Research in Pharmaceutical Sciences, June 2026; 21(3): 364-374
Received: 05-01-2025
Peer Reviewed: 09-03-2025
Revised: 09-07-2025
Accepted: 15-07-2025
Published: 10-05-2026

School of Pharmacy & Pharmaceutical Sciences
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Original Article

Caffeic acid phenethyl ester protects mice against nicotine-induced seizures by attenuating oxidative stress and inflammation

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Abstract

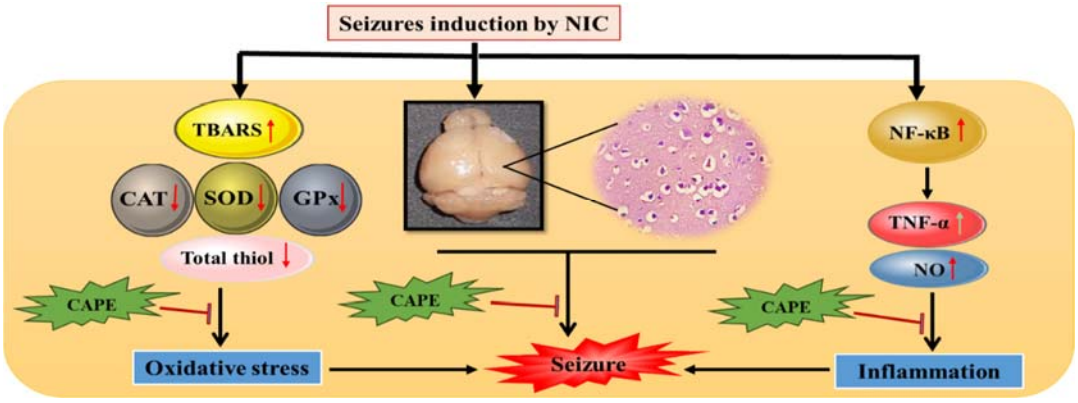
Background and purpose: A seizure is a neurological disorder in the brain that is caused by changes in the function of brain neurons. Caffeic acid phenetyl ester (CAPE), as a polyphenol, has antioxidant, anti-inflammatory, and anticancer effects. Since the effects of CAPE on the neurotoxins and neurotoxic medicinal agents have not been widely investigated, this study aimed to investigate the effect of CAPE on the nicotine (NIC)-induced seizures in mice.

Experimental approach: Thirty-three male mice were divided into five groups of 6-8 as follows: sham group (normal saline), NIC group (5 mg/kg single dose on day 7), treatment groups (CAPE at 4 and 8 mg/kg for 7 days), and diazepam group (1 mg/kg single dose on day 7). At the end, the animals were anesthetized, and mortality, convulsive behavior, total thiol, thiobarbituric acid reactive substances (TBARS), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), and the expression of nuclear factor kappa B (NF-κB) protein in the brain frontal cortex were measured, and histological studies were performed.

Findings/Results: Treatment with CAPE decreased the levels of TBARS, TNF-α, and NO and increased the levels of total thiol, CAT, SOD, GPx, and NF-κB protein expression compared to the NIC group. Seizure behavioral tests and histopathological investigations confirmed these results.

Conclusion and implications: According to the antioxidant effects of CAPE in various studies, it seems that CAPE can improve seizures by reducing inflammation and inhibiting oxidative stress.

Keywords: Caffeic acid phenethyl ester; Inflammation; Nicotine; Oxidative stress; Seizures.



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Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/RPS.RPS_3_25

INTRODUCTION

A seizure is a neurological disorder in the brain that is caused by changes in the function of brain neurons (1-3). In this disease, due to an imbalance between the inhibitory and excitatory systems in the central nervous system, the excitability of neurons is permanently increased. Brain common areas involved in seizures are the hippocampus, amygdala, frontal cortex, temporal cortex, and olfactory cortex. Seizures lead to anatomical changes in these areas (4). The main causes of seizures include destruction of inhibitory mechanisms, especially synaptic inhibition by gamma-aminobutyric acid (GABA), enhancement of synaptic excitatory mechanisms by the N-methyl-D-aspartate (NMDA) receptor, dysfunction of potassium, sodium, and calcium ion channels, an increase of inflammatory factors and oxidative factors (5). Seizures induce an increase in antioxidants, free radicals, and oxidative stress in the brain (6). The mechanism of action of anticonvulsant drugs is to block Na+ or Ca^{2+} channels, stimulate GABAergic, and reduce glutamatergic neurotransmission (7). In addition to the side effects of chemical anticonvulsants, including depression, ischemia, and cognitive disorders (8), 20-30% of patients have refractory seizures (9). Therefore, the choice of anticonvulsant drug depends on its efficacy, tolerability, and safety (10). Despite the abundance of these drugs, treatment is still unsatisfactory (11). There are several models for the induction of seizures (12). One of them is the overstimulation of nicotinic acetylcholine receptors (nAChRs) by nicotine (NIC), which can lead to the onset of seizures (13). NIC is the primary alkaloid in the leaves of the tobacco plant that initially stimulates the nervous system and suppresses this system if used continuously (14). NIC leads to the activation of nAChRs and then stimulation of glutamate release. Increased glutamate release leads to activation of NMDA receptors and ultimately seizures (15). The anticonvulsant effect of NIC can be due to inhibition of glycine or stimulation of the motor centers of the nervous system (16). Low levels of NIC directly excite neurons, facilitating the transmission of nerve impulses and leading to tremors. High levels of NIC lead to suppression of nerve impulses and seizures. Several studies have emphasized that specific ligands of nAChRs are effective in regulating the release of inflammatory cytokines (17, 18). In recent studies, mecamylamine (an antagonist of nAChRs), diazepam (Diaz, a GABA receptor modulator), and amantadine (an antagonist of NMDA receptors) reduced NIC-induced seizures (19). In this study, Diaz was used as a positive control. Diaz is a benzodiazepine (BZD) that is used as a treatment of choice for severe seizures in adults and children (20). BZDs are positive allosteric modulators of GABA receptors. The binding of BZDs to this receptor complex leads to enhanced GABA binding. As a result, the membrane potential of the neuron is hyperpolarized, and an increase in the potential difference between the resting and threshold states leads to neuronal firing. Because of the burst in these neurons, various changes occur at the cell surface, including activation of glutamate receptors and changes in GABA receptors (21,22). Research has shown that antioxidants protect the human body against free radicals and the effects of reactive oxygen species (ROS) (23). Herbal medicines can play a fundamental role in the development of new anticonvulsant drugs due to their antioxidant effects (18). Flavonoid compounds and phenolic acids are among the secondary and active metabolites of plants (24). These compounds have immunomodulatory and anti-inflammatory effects, and their antioxidant effects are known to scavenge ROS caused by lipid peroxidation, cellular damage, and oxidative stress (25). Caffeic acid phenethyl ester (CAPE) is a natural polyphenol obtained from the propolis in the bark of coniferous trees and bee hives. It is also widely present in vegetables, fruits, and coffee (26-28). The chemical structure of CAPE consists of a catechol ring and two hydroxyl groups. The catechol ring is considered responsible for the therapeutic properties of CAPE (29). The structure of CAPE is an effective trap for radicals. The combination of an aromatic nucleus with a conjugated side chain allows for easy transfer of unpaired electrons. By donating hydrogen to quench radicals, CAPE acts as a precursor (30). CAPE has anti-inflammatory, anticonvulsant, anticancer, antioxidant, and

immune-boosting effects. Studies have also shown that CAPE can penetrate the blood-brain barrier (BBB) and exert a neuroprotective function (31). The study by Stähli et al. showed that CAPE exerts its antioxidant effects through the nuclear factor erythroid 2-related factor 2 (Nrf-2)-mediated heme oxygenase-1 (HO-1) pathway and its anti-inflammatory effects through inhibition of nuclear factor kappa B (NF-κB) (32). According to the study by Zhang et al. CAPE inhibits microglia-induced neuroinflammation and oxidative stress and reduces neuronal tissue damage (33). Since long-term use of chemical anticonvulsants is associated with side effects and sometimes toxicity, if we can use herbal medicines to modulate seizures, we can reduce the side effects. Since the effects of CAPE on neurotoxins and neurotoxic pharmacological agents have not been extensively investigated, this study aimed to investigate the effect of CAPE on NIC-induced seizures in mice.

MATERIALS AND METHODS

Chemicals and reagents

CAPE (CAS No. 104594-70-9, purity ≥ 97%), NIC (CAS No. 54-11-5, purity ≥ 99%), thiobarbituric acid reactive substances (TBARS), Bradford reagent, and bovine serum albumin (BSA) were purchased from Sigma Aldrich Company (USA). Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) assay kits were purchased from the ZellBio Company (Germany). Tumor necrosis factor alpha (TNF-α) and nitric oxide (NO) kits were purchased from Sun Long Biotech Co., Ltd (Shanghai, China).

Animals

Thirty-three male NMRI mice, aged 6-8 weeks, weighing 25 ± 2 g, were maintained at 23 ± 2 °C with a 12/12-h light/dark cycle, relative humidity, and free access to food and water. Animals were purchased from the Animal Breeding Laboratory Center of Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran. The study was performed in accordance with the ethical guidelines set by the Ethical Committee on Animal Experimentation at AJUMS Protocols (Ethical approval ID: IR.AJUMS.ABHC.REC.1401.080), which was in accordance with the National Institutes of Health.

Experimental design and convulsive behavior assessment

The animals were designated into five groups of 6-8 as follows: sham group (normal saline), NIC group (10 mL/kg of normal saline intraperitoneally + 5 mg/kg NIC intraperitoneally single dose on the seventh day), treatment groups (CAPE at 4 and 8 mg/kg intraperitoneally for seven days + NIC (30 min later on the seventh day), and Diaz (1 mg/kg intraperitoneally single dose) + NIC (30 min later on the seventh day) group. The CAPE and NIC doses were selected based on previous research (34, 35). After NIC injection, each animal was placed in a separate cage and monitored by an observer for seizure behavior (seizure onset time, duration, and intensity, and number of deaths due to seizure). In order to determine seizure onset time, the time between the injection of NIC and the observation of the first signs of tremor was measured. To measure the intensity of the seizure, the animal was placed on the table. If the animal's movements were normal, the animal was given a score of zero. If the animal's head and jaw moved slowly, a score of 1 was given; if the head and jaw were shaking strongly, a score of 2 was given; if the animal's body was shaking slowly, a score of 3 was given; and if the body was shaking strongly, a score of 4 was given. To study seizure duration, a stopwatch measured the time between the onset of the first seizure symptoms and the complete disappearance of the seizure. To study the mortality rate, the animals were monitored after the experiment to determine the mortality rate of the animals. On the seventh day of the study, the animals were anesthetized with ketamine (90 mg/kg)/xylazine (10 mg/kg) intraperitoneally, and their heads were separated with a guillotine. The frontal cortex of the brain was extracted and washed with normal saline. A portion of the frontal cortex was placed in 10% formalin for histopathological examination. The other portion was stored at -70 °C for the measurement of antioxidative/oxidative stress factors, inflammatory markers, and NF-κB protein expression.

A schematic diagram of the study design is shown in Fig. 1. Mice were treated with CAPE (4 and 8 mg/kg, ip, daily) or normal saline for 7 days. NIC (5 mg/kg, ip, single dose) or normal saline was injected on the seventh day.

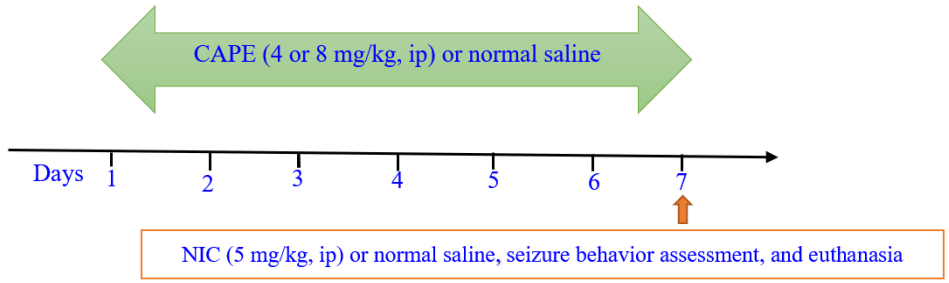

Fig. 1. Schematic diagram of the study design for investigating the effect of the CAPE on NIC-induced convulsion in mice. Mice were treated with CAPE (4 and 8 mg/kg, ip, daily) or normal saline for 7 days. NIC (5 mg/kg, ip, single dose) or normal saline was injected on the seventh day. CAPE, caffeic acid phenethyl ester; NIC, nicotine.

Preparation of tissue supernatant

For this purpose, the frontal cortex of the brain was homogenized and centrifuged at 4000 *g* for 20 min at 4 °C. The supernatants were separated and stored in a freezer at -70 °C to measure tissue factors (TBARS, total thiol, CAT, SOD, GPx, TNF-α, NO, and expression of NF-κB protein).

Protein content

Bradford's method was used to measure protein concentration in the supernatant. For this purpose, 40 µL of Bradford's reagent and 140 µL of distilled water were added to 20 µL of the samples. The absorbance was measured with a spectrophotometer at 595 nm. BSA was used as a standard for plotting the standard curve (36).

Total thiol

Ellman's reagent or 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) was used to measure total thiol level. The intensity of the yellow color was read with a spectrophotometer at 412 nm, and the results were expressed as nmol/mg protein (37).

Lipid peroxidation

To evaluate lipid peroxidation, the level of TBARS was measured using the Kei method (38). The colored complex absorbance resulting from the interaction of TBARS and lipid peroxides was read with a spectrophotometer at 532 nm, and expressed as nmol/mg protein.

Activity of antioxidant enzymes

To measure the activity of SOD, CAT, and GPx enzymes, ZellBio assay kits were used according to the manufacturer's instructions, and the results were reported as U/mg protein.

Inflammatory markers

The levels of pro-inflammatory markers of TNF-α and NO were evaluated using ELISA kits and were recorded as pg/mg protein and nmol/mg protein, respectively.

Western blotting

This technique was applied to measure the expression of the NF-κB protein. The brain frontal cortex samples were homogenized and centrifuged. The amount of 20 µg protein for each group was electrophoresed on a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membranes were blocked with 5% skim milk for 2 h at 4 °C. The membranes were probed with the primary antibody, NF-κB (1:500, Cat No. 8242, Cell Signaling Technology, USA) for 1 h at 4 °C. The membranes were exposed to anti-rabbit IgG-HRP (1:1000, Cat No. Sc-2357, Santa Cruz Biotechnology, Inc.) for 1 h. An electrochemiluminescence (ECL) kit (Abcam, 133408, USA) was used to visualize protein bands, and Image Lab™ Touch software (Bio-Rad, USA) was used to quantify the bands. GAPDH enzyme (1:500, Cat No. 5174, Cell Signaling Technology, USA) was used as a loading control.

Histopathological analysis

The frontal cortex of the mouse brain was immediately removed and fixed in formalin solution, and embedded in paraffin blocks. Sections of 4-6 µm were prepared from the frontal cortex tissue and stained with hematoxylin and eosin (H&E) (39). For each mouse, three microscopic slides in six fields were examined by an optical microscope (Olympus, BH-2, Japan).

Statistical analysis

Statistical analysis was performed by GraphPad Prism version 9 statistical software, and the normality of the data was confirmed using the Kolmogorov-Smirnov test. The data are reported as mean ± SEM, and finally, the results were analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. *P*-values < 0.05 were considered statistically significant.

RESULTS

The effect of CAPE on the mortality and seizure behavior

Statistical analysis was performed between groups for mortality and seizure behavior. The results showed that NIC injection led to seizures in all animals. According to Table 1, two mice in the NIC group (33.33%) and one mouse in the CAPE 4 + NIC group (16.66%) died. There were no deaths in the sham, CAPE 8 + NIC, and Diaz + NIC groups. In this Table, the highest seizure severity was observed in the NIC group. The groups of the Diaz + NIC and the CAPE 8 + NIC had the lowest seizure severity. The NIC group had the longest extended seizure duration, and the lowest seizure duration was observed in the Diaz + NIC group. The severity and duration of seizure decreased in the groups that received CAPE 4 + NIC and CAPE 8 + NIC, and the onset time of the first seizure increased in these groups in comparison with the NIC-treated group. In general, the anticonvulsant effects were better in the Diaz + NIC group than in the CAPE groups.

The effect of CAPE on the oxidative stress parameters

The results related to the oxidative/anti-oxidative levels of total thiol, TBARS, CAT, SOD, and GPx in the brain frontal cortex are presented in Fig. 2. The levels of total thiol, CAT, SOD, and GPx in the NIC group were significantly lower than those in the sham group. However, in the group receiving CAPE + NIC, the levels of activity CAT, SOD, and GPx were significantly higher than in the NIC group. NIC led to an increase in the TBARS level compared to the sham group. However, in the group receiving CAPE + NIC, compared to the NIC group, the level of TBARS significantly decreased. In the group receiving Diaz + NIC, the levels of total thiol, CAT, SOD, and GPx were significantly higher than those of the NIC group, and the level of TBARS was significantly lower relative to the NIC group.

The effect of CAPE on the inflammatory markers

The effect of CAPE on the inflammatory markers NO and TNF-α is shown in Fig. 3. These markers significantly increased in the NIC group compared to the sham group. CAPE treatment resulted in a significant reduction in NO and TNF-α levels compared to the NIC group. In the CAPE 8 + NIC group compared to the CAPE 4 + NIC group, a more significant reduction was observed in the level of TNF-α, which indicates a better effect of CAPE 8 compared to CAPE 4 in mice. The levels of NO and TNF-α in the group receiving Diaz + NIC were significantly lower than in the NIC group.

Table 1. The effect of CAPE on the mortality and seizure behavior in NIC-induced convulsion mice. Data are expressed as mean ± SEM, n = 6. ***P < 0.001 indicates significant differences compared to the sham group; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01, $^{\#\#\#}P$ < 0.001 versus the NIC group.

Groups	Seizure mortality (%)	Seizure onset time (min)	Seizure duration (min)	Seizure severity (degree)
Sham	0	0	0	0
NIC	33.33^{***}	10^{***}	90^{***}	4^{***}
CAPE 4 + NIC	$16.66^{\#}$	$24^{\#}$	$30^{\#}$	$2^{\#}$
CAPE 8 + NIC	$0^{\#\#\#}$	$28^{\#\#}$	$20^{\#\#}$	$1^{\#\#}$
Diaz + NIC	$0^{\#\#\#}$	$36^{\#\#\#}$	$15^{\#\#\#}$	$1^{\#\#}$

CAPE, Caffeic acid phenethyl ester; NIC, nicotine; Diaz, diazepam.

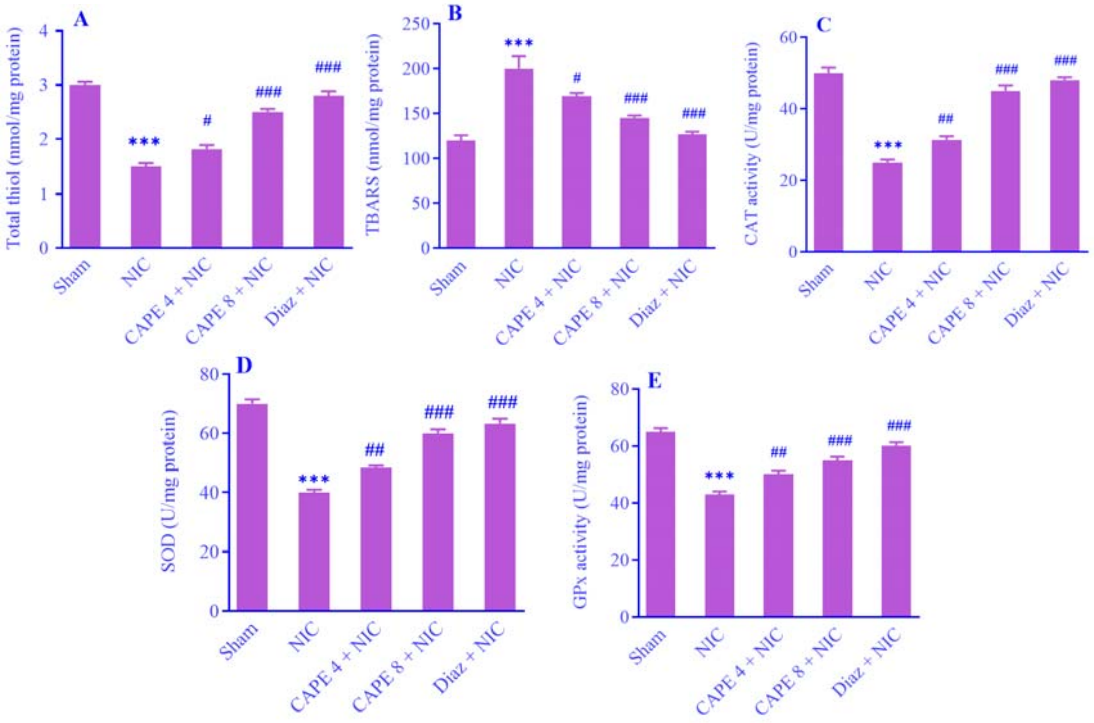

Fig. 2. The effect of CAPE on the oxidative stress parameters (A) total thiol, (B) TBARS, (C) CAT, (D) SOD, and (E) GPx in the brain frontal cortex of NIC-induced convulsive mice. Data are expressed as mean ± SEM, n = 6. ***$P < 0.001$ indicates significant differences compared to the sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus the NIC group.. CAPE, caffeic acid phenethyl ester; NIC, nicotine; Diaz, diazepam; TBARS, thiobarbituric acid reactive substances; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase.

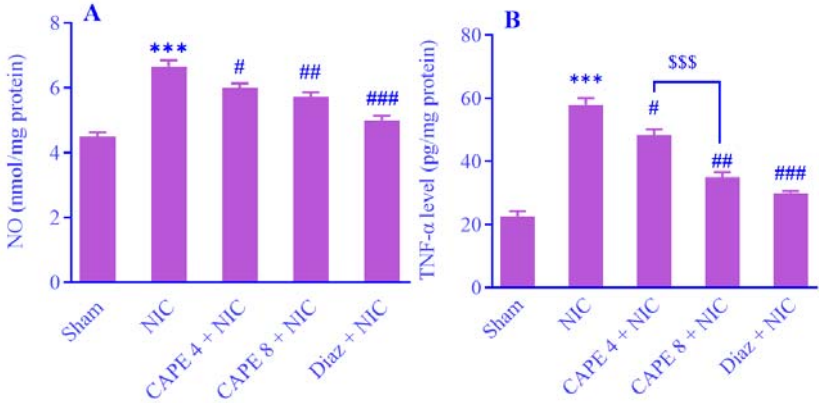

Fig. 3. The effect of CAPE on the (A) NO and (B) TNF-α in the brain frontal cortex of NIC-induced convulsive mice. Data are expressed as mean ± SEM, n = 6. ***$P < 0.001$ indicates significant differences compared to the sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus the NIC group. CAPE, caffeic acid phenethyl ester; NIC, nicotine; Diaz, diazepam; NO, nitric oxide; TNF-α, tumor necrosis factor-alpha

The effect of CAPE on the expression of NF-κB protein

As shown in Fig. 4, the NF-κB protein level significantly increased in the NIC group compared to the sham group. Co-treatment with CAPE at 4 and 8 mg/kg significantly reduced the expression of NF-κB protein compared to the NIC group. These findings indicate that CAPE can reverse the increase in NF-κB protein expression induced by NIC. The expression of NF-κB protein in the Diaz + NIC group was significantly lower than that in the NIC group.

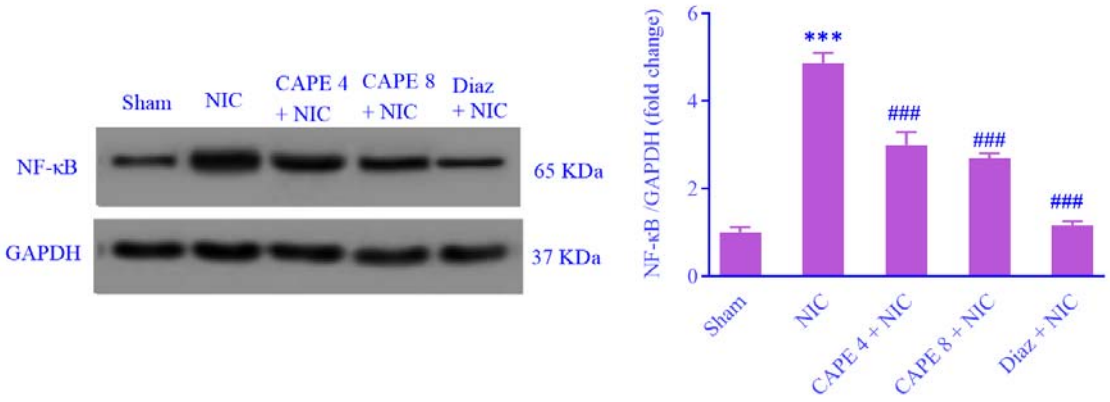

Fig. 4. The effect of CAPE on the expression of NF-κB protein in the brain frontal cortex of NIC-induced convulsive mice. Data are expressed as mean ± SEM, n = 6. ***$P < 0.001$ indicates significant differences compared to the sham group; $^{###}P < 0.001$ versus the NIC group. CAPE, caffeic acid phenethyl ester; NIC, nicotine; Diaz, diazepam; NF-κB, nuclear factor kappa B.

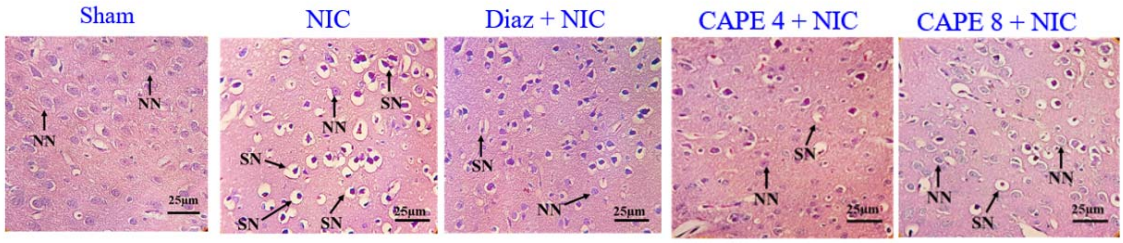

Fig. 5. Light microscopy examination of the brain frontal cortex tissue sections stained by H&E. Arrows show normal neurons (NN) with central large vesicular nuclei, and neurons with dystrophic changes in the form of shrunken hyperchromatic nuclei (SN). CAPE, Caffeic acid phenethyl ester; NIC, nicotine; Diaz, diazepam. Magnification: 250×.

Histopathological examination

The results of H&E staining of the brain frontal cortex in the study groups are shown in Fig. 5. Accordingly, the tissue structure of the frontal cortex in the sham group was normal. The nuclei of the nerve cells in this group were bright, large, and vesicular. In the NIC group, serious damage was observed in the frontal cortex. So that the nerve cells were destroyed, the cell nuclei became small and dark, and the cells became hypertrophied. However, in the groups receiving CAPE (4 and 8 mg/kg), these damages were repaired, and the tissue structure of the frontal cortex returned to its normal form to some extent.

DISCUSSION

A seizure is a neurological disorder in the brain caused by changes in the function of brain neurons. Since seizures lead to increased free radicals and oxidative stress in the brain, and CAPE can penetrate the BBB and exert neuroprotective functions, in this study, the protective effect of CAPE on NIC-induced seizures was investigated. Previous studies have shown that oxidative stress and free radicals are involved in many acute and chronic neurological disorders and seizure-induced brain injury (40,41). Excessive activation of excitatory amino acid receptors is a key factor in the development of seizures (42). NIC, through excessive stimulation of nAChRs, leads to the stimulation of glutamate release and the development of seizures (14). In this regard, the convulsive effect of NIC could be due to inhibition of glycine or stimulation of motor centers of the CNS (20). In this study, NIC administration induced seizures in all mice. Administration of CAPE before NIC increased seizure onset time, a decrease in seizure severity and duration, and ultimately a decrease in mortality and an improvement in seizure parameters. This improvement was greater in the CAPE 8 mg/kg treatment group compared to the CAPE 4 mg/kg, indicating a better effect

of the CAPE 8 mg/kg. Our results were consistent with previous studies (43). The state of imbalance between oxidant and antioxidant factors is called oxidative stress. The level of oxidative stress is determined by measuring the antioxidant parameters, including total thiol, CAT, SOD, and GPx, and the oxidant parameter TBARS (44). The antioxidant activity of CAPE has been shown in various studies (31). In this study, the effect of CAPE on oxidative stress is quite significant. NIC led to a decrease in total thiol levels and in the activities of the antioxidant enzymes CAT, SOD, and GPx, whereas these parameters were significantly improved in the CAPE treatment groups. SOD is an enzyme that converts O_2^- to H_2O_2. In the study by Cigremis *et al*., the activity of this enzyme increased in the CAPE treatment group compared to the other groups (45). This increase indicates the effectiveness of CAPE in reducing oxidative damage and neurotoxicity in brain tissue. These results were consistent with our study. The study by Mustafa Iraz et al., showed that CAPE may prevent oxidative changes by enhancing the antioxidant defense system via reducing ROS, and increasing the activities of antioxidant enzymes CAT, SOD, and GPx (46). These results were in line with the findings of the present study, in which NIC led to an increase in the oxidative parameter TBARS, which is due to increased oxidative stress, mitochondrial dysfunction, and the involvement of ROS in NIC-induced seizures. The antioxidant effects of polyphenolic compounds in scavenging ROS caused by lipid peroxidation, cellular damage, and oxidative stress are well known (27). The chemical structure of CAPE consists of an aromatic nucleus with a conjugated side chain that allows for easy transfer of unpaired electrons and traps free radicals (30). In this study, CAPE significantly reduced the level of TBARS in brain tissue due to its ability to scavenge ROS. According to previous studies, TNF-α induces the synthesis of neurotransmitters and plays a role in inflammatory mechanisms (47). In this study, the inflammatory markers NO and TNF-α increased in the NIC group. Previous studies have shown that CAPE has anti-inflammatory, neuroprotective, and immunomodulatory properties (48, 49). In this study, CAPE treatment significantly inhibited the overproduction of these inflammatory markers. The study by Al-Hariri et al. showed that CAPE led to a decrease in inflammatory cytokines (50), which are consistent with our study. Previous studies have indicated that neuronal death caused by increased NO can be due to apoptosis (51). NF-κB is a critical molecule that plays a role in immune responses, inflammatory reactions, control of cell division, and apoptosis (52). In this study, NF-κB protein expression was increased in the NIC group and significantly decreased in the CAPE-treated groups. According to previous studies, CAPE has antioxidant activity and is a lipoxygenase inhibitor (53). Since ROS is involved in the signal transduction pathways that lead to NF-κB activation, CAPE prevents NF-κB activation by reducing ROS production in the brain tissue during seizures (54). In the study by Kumar et al., CAPE treatment resulted in a decrease in TNF-α and NF-κB (55). The aforementioned findings align with the observations made in our investigation. Histopathological analysis of brain tissue showed significant damage in the NIC group. In this group, the nuclei of the cells were small and dark, and the cells were hypertrophic. However, in the CAPE-treated groups, these damages were repaired, the number of hyperchromatic cells was reduced, and the brain tissue structure had somewhat returned to normal. According to the antioxidant effects of CAPE in various studies, it appears that CAPE can improve seizures by reducing inflammation and inhibiting oxidative stress.

CONCLUSION

The present study demonstrates that CAPE effectively mitigates NIC-induced seizures in mice by strengthening cellular antioxidant defenses. This is evidenced by increased antioxidant parameters, total thiol, CAT, SOD, and GPx, reduced oxidant parameter TBARS, inflammatory factors NO and TNF-α, and the expression of NF-κB protein. Further preclinical investigations are warranted to evaluate the potential of CAPE in neuroinflammation models.

Acknowledgements

This research was funded by the Student Research Committee of Ahvaz Jundishapur University of Medical Sciences through Grant No. 01s71. We would like to express our sincere gratitude to the Deputy Director of Research of AJUMS for supporting this research.

Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

The study's conceptualization and design were jointly developed by MS. Badiee and MJ. Khodayar. Methodological frameworks were established by MS. Badiee and MJ. Khodayar. Formal analysis and investigative procedures were carried out by MS. Badiee and MJ. Khodayar. The initial manuscript draft was prepared by MS. Badiee, MJ. Khodayar, and F. Fakhredini, while subsequent review and editing were performed by MS. Badiee and MJ. Khodayar. Funding acquisition was managed by MS. Badiee. Resources were provided by MS. Badiee, MJ. Khodayar, and A. Vadizadeh. The study was supervised by MJ. Khodayar, F. Fakhredini, M. Moosavi, H. Kalantar, and S. Rafiei Asl. All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

AI declaration

The authors did not use any AI-assisted technologies in the preparation of this manuscript.

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