for neuroinflammation and associated memory loss. The test's parameters provide a way to quantify object recognition memory, which may then be used to investigate memory's neurological bases. Time spent with a NO parallel to a FO by LPS-induced rats increased after treatment with LEVE, providing strong evidence for the recovery of cognitive function lost as a result of neuroinflammation. Furthermore, the discrimination capability as DI was also significantly altered by LEVE with a dose of 200 mg/kg. The more time spent by an NO and the higher percentage of DI reveals an animal's retention capacity, recognition of novelty, and discrimination ability between NO and FO (Mani et al., 2022a; 2022b). Early, LEVE improved the mean discrimination index between the objects in NOR test on days 14 and 28 after the bilateral common carotid artery stenosis (BCAS) procedures (Inaba et al., 2019).

The Y-maze procedure was used to examine participants' capacity for spatial working memory. Most of the brain area is used in this analysis, including the hippocampus, the prefrontal cortex, and the basal forebrain. Particularly highlighted are prefrontal cortex activities such as remembering which arms have already been visited and displaying a preference to enter a less recently visited arm (Mani et al., 2022a; 2022b; Liet et al., 2015). The medial prefrontal cortex (MPFC) is crucial to the intellectual process, and dysfunction of the MPFC has been connected to a range of disorders including Parkinson's, schizophrenia, and AD (Xu et al., 2019). Rats given LPS had lower values of NEKA than the control group. The NEKA by LPS-induced rats was lower than that of the control group. Furthermore, LEVE (200 mg/kg, p.o.) treatment led to an extensive improvement in comparison to LPS-induced rats. Treatment with LEVE (100 mg/kg, p.o.) did not alter NEKA in the test session. On the other hand, the NENA by LPS-induced rats was lesser than that of the control. The stay duration in the novel arm was shorter, and this difference was statistically significant when compared to the control group. All the doses of LEVE countered memory impairment compared to LPS-induced rats. The lower in both NEKA and NENA followed by LPS induction clarified the weakening of spatial memory in rats. However, the improvement of both arm entries supported the spatial memory improvement with LEVE treatment. Additionally, the amount of time spent by the animals in the novel arm against total time expressed the anxiety and coping-like behavior of rats in the new location (Mani et al., 2022a; 2022b). Both doses of LEVE treatment improved the anti-anxiety and coping-like behavior of the LPS-induced rats by significantly increasing the TSNA (%). In extension, our previous experiments strongly supported the present results of LEVA at the same dose levels by improving the parallel behavior targets like NEKA, NENA, and time spent in the novel arm in the doxorubicin-induced rat model (Mani et al., 2022a). Besides, LEVE selectively altered the arm alteration rate percentage, but not in total arm entry in BCAS mice (Inaba et al., 2019).

In this experiment, the EPM task was employed as a method for analyzing the rats' behavior and cognition. Shortening the TL of the second day in the EPM test reflected the retention capability of a learned task and was considered as memory (Mani et al., 2022a; 2022b). In LPS-induced rats, higher TL values compared to control animals revealed memory impairments. Interestingly, this stretch in TL duration was shortened in LEVE-treated groups. These results with LEVE were dosage-dependent. The higher dosage of LEVE may have had a more robust protective impact on hippocampal neurons, which might explain the finding (Salaka et al., 2022; Mani et al., 2022a). Accordingly, the findings suggested that increasing the availability of ACh during the execution of the behavioral tasks would lower the anxious behavior of the animals while the performance of the task, which, in turn, would enhance memory performance (Mani et al., 2022a; 2022b). Likewise, the usefulness of LEVE in the reversal of cognitive deficits was also reported early. Administration of LEVE augmented radial arm maze task memory functions of the ketamine-exposed rats with schizophrenia (Koh et al., 2018). Early, LEVE treatment protected the excessive hippocampus CA3 region activity, which is linked to age-related memory impairment, and also the treatment resulted spatial memory improvement in aged rats using Morris water maze (MWM) tasks (Koh et al., 2010). Similarly, chronic administration of LEVE to human APP transgenic mice reversed learning and memory deficiency by restoring synaptic and neuronal network dysfunctions. For analyzing behavioral targets, open field, EPM, MWM, and NOR models were employed in this report (Sanchez et al., 2012).

One of the pathogenic reasons for neurodegeneration is inflammation in the brain, which often occurs before the onset of neurodegenerative diseases like AD. Considers, the development of inflammations in neurons to have a critical role in the pathogenesis of AD, and this has been supported by several reports from both basic cellular neuroscience and human genetics (Heppner et al., 2015; Leng and Edison, 2021). LPS is a useful model for inducing inflammation via a variety of methods. There are several in vivo and *in vitro* techniques that use LPS. Not only is this substance used to stimulate cell cultures, but it might also be injected once or multiple times into the CNS directly by the intracerebroventricular method or peripherally by the intraperitoneal method, which induces neuroinflammation, cholinergic deficiency, and cognitive impairment. Besides, the $A\beta_{1\text{-}42}$ levels were reported to be elevated in LPS-treated brains, supporting that induction of neuronal inflammation by LPS might be suitable for investigating the pathogenesis of AD (Zhao et al., 2019). Additionally, LPS has been shown to promote harm to brain tissues by aggravating oxidative stress and initiating cellular apoptosis (Daroi et al., 2022). In this regard, LPS-based experimental models help comprehend the disease process and certain events that take place in actual AD since they provide a connection to neuroinflammation, cholinergic deficit, oxidative stress, and cellular apoptosis.

A deficit in the cholinergic nervous system is thought to have a pivotal role in cognitive decline since ACh is a key excitatory neurotransmitter that facilitates higher behaviors including normal learning and memory functions. The central cholinergic nerve system may influence ACh concentrations by controlling ACh production and release. Clinical research from the last few years suggested that the cholinergic system in people with AD is severely damaged following injury, with neurodegeneration, a loss of cholinergic neurons, and a lack of ACh all being commonplace in their brains (Hampel et al., 2019). Those with AD who lack ACh suffer from a severe decline in mental and behavioral abilities over time (Hung and Fu, 2017). Our findings on the connection between neuroinflammation and brain ACh levels demonstrated that after LPS induction declined the ACh levels in the brain. In this study, neuroinflammation caused a decrease in the level of ACh in the brain. The decreased ACh was restored with LEVE therapy (200 mg/kg, p.o.). A parallel study, after induction of doxorubicin, resulted in the elevation of brain AChE enzyme that is known to decrease the ACh transmitter. It has been shown that decreasing the level of the AChE enzyme leads to an increase in ACh by using LEVE therapy (Mani et al., 2022a).

Arachidonic acid plays a crucial part in the inflammatory cascade, and arachidonic acid is transformed into bioactive prostanoids by the COX which is identified as the prostaglandin H synthase enzyme. Furthermore, COX-2 triggers inflammation by releasing cytokines, suggesting that targeted COX-2 inhibition might reduce inflammation and considers a potential anti-inflammatory target (Fitzpatrick, 2004). In this experiment, COX-2 in LPS-induced rats was elevated in contrast to the control. Further, LEVE showed a significant reduction in COX-2 at two dose levels compared to LPS-induced rats. Besides, the transcription factor NF-κB is crucial for the stimulation of inflammations in chronic

diseases (Kunnumakkara et al., 2020). In glial cells, the activation of NF-κB is a key role in the process of inflammation, resulting in neurodegeneration (Shabab et al., 2017). The stimulation of NF-κB synthesis through activation of the toll-like receptor 4 (TLR4), by binding of LPS, leads to the expression of chemokines, proinflammatory cytokines, and other vulnerable enzymes like COX-2 and iNOS. Altogether, these cause tissue injuries and neuroinflammation (Shabab et al., 2017). Presently, the administration of LEVE with 200 mg/kg highlighted a decline in NF-κB levels after LPS-induced supports its neuroprotective role. In parallel, our earlier report highlighted that LEVE treatment declined the collection of inflammatory markers like COX-2, NF-κB, prostaglandin E2 (PGE2), and TNF-α in a doxorubicin-induced chemobrain (Mani et al., 2022a). According to a recent study, LEVE has an impact on the augmentation of brain inflammation by preventing monocyte and neutrophil infiltrations into the hippocampus and the occurrence of spontaneous recurrent seizures caused by SE (Matsuo et al., 2022).

The Aβ accumulation triggers inflammatory responses in microglia and astrocytes, leading to the production of inflammatory-related factors such as chemokines, complement factors, and cytokines like IL-6, IL-1β, and TNF-α. Furthermore, in AD, a chronic neuroinflammatory environment due to excessive Aβ synthesis leads to pathological levels of cytokines that activate an APP which favors the liberation of Aβ (Minter et al., 2016). Proinflammatory cytokines including IL-6 and TNF-α are higher in human and rodent brains concerning AD. Moreover, anti-inflammatory cytokines IL-10 as well as TGF-β1 have decreased the cellular immune response, resulting in further anti-inflammatory benefits (Shen et al., 2019).

Earlier, it has been shown that LEVE reduces microglial activation and decreases TNF-α and Il-1 levels in the ischemic brain (Yao et al., 2021). Moreover, reduction in hippocampal IL-1β and TNFα levels in status epileptics and IL-1β, TNF- α, IL-4, and IL-6 levels in traumatic brain injury have been also reported after LEVE treatment (Matsuo et al., 2022; Bayhan et al., 2020). Presently, the levels of pro- and anti-inflammatory mediators were determined to confirm the cytokine expressions after LPS treatment. To support LEVE's anti-inflammatory actions in lowering the inflammatory response, groups of cytokines were measured. LEVE led to a decrease in the level of the pro-inflammatory cytokines (IL-6 and TNF-α) and increased the anti-inflammatory cytokines TGF-β1 in both doses, and IL-10 levels in a higher dose (200 mg/kg).

Evidence of neuronal cell death with apoptotic morphological futures as well as expression of pro-apoptotic factors in the autoptic brain of neurodegenerative patients supports the implication of apoptosis mechanisms in the neurodegenerative process (Mattson, 2000). In neurons, the promotion or suppression of apoptosis is determined by the number of proteins. Particularly, caspases mediate cell death by apoptosis, and also non-apoptotic pathways like necroptosis, pyroptosis, and autophagy (Shalini et al., 2015). Caspases are implicated in two distinct mechanisms for apoptotic cell death that are referred to as "death receptor-mediated (extrinsic)" and "mitochondria-dependent (intrinsic)". The TNF superfamily from outside the cells triggers the extrinsic pathway. Whereas the intrinsic pathway depends on the liberation of apoptotic factors from mitochondria inside the cells (Radi et al., 2014). In addition, proteins of the BCL-2 family regulate the mitochondrial apoptotic pathways. The BCL-2 family member BAX serves as a pro-apoptotic factor that initiates the apoptotic process by releasing cytochrome complex (cyt c) as a result of making pores on the outer membrane of the mitochondrial. Then, the caspases are activated by the liberation of cyt c, which results in more cell destruction (Westphal et al., 2011). Interestingly, Bcl-2 defends and maintains the stability of the pore opening in the mitochondrial membrane and alleviates its functions, and additionally suppresses oxidative stress (Radi et al., 2014; Lindsay et al., 2011). After LPS induction, Bax and caspase-3 levels were activated in the brain, and therapy with LEVE successfully reduced these effects. By contrast, Bcl-2 levels declined in LPS induction, but neither of the LEVE treatment doses significantly altered these changes.

In normal physiology, insignificant variations in the steady-state levels of free radicals may even support intracellular signaling; however, oxidative stress with lipid, protein, and DNA damage arises when free radical synthesis is uncontrolled and outpaces the capability of antioxidant defenses to control it (Bayir and Kagan, 2008). Still, many cellular antioxidant mechanisms, including glutathione peroxidase (GPx), catalase, superoxide dismutase (SOD), GSH, and also vitamins C and E, avert the severity of this oxidative stress (Jelinek et al., 2021). In the present study, after LPS induction, an elevation in MDA levels and a decline in catalase and GSH levels were recorded. MDA is a consequence of cellular polyunsaturated fatty acid peroxidation and is produced excessively in response to an increase in free radicals (Del Rio et al., 2005). Further, in oxidative defense mechanisms, the SOD converts the oxygen free radicals to hydrogen peroxide (H_2O_2). These H_2O_2 molecules are then converted to oxygen and water by catalase, and a different system, GPx, utilizes GSH to convert H_2O_2 to water (Jelinek et al., 2021). Interestingly, LEVE protected LPS-induced oxidative vulnerability by reducing MDA levels and restoring antioxidant activity by increasing catalase levels in brain tissues, but there was no significant alteration of GSH levels. According to an earlier study, the oxidative and antioxidant parameters were potentially altered in traumatic brain injury by LEVE (Bayhan et al., 2020). Furthermore, LEVE attenuated PTZ-induced oxidative stress by reducing MDA and improving SOD, catalase, and GPx levels in the brain (Rehman et al., 2022).

5. Conclusion

The current study found that LEVE treatment improved the LPS-induced cognitive debits in numerous maze models. Continuous administration of LEVE for 30 days resulted in lower TL time in the EPM test, higher exploration time and discrimination capability of a novel object in the NOR test, and a higher number of entries and prolonged time spent at the novel arm in the Y-maze test. Further, LEVE improved cholinergic function by elevating ACh transmission and reduced neuroinflammation, oxidative vulnerability, and neuronal apoptosis in rats' brains. Together, the listed results could support the neuroprotective effect of LEVE in neuroinflammatory-induced neuronal degeneration. Further investigations of LEVE with additional parameters of the same line mechanisms and exploration of additional inflammatory-related mechanisms would strengthen the possibility of clinical utility.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ahmad, M.A., Kareem, O., Khushtar, M., Akbar, M., Haque, M.R., Iqubal, A., Haider, M. F., Pottoo, F.H., Abdulla, F.S., Al-Haidar, M.B., Alhajri, N., 2022. Neuroinflammation: A potential risk for dementia. Int. J. Mol. Sci. 23, 616. https://doi.org/10.3390/ijms23020616.

Batista, C.R.A., Gomes, G.F., Candelario-Jalil, E., Fiebich, B.L., de Oliveira, A.C.P., 2019. Lipopolysaccharide-induced neuroinflammation as a bridge to understand neurodegeneration. Int. J. Mol. Sci. 20, 2293. https://doi.org/10.3390/ijms20092293.

Bayhan, I., Turtay, M.G., Ciftci, O., Cetin, A., Basak, N., Namık Oztanır, M., Oguzturk, H., Gurbuz, S., Guven, T., 2020. Comparison of immunological, histological and oxidative effects of felbamate and levetiracetam in traumatic brain injury. Eur. Rev. Med. Pharmacol. Sci. 24, 7083–7091. https://doi.org/10.26355/eurrev_202006_21702.

Bayir, H., Kagan, V.E., 2008. Bench-to-bedside review: Bench-to-bedside review: Mitochondrial injury, oxidative stress and apoptosis – there is nothing more practical than a good theory. Crit. Care 12, 206. https://doi.org/10.1186/cc6779.

Bison, S., Carboni, L., Arban, R., Bate, S., Gerrard, P., Razzoli, M., 2009. Differential behavioral, physiological, and hormonal sensitivity to LPS challenge in rats. Int. J. Interferon Cytokine Mediat. Res. 1, 1–13. https://doi.org/10.2147/IJICMR.S4273.

Bluthé, R.M., Dantzer, R., Kelley, K.W., 1992. Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat. Brain Res. 573, 318–320. https://doi.org/10.1016/0006-8993(92)90779-9.

Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., Tracey, K.J., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405, 458–462. https://doi.org/10.1038/35013070.

Chowdhary, N., Barbui, C., Anstey, K.J., Kivipelto, M., Barbera, M., Peters, R., Zheng, L., Kulmala, J., Stephen, R., Ferri, C.P., Joanette, Y., Wang, H., Comas-Herrera, A., Alessi, C., Suharya Dy, K., Mwangi, K.J., Petersen, R.C., Motala, A.A., Mendis, S., Prabhakaran, D., Bibi Mia Sorefan, A., Dias, A., Gouider, R., Shahar, S., Ashby-Mitchell, K., Prince M, Dua, T., 2022. Reducing the risk of cognitive decline and dementia: WHO recommendations. Front. Neurol. 12, 765584. https://doi.org/10.3389/fneur.2021.765584

Daroi, P.A., Dhage, S.N., Juvekar, A.R., 2022. p-Coumaric acid mitigates lipopolysaccharide induced brain damage via alleviating oxidative stress, inflammation and apoptosis. J. Pharm. Pharmacol. 74, 556–564. https://doi.org/10.1093/jpp/rgab077.

de Souza, A.G., Chaves Filho, A.J.M., Souza Oliveira, J.V., de Souza, D.A.A., Lopes, I.S., de Carvalho, M.A.J., de Lima, K.A., Florenço Sousa, F.C., Mendes Vasconcelos, S. M., Macedo, D., de França Fonteles, M.M., 2019. Prevention of pentylenetetrazole-induced kindling and behavioral comorbidities in mice by levetiracetam combined with the GLP-1 agonist liraglutide: Involvement of brain antioxidant and BDNF upregulating properties. Biomed. Pharmacother. 109, 429–439. https://doi.org/10.1016/j.biopha.2018.10.066.

Del Rio, D., Stewart, A.J., Pellegrini, N., 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr. Metab. Cardiovasc. Dis. 15, 316–328. https://doi.org/10.1016/j.numecd.2005.05.003.

Deshpande, L.S., Delorenzo, R.J., 2014. Mechanisms of levetiracetam in the control of status epilepticus and epilepsy. Front. Neurol. 5, 11. https://doi.org/10.3389/fneur.2014.00011.

Devi, L., Ohno, M., 2013. Effects of levetiracetam, an antiepileptic drug, on memory impairments associated with aging and Alzheimer's disease in mice. Neurobiol. Learn. Mem. 102, 7–11. https://doi.org/10.1016/j.nlm.2013.02.001.

Eyal, S., Yagen, B., Sobol, E., Altschuler, Y., Shmuel, M., Bialer, M., 2004. The activity of antiepileptic drugs as histone deacetylase inhibitors. Epilepsia 45, 737–744. https://doi.org/10.1111/j.0013-9580.2004.00104.x.

Fitzpatrick, F.A., 2004. Cyclooxygenase enzymes: regulation and function. Curr. Pharm. Des. 10, 577–588. https://doi.org/10.2174/1381612043453144.

Gamage, R., Wagnon, I., Rossetti, I., Childs, R., Niedermayer, G., Chesworth, R., Gyengesi, E., 2020. Cholinergic modulation of glial function during aging and chronic neuroinflammation. Front. Cell. Neurosci. 14,. https://doi.org/10.3389/fncel.2020.577912 577912.

Haberman, R.P., Branch, A., Gallagher, M., 2017. Targeting neural hyperactivity as a treatment to stem progression of late-onset Alzheimer's disease. Neurotherapeutics 14, 662–676. https://doi.org/10.1007/s13311-017-0541-z.

Haghikia, A., Ladage, K., Hinkerohe, D., Vollmar, P., Heupel, K., Dermietzel, R., Faustmann, P.M., 2008. Implications of antiinflammatory properties of the anticonvulsant drug levetiracetam in astrocytes. J. Neurosci. Res. 86, 1781–1788. https://doi.org/10.1002/jnr.21639.

Hampel, H., Mesulam, M.M., Cuello, A.C., Khachaturian, A.S., Vergallo, A., Farlow, M. R., Snyder, P.J., Giacobini, E., Khachaturian, Z.S., 2019. Revisiting the cholinergic hypothesis in Alzheimer's disease: Emerging evidence from translational and clinical research. J. Prev Alzheimers Dis. 6, 2–15. https://doi.org/10.14283/jpad.2018.43.

Heppner, F.L., Ransohoff, R.M., Becher, B., 2015. Immune attack: the role of inflammation in Alzheimer disease. Nat. Rev. Neurosci. 16, 358–372. https://doi.org/10.1038/nrn3880.

Himmerich, H., Bartsch, S., Hamer, H., Mergl, R., Schönherr, J., Petersein, C., Munzer, A., Kirkby, K.C., Bauer, K., Sack, U., 2013. Impact of mood stabilizers and antiepileptic drugs on cytokine production in-vitro. J. Psychiatr. Res. 47, 1751–1759. https://doi.org/10.1016/j.jpsychires.2013.07.026.

Hung, S.Y., Fu, W.M., 2017. Drug candidates in clinical trials for Alzheimer's disease. J. Biomed. Sci. 24, 47. https://doi.org/10.1186/s12929-017-0355-7.

Inaba, T., Miyamoto, N., Hira, K., Ueno, Y., Yamashiro, K., Watanabe, M., Shimada, Y., Hattori, N., Urabe, T., 2019. Protective role of levetiracetam against cognitive impairment and brain white matter damage in mouse prolonged cerebral hypoperfusion. Neuroscience 414, 255–264. https://doi.org/10.1016/j.neuroscience.2019.07.015.

Iqubal, A., Sharma, S., Sharma, K., Bhavsar, A., Hussain, I., Iqubal, M.K., Kumar, R., 2018. Intranasally administered pitavastatin ameliorates pentylenetetrazol-induced neuroinflammation, oxidative stress and cognitive dysfunction. Life Sci. 211, 172–181. https://doi.org/10.1016/j.lfs.2018.09.025.

Itoh, K., Ishihara, Y., Komori, R., Nochi, H., Taniguchi, R., Chiba, Y., Ueno, M., Takata-Tsuji, F., Dohgu, S., Kataoka, Y., 2016. Levetiracetam treatment influences blood-brain barrier failure associated with angiogenesis and inflammatory responses in the acute phase of epileptogenesis in post-status epilepticus mice. Brain Res. 1652, 1–13. https://doi.org/10.1016/j.brainres.2016.09.038.

Itoh, K., Taniguchi, R., Matsuo, T., Oguro, A., Vogel, C.F.A., Yamazaki, T., Ishihara, Y., 2019. Suppressive effects of levetiracetam on neuroinflammation and phagocytic microglia: A comparative study of levetiracetam, valproate and carbamazepine. Neurosci. Lett. 708,. https://doi.org/10.1016/j.neulet.2019.134363 134363.

Jelinek, M., Jurajda, M., Duris, K., 2021. Oxidative Stress in the brain: Basic concepts and treatment strategies in stroke. Antioxidants 10, 1886. https://doi.org/10.3390/antiox10121886.

Kamdi, S.P., Raval, A., Nakhate, K.T., 2021. Phloridzin attenuates lipopolysaccharide-induced cognitive impairment via antioxidant, anti-inflammatory and neuromodulatory activities. Cytokine 139,. https://doi.org/10.1016/j.cyto.2020.155408 155408.

Keymoradzadeh, A., Hedayati Ch, M., Abedinzade, M., Gazor, R., Rostampour, M., Taleghani, B. K., 2020. Enriched environment effect on lipopolysaccharide-induced spatial learning, memory impairment and hippocampal inflammatory cytokine levels in male rats. Behav. Brain Res. 394, 112814. https://doi.org/10.1016/j.bbr.2020.112814

Kim, J.E., Choi, H.C., Song, H.K., Jo, S.M., Kim, D.S., Choi, S.Y., Kim, Y.I., Kang, T.C., 2010. Levetiracetam inhibits interleukin-1 beta inflammatory responses in the hippocampus and piriform cortex of epileptic rats. Neurosci. Lett. 471, 94–99. https://doi.org/10.1016/j.neulet.2010.01.018.

Koh, M.T., Haberman, R.P., Foti, S., McCown, T.J., Gallagher, M., 2010. Treatment strategies targeting excess hippocampal activity benefit aged rats with cognitive impairment. Neuropsychopharmacology 35, 1016–1025. https://doi.org/10.1038/npp.2009.207.

Koh, M.T., Shao, Y., Rosenzweig-Lipson, S., Gallagher, M., 2018. Treatment with levetiracetam improves cognition in a ketamine rat model of schizophrenia. Schizophr. Res. 193, 119–125. https://doi.org/10.1016/j.schres.2017.06.027.

Kunnumakkara, A.B., Shabnam, B., Girisa, S., Harsha, C., Banik, K., Devi, T.B., Choudhury, R., Sahu, H., Parama, D., Sailo, B.L., Thakur, K.K., Gupta, S.C., Aggarwal, B.B., 2020. Inflammation, NF-κB, and chronic diseases: How are they linked? Crit. Rev. Immunol. 40, 1–39. https://doi.org/10.1615/CritRevImmunol.2020033210.

Lee, J., Chan, S.L., Mattson, M.P., 2002. Adverse effect of a presenilin-1 mutation in microglia results in enhanced nitric oxide and inflammatory cytokine responses to immune challenge in the brain. NeuroMol. Med. 2, 29–45. https://doi.org/10.1385/NMM:2:1:29.

Leng, F., Edison, P., 2021. Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? Nat. Rev. Neurol. 17, 157–172. https://doi.org/10.1038/s41582-020-00435-y.

Liet, C., Amenouche, F., Freret, T., Boulouard, M., Mauvieux, B., Lelong-Boulouard, V., Bocca, M.L., 2015. Effects of acute administration of melatonin on attentional, executive, and working memory processes in rats. Fundam. Clin. Pharmacol. 29, 472–477. https://doi.org/10.1111/fcp.12134.

Lindsay, J., Esposti, M.D., Gilmore, A.P., 2011. Bcl-2 proteins and mitochondria—Specificity in membrane targeting for death. BBA 1813, 532–539. https://doi.org/10.1016/j.bbamcr.2010.10.017.

Mani, V., Arfeen, M., Ali, H.M., Abdel-Moneim, A.H., Aldubayan, M., Alhowail, A., 2021. Neuroprotective effect of clobenpropit against lipopolysaccharide-induced cognitive deficits via attenuating neuroinflammation and enhancing mitochondrial functions in mice. Brain Sci. 11, 1617. https://doi.org/10.3390/brainsci11121617.

Mani, V., Arfeen, M., Rabbani, S.I., Shariq, A., Amirthalingam, P., 2022a. Levetiracetam ameliorates doxorubicin-induced chemobrain by enhancing cholinergic transmission and reducing neuroinflammation using an experimental rat model and molecular docking study. Molecules 27, 7364. https://doi.org/10.3390/molecules27217364.

Mani, V., Arfeen, M., Sajid, S., Almogbel, Y., 2022b. Aqueous Ajwa dates seeds extract improves memory impairment in type-2 diabetes mellitus rats by reducing blood glucose levels and enhancing brain cholinergic transmission. Saudi J. Biol. Sci. 29, 2738–2748. https://doi.org/10.1016/j.sjbs.2021.12.060.

Mani, V., Arfeen, M., Ali, H.M., Hafez Abdel-Moneim, A.M., Aldubayan, M., Dhanasekaran, M., Alhowail, A., 2022c. Ciproxifan attenuates the memory impairment induced by lipopolysaccharide through modulation of cholinergic transmission in the mouse brain. Eur. Rev. Med. Pharmacol. Sci. 26, 1897–1905. https://doi.org/10.26355/eurrev_202203_28335.

Matsuo, T., Komori, R., Nakatani, M., Ochi, S., Yokota-Nakatsuma, A., Matsumoto, J., Takata, F., Dohgu, S., Ishihara, Y., Itoh, K., 2022. Levetiracetam suppresses the infiltration of neutrophils and monocytes and downregulates many inflammatory cytokines during epileptogenesis in pilocarpine-induced status epilepticus mice. Int. J. Mol. Sci. 23, 7671. https://doi.org/10.3390/ijms23147671.

Mattson, M., 2000. Apoptosis in neurodegenerative disorders. Nat. Rev. Mol. Cell Biol. 1, 120–130. https://doi.org/10.1038/35040009.

Minter, M.R., Taylor, J.M., Crack, P.J., 2016. The contribution of neuroinflammation to amyloid toxicity in Alzheimer's disease. J. Neurochem. 136, 457–474. https://doi.org/10.1111/jnc.13411.

Mirahmadi, S.M., Shahmohammadi, A., Rousta, A.M., Azadi, M.R., Fahanik-Babaei, J., Baluchnejadmojarad, T., Roghani, M., 2018. Soy isoflavone genistein attenuates lipopolysaccharide-induced cognitive impairments in the rat via exerting anti-oxidative and anti-inflammatory effects. Cytokine 104, 151–159. https://doi.org/10.1016/j.cyto.2017.10.008.

Ozkul, A., Akyol, A., Yenisey, C., Arpaci, E., Kiylioglu, N., Tataroglu, C., 2007. Oxidative stress in acute ischemic stroke. J. Clin. Neurosci. 14, 1062–1066. https://doi.org/10.1016/j.jocn.2006.11.008.

Radi, E., Formichi, P., Battisti, C., Federico, A., 2014. Apoptosis and oxidative stress in neurodegenerative diseases. J. Alzheimers Dis. 42, S125–S152. https://doi.org/10.3233/JAD-132738.

Rahim, N.S., Lim, S.M., Mani, V., Hazalin, N.A.M.N., Majeed, A.B.A., Ramasamy, K., 2021. Virgin coconut oil-induced neuroprotection in lipopolysaccharide-challenged rats is mediated, in part, through cholinergic, anti-oxidative and anti-inflammatory pathways. J. Diet. Suppl. 18, 655–681. https://doi.org/10.1080/19390211.2020.1830223.

Rehman, Z., Farooq, T., Javaid, S., Ashraf, W., Fawad Rasool, M., Samad, N., Tariq, M., Muhammad Muneeb Anjum, S., Sivandzade, F., Alotaibi, F., Alqahtani, F., Imran, I., 2022. Combination of levetiracetam with sodium selenite prevents pentylenetetrazole-induced kindling and behavioral comorbidities in rats. Saudi Pharm. J. 30, 494–507. https://doi.org/10.1016/j.jsps.2022.03.003

Salaka, R.J., Nair, K.P., Sasibhushana, R.B., Udayakumar, D., Kutty, B.M., Srikumar, B. N., Shankaranarayana Rao, B.S., 2022. Differential effects of levetiracetam on hippocampal CA1 synaptic plasticity and molecular changes in the dentate gyrus in epileptic rats. Neurochem. Int. 158,. https://doi.org/10.1016/j.neuint.2022.105378 105378.

Sanchez, P.E., Zhu, L., Verret, L., Vossel, K.A., Orr, A.G., Cirrito, J.R., Devidze, N., Ho, K., Yu, G.Q., Palop, J.J., Mucke, L., 2012. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. PNAS 109, E2895–E2903. https://doi.org/10.1073/pnas.1121081109.

Shabab, T., Khanabdali, R., Moghadamtousi, S.Z., Kadir, H.A., Mohan, G., 2017. Neuroinflammation pathways: a general review. Int. J. Neurosci. 127, 624–633. https://doi.org/10.1080/00207454.2016.1212854.

Shalini, S., Dorstyn, L., Dawar, S., Kumar, S., 2015. Old, new and emerging functions of caspases. Cell Death Differ. 22, 526–539. https://doi.org/10.1038/cdd.2014.216.

Shen, X.N., Niu, L.D., Wang, Y.J., Cao, X.P., Liu, Q., Tan, L., Zhang, C., Yu, J.T., 2019. Inflammatory markers in Alzheimer's disease and mild cognitive impairment: a meta-analysis and systematic review of 170 studies. J. Neurol. Neurosurg. Psychiatry 90, 590–598. https://doi.org/10.1136/jnnp-2018-319148.

Silvers, J.M., Harrod, S.B., Mactutus, C.F., Booze, R.M., 2007. Automation of the novel object recognition task for use in adolescent rats. J. Neurosci. Methods 166, 99–103. https://doi.org/10.1016/j.jneumeth.2007.06.032.

Srivastava, A., Dixit, A.B., Banerjee, J., Tripathi, M., Sarat Chandra, P., 2016. Role of inflammation and its miRNA based regulation in epilepsy: Implications for therapy. Clin. Chim. Acta 452, 1–9. https://doi.org/10.1016/j.cca.2015.10.023.

Stefanko, D.P., Barrett, R.M., Ly, A.R., Reolon, G.K., Wood, M.A., 2009. Modulation of long-term memory for object recognition via HDAC inhibition. PNAS 106, 9447–9452. https://doi.org/10.1073/pnas.0903964106.

Tang, S., Li, C., Suo, Z., Xu, Y., Wei, K., Zhao, L., Huang, H., Liu, X., Liu, D., Li, X., 2023. Neuroprotective effects of savinin on LPS-induced neuroinflammation in vivo via regulating MAPK/NF-κB pathway and NLRP3 inflammasome activation. Molecules 28, 1575. https://doi.org/10.3390/molecules28041575.

Tyagi, E., Agrawal, R., Nath, C., Shukla, R., 2007. Effect of anti-dementia drugs on LPS induced neuroinflammation in mice. Life Sci. 80, 1977–1983. https://doi.org/10.1016/j.lfs.2007.02.039.

Tyagi, E., Agrawal, R., Nath, C., Shukla, R., 2008. Influence of LPS-induced neuroinflammation on acetylcholinesterase activity in rat brain. J. Neuroimmunol. 205, 51–56. https://doi.org/10.1016/j.jneuroim.2008.08.015.

Westphal, D., Dewson, G., Czabotar, P.E., Kluck, R.M., 2011. Molecular biology of Bax and Bak activation and action. BBA 1813, 521–531. https://doi.org/10.1016/j.bbamcr.2010.12.019.

Wong, W., 2020. Economic burden of Alzheimer disease and managed care considerations. The Am. J. Manag. Care. 26, S177–S183. https://doi.org/10.37765/ajmc.2020.88482.

Xu, P., Chen, A., Li, Y., Xing, X., Lu, H., 2019. Medial prefrontal cortex in neurological diseases. Physiol. Genomics 51, 432–442. https://doi.org/10.1152/physiolgenomics.00006.2019.

Yao, X., Yang, W., Ren, Z., Zhang, H., Shi, D., Li, Y., Yu, Z., Guo, Q., Yang, G., Gu, Y., Zhao, H., Ren, K., 2021. Neuroprotective and angiogenesis effects of levetiracetam following ischemic stroke in rats. Front. Pharmacol. 12,. https://doi.org/10.3389/fphar.2021.638209 638209.

Zhao, J., Bi, W., Xiao, S., Lan, X., Cheng, X., Zhang, J., Lu, D., Wei, W., Wang, Y., Li, H., Fu, Y., Zhu, L., 2019. Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. Sci. Rep. 9, 5790. https://doi.org/10.1038/s41598-019-42286-8.