Neurochemical Research (2019) 44:2556–2565
https://doi.org/10.1007/s11064-019-02874-6

ORIGINAL PAPER

Anticonvulsant and Neuroprotective Effects of Paeonol in Epileptic Rats

Dong-Hai Liu[1] · Elvis Agbo[2] · Shu-Hong Zhang[3] · Jin-Ling Zhu[3]

Received: 7 May 2019 / Revised: 6 September 2019 / Accepted: 9 September 2019 / Published online: 13 September 2019

Abstract

Paeonol is the main active compound in the root bark extract of the peony tree, and it has antioxidative and anti-inflammatory effects. Recent studies have reported the neuroprotective effects of paeonol including its capacity in improving impaired memory. However, the effect of paeonol on epilepsy is yet to be demystified. We aimed to investigate the therapeutic effect of paeonol in epilepsy and its relationship with oxidative stress damage and neuronal loss in the rat brain to reveal the underlying mechanisms of epileptic seizures. A rat model for chronic epilepsy was established, and the seizure scores of the rats in different groups were recorded. The seizure duration and the seizure onset latency were used to evaluate the anticonvulsant effects of paeonol. Terminal deoxynucleotidyl transferase dUTP nick end-labeling staining, Nissl staining and H/E staining were used to evaluate the effects of paeonol on neuronal loss and apoptosis in epileptic rats. The colorimetric assessment of malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, catalase activity and total antioxidant capacity of paeonol were used in assessing paeonol's effect on oxidative stress in epileptic rats. Evaluation of Caspase-3 mRNA and protein expression levels were determined using western blot and quantitative real-time (RT-q)PCR. In this study, we found that paeonol reduced the seizure scores of epileptic rats and attenuated the duration and onset latency of seizures. Paeonol can also increase the activities of total antioxidant capacity, SOD and catalase activity and reduce MDA content as well. This suggests that paeonol can improve the level of oxidative stress in rats. More significantly, paeonol can improve neuronal loss and apoptosis in epileptic rats. These results indicate that paeonol has anticonvulsant and neuroprotective effects in epileptic rats. This effect may be caused by reducing oxidative stress.

Keywords Paeonol · Epilepsy · Oxidative stress · Anticonvulsant · Neuroprotective · Apoptosis

Introduction

Epilepsy is one of the most common neurological diseases, and it is characterized by spontaneous seizures, abnormal electrical discharges in the brain, and convulsions. According to statistics, epilepsy afflicts more than 65 million people worldwide, and over 100,000 new cases appear each year [1]. There have been approximately 15 anti-epileptic drugs (ASDs) in the clinical treatment of epilepsy within the past 20 years [2]. Although numerous ASDs have been used to treat epilepsy, approximately one-third of epileptic cases still cannot be controlled [3, 4]. In addition, despite the introduction of a new generation of ASDs, the substantial side effects are still the biggest challenges in the treatment of epilepsy [4]. Therefore, there is the need in elucidating the molecular mechanisms of epileptogenesis in order to develop new antiepileptic drugs which will alter the progression of epilepsy.

Accumulating evidence from experimental and clinical studies indicates that oxidative stress might be a consequence or cause of epilepsy [5]. On one hand, the activity of free radical scavengers, such as superoxide dismutase (SOD), catalase activity (CAT), and glutathione peroxidase (GSH-Px), is reduced in the hippocampus of epileptic animals [6]. Similarly, these free radical scavengers

✉ Jin-Ling Zhu
2746208403@qq.com

[1] School of Basic Medicine, Jiamusi University, Jiamusi 154007, Heilongjiang, People's Republic of China

[2] Department of Anatomy, School of Basic Medicine, Jiamusi University, Jiamusi 154007, Heilongjiang, People's Republic of China

[3] Department of Biology, School of Basic Medicine, Jiamusi University, Jiamusi 154007, Heilongjiang, People's Republic of China

are also reduced in the cerebrospinal fluid or serum of epileptic patients [7]. On the other hand, there has been some break through in reducing the damage of neurons following seizures by targeting oxidative stress with small molecules in epileptic animal models [8, 9]. The implication of oxidative stress as a novel mechanism suggests a potential disease-modifying therapeutic approach to target it. As an important platform in the regulation of oxidative stress responses, anti-oxidants have attracted increasing attention in various CNS disorders [10, 11]. Systems of anti-oxidation are divided into non-enzymatic and enzymatic antioxidant groups. The enzymatic group comprises of various enzymes like SOD and CAT [12]. SOD works with Mn in the mitochondria and Cu/Zn in the cytosol, and catalyzes the breakdown of superoxide anions into H_2O_2 and oxygen [13, 14]. In the presence of Fe or Mn cofactors, CAT catalyzes the conversion of H_2O_2 to oxygen and water [15]. The nonenzymatic group is made up of various antioxidants acting directly on agents of oxidation and are obtained from dietary sources. The nonenzymatic group includes vitamin E, carotenoids, vitamin C, polyphenols, flavonoids etc. A recent study showed that oxidative stress induced by epilepsy is capable of eliciting a chain reaction that causes extensive damage to neurons in the brain and thus triggers epileptic seizures [16]. Pitkänen et al. found that the hippocampi in the brains of epileptic patients eventually decrease in size [17], and this loss of neurons can subsequently lead to cognitive impairment [18] and an increase in the severity of seizures [19]. Several studies have shown that neuronal loss is directly associated with the onset of epilepsy [20], with apoptosis being an important reason for the induction of neuronal loss [21, 22]. These results indicate that in the physiopathology of epilepsy, oxidative stress-mediated apoptosis processes might be an important mediator.

Paeonol (2′-hydroxy-4′-methoxyacetophenone, C9H10O3) is one of the main active compounds in the root-bark extract of the peony tree and is used in Chinese traditional medicine for improving blood circulation, amenorrhea, dysmenorrhea, fever, etc. [23, 24]. Paeonol has antioxidative and free radical scavenging effects [25, 26], with recent studies showing that paeonol exerts a neuroprotective role against Alzheimer's disease, cerebral infarction, Parkinson's disease and acute ischemic stroke [27–29]. However, the effect of paeonol on epilepsy has yet to be elucidated. These studies provide a useful basis for the potential use of paeonol in preventing and treating epilepsy. We used paeonol as an intervention for epileptic rats to explore the therapeutic potential of paeonol for epilepsy. In addition, the relationship between oxidative stress damage and neuronal loss in the brains of rats was explored in order to elucidate potential mechanisms of oxidative stress in epilepsy treatment.

Materials and Methods

Animals

30 male Wistar rats (180–200 g, 8–10 weeks) were bought from the Animal Experimental Center of Harbin Medical University and were housed at the Animal Experimental Center of Jiamusi University. All rats were put in clean cages. Three rats were placed in each cage that has a dimension of 400 mm (length) × 280 mm (width) × 200 mm (height). The rats were kept at a humidity of 40% and a temperature of 22–24 °C. Animals were kept on a 12-h dark/light cycle with free access to water and food. Rats were acclimated to the new environment for 1 week prior to the start of the study. All animal care and laboratory procedures were performed by adhering to the National Institute of Health's approved guidelines for the use and care of laboratory animals and guidelines approved by the Animal Ethics Committee of Jiamusi University. We made all efforts to keep animal pain and number minimal in our study.

Treatments

Thirty male Wistar rats were randomly divided into five groups: normal control group (NC), epilepsy group (EP), low-dose paeonol-treated group (LP), medium-dose paeonol-treated group (MP) and high-dose paeonol-treated group (HP). Each group consisted of six rats. The NC group rats were intraperitoneally injected with 0.5% sodium carboxymethylcellulose(C8621; Solaibro, China)solution once daily for 28 days; EP group rats were intraperitoneally injected with 35 mg/kg of Pentylenetetrazole (PTZ) (Sigma Aldrich, Germany) once daily for 28 days [30] to induce epilepsy; the LP, MP, and HP groups were first intraperitoneally injected with 20 mg/kg, 40 mg/kg and 60 mg/kg of paeonol (AB22173, ShangHai YuanYe Biological, China) (Paeonol was dissolved in 0.5% sodium carboxymethylcellulose solution and prepared into 20 mg/kg, 40 mg/kg and 60 mg/kg paeonol suspension) respectively and then with an intraperitoneal injection of PTZ after 30 min once daily for 28 days. We didn't use a positive control in order to save cost and time since the anticonvulsant activities of drugs such as sodium valproate, oxcarbazepine, lamotrigine and diazepam in PTZ-induced kindling have been previously reported [31–34] (Table 1).

PTZ Kindling

At the end of injection of rats with PTZ, the rats were observed for a period of 30 min. The evaluation of convulsion intensity was classified as follows [35]: stage 0, no

Table 1 Drugs and dosages used in the 5 model rat groups

Experimental group	0.5% sodium carboxymethylcellulose	PTZ (mg/kg)	Paeonol (mg/kg)
NC	Yes	No	No
EP	No	35	No
LP	No	35	20
MP	No	35	40
HP	No	35	60

In the LP, MP, and HP groups, paeonol was administered 30 min prior to PTZ administration

response; stage 1, facial movements and ear and whisker twitching; stage 2, myoclonic convulsions without rearing; stage 3, myoclonic convulsions with rearing; stage 4, tonic–clonic convulsions; stage 5, generalized tonic–clonic seizures with loss of postural control; and stage 6, death. We considered rats with three consecutive stage 4 seizures as being fully kindled [35]. Seizure scores, durations, and onset latencies were recorded.

Brain Tissue Preparation

All rats were anesthetised by injection of xylazine (10 mg/kg) intraperitoneally. The hearts of three rats from each group were first perfused with cold saline (500 ml) followed by 200 ml of 4% paraformaldehyde (PFA), and then brain tissues were fixed with 4% PFA for 24 h. The brains were then paraffin-embedded, and 4 μm hippocampal sections were obtained 4 mm from the bregma for later use [36]. The other three deeply anesthetized rats were directly sacrificed, and their hippocampi were immediately isolated and stored in liquid nitrogen.

H&E Staining

Slices containing rat hippocampal sections were first dewaxed in xylene and ethanol. The sections were then stained with hematoxylin dye solution for 10 min, soaked in 55 °C warm water for 5 min, placed in 1% alcoholic HCl for 3 s and then finally placed in eosin dye solution for 1 min. The stained sections were washed and immersed in 95% ethanol for 2 min, soaked in dimethyl benzene for 3 min and finally covered with a neutral resin. HE-stained slices (3 slides per rat and 3 rats per group) were observed using an OLYMPUS DM4000 optical microscope and damaged neurons per 1 mm length of the medial CA1 region were counted using Image-Pro Plus 6.0 (Media Cybernetics, USA). An investigator in a blinded fashion counted the number of surviving pyramidal neurons per 1 mm length of the medial CA1 region [37] since damage to neurons was more uniform and obvious than that in the lateral CA1 region [38].

Nissl Staining

Deparaffinization of Paraffin sections were done with xylene and gradient alcohol followed by staining with 5% toluidine blue staining solution for 10 min. The stained sections were washed and immersed in 95% ethanol for 2 min, soaked in dimethyl benzene for 3 min and finally covered with a neutral resin. Nissl stained slices (3 slides per rat and 3 rats per group) were observed using an OLYMPUS optical microscope and neurons per 1 mm length of the medial CA1 region were counted using Image-Pro Plus 6.0 (Media Cybernetics, USA).

TUNEL Staining

Analysis of the extent of tissue damage in the rat hippocampus was done using a TUNEL apoptosis assay kit based on fluorescence (Cat. Wla030a; Wanlei, China) by strictly following the manufacturer's instructions. Briefly, dewaxing of rat hippocampus slices was achieved using xylene and ethanol. Then, the samples were bathed in citrate buffer (pH 6.0, 97 °C) for 5 min, incubated with DNase-free proteinase K, rinsed using PBS buffer, incubated using 3% H_2O_2, added to 50 μl TUNEL detection solution and placed in the dark for 70 min at 37 °C; samples were then rinsed using PBS buffer, incubated with DAPI, and washed using PBS buffer. The slices (3 slides per rat and 3 rats per group) were scanned using an OLYMPUS laser confocal microscope and neurons were counted using Image-Pro Plus 6.0 (Media Cybernetics, USA). Apoptotic percentage was calculated as the cell number of TUNEL-positive green nuclei divided by the total number of cells across the viewed areas, and multiplied by 100.

Measurement of SOD, CAT, Total Antioxidant Capacity (T-AOC), and Malondialdehyde (MDA)

0.1 g of hippocampal tissues was put in a 1.5 ml microtube and homogenization of hippocampal tissues was done in tissue lysis buffer (Beyotime Biotechnology, China). They were then centrifuged for 10 min at 18,759×*g* and at a temperature of 4 °C. The concentration of protein was evaluated using a BCA protein assay kit (Beyotime Biotechnology, China). Measurement of the activity or content of SOD (U/mg protein), CAT (U/mg protein), MDA (nmol/g protein), and T-AOC (U/mg protein) [39, 40] were done with MDA kit (A003-1, Nanjing Jiancheng Bioengineering Institute), SOD kit (A001-3, Nanjing Jiancheng Bioengineering Institute) and T-AOC kit (A015-2, Nanjing Jiancheng Bioengineering Institute) by following the manufacturer's instructions using spectrophotometry.

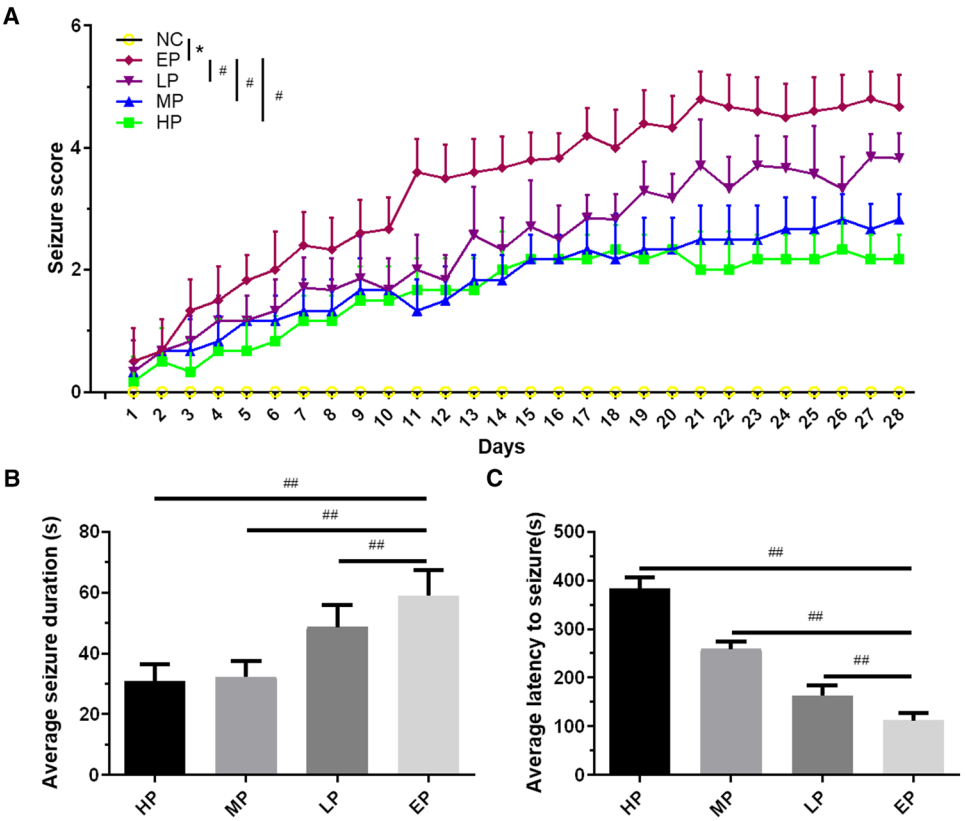


Fig. 1 The effect of paeonol on PTZ-induced seizures. **a** Statistical results showed that paeonol lowered the seizure score in a dose-dependent fashion. **b** Statistical results showing that paeonol decreased the duration of generalized seizures. **c** Statistical results showing that paeonol increased the latency to generalized seizures. Data are expressed as the mean ± SEM. n = 6. *P < 0.05 versus NC;**P < 0.01 versus NC; #P < 0.05 versus EP; and ##P < 0.01 versus EP

Quantitative RT-PCR Analysis

First, 0.1 g of hippocampal tissue frozen in liquid nitrogen was placed in a tube and grinded. We employed the use of Takara MiniBEST Universal RNA extraction kit in extracting the total RNA samples according to the manufacturer's manual (Cat. 9769; Takara, China). The synthesis of cDNA was performed using the Prime Script RT Master Mix kit according to the manufacturer's (RR036a; Takara, China) instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was then performed with TB Green™ Premix Ex Taq™ II (RR820a; Takara, China) by strictly following the manufacturer's manual. The expression of GAPDH served as the internal control. Primer sequences used are: Caspase-3 forward: 5′-CAA GTTCAACGGCACGTCAA-3′; Caspase-3 reverse, 5′-TGGTGAAGACGCCAGAGACTC-3′; GAPDH forward: 5′-ACTCCCATTCTTCCACCTTTG-3′; GAPDH reverse: 5′-CCCTGTTGCTGTAGCCATATT-3′.

Western Blot

Caspase-3, a cysteine-aspartic protease, is a heterodimer consisting of two subunits of 17.12 kD produced by enzymatic hydrolysis of 28 kD zymogen. When the cells are stimulated by various external harmful factors, Caspase-3 is converted into active caspase-3, also known as cleaved-caspase-3. Cleaved-caspase-3 has matured enzymatic properties and can participate in apoptosis through enzymatic cleavage of specific substrates, and DNA-dependent protein kinases. Therefore, cleaved caspase-3 is often used as an important indicator for determining apoptosis [41–43]. Hippocampal tissues frozen in liquid nitrogen (0.1 g) were placed in western blot cell lysis buffer (Beyotime Biotechnology, China) and homogenized with a tissue grinder. Proteins were boiled at 97 °C for 10 min in 5 × SDS-PAGE protein loading buffer (Beyotime Biotechnology, China). Separation of 30 μg protein samples were done using an SDS polyacrylic gel and transferred to a PVDF membrane. Skimmed milk powder (5%; 37 °C; TBS dissolved) was used for blocking the membranes. Samples were subsequently incubated with Cleaved caspase-3 (1:500, AF7022, Affinity, USA) and beta-Actin (1:3000, AF7018, Affinity, USA) primary antibodies. They were then incubated with HRP-conjugated secondary antibody. The blots were developed using enhanced chemiluminescence (ECL) solution (Beyotime Biotechnology, China).The optical density of protein bands was finally analyzed using the Tanon Protein Analysis System and the final results expressed as the optical density

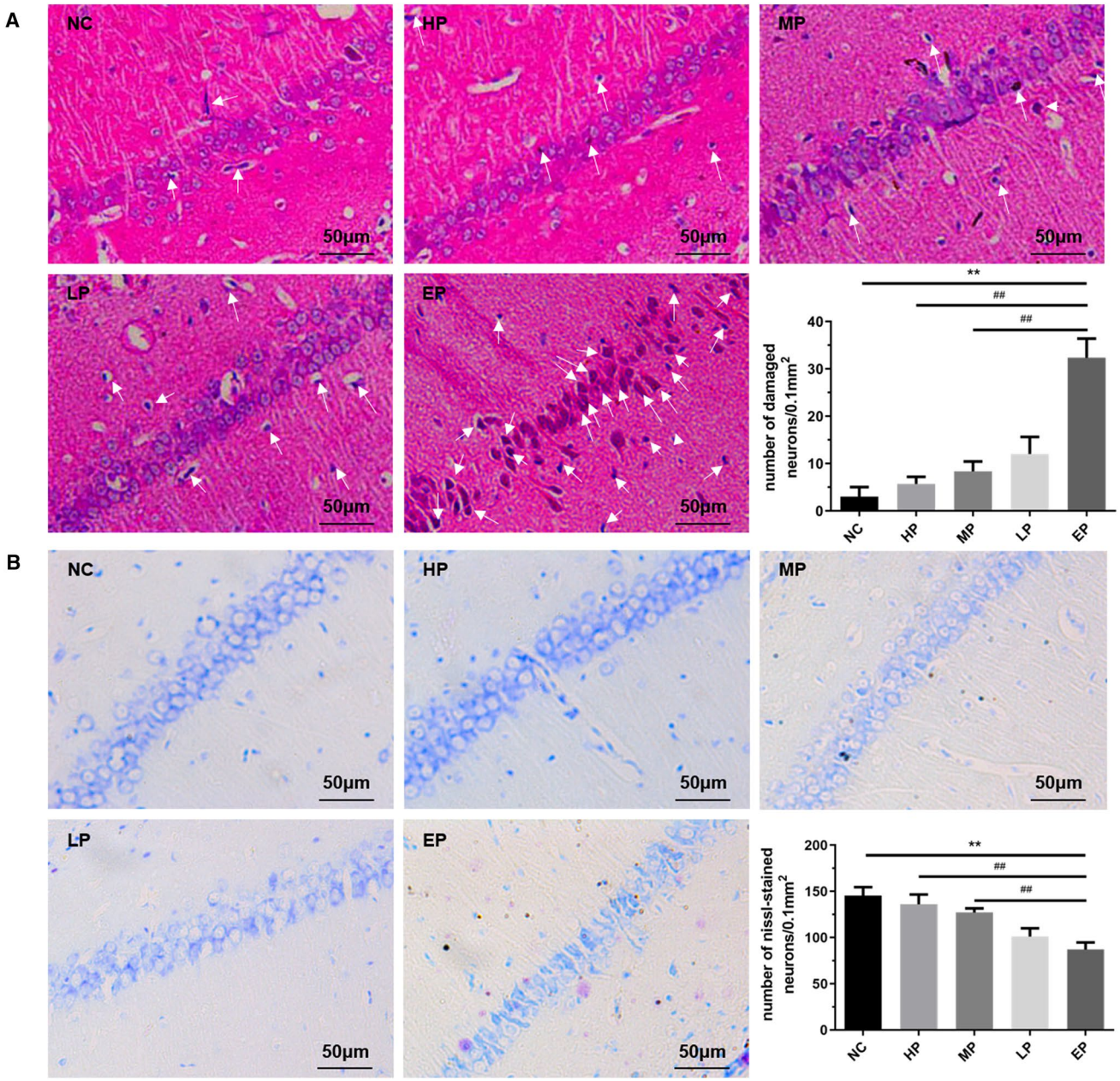


Fig. 2 Effects of paeonol on hippocampal neuronal damage elicited by PTZ kindling. **a** Histological examination by H&E staining showing that paeonol blocked hippocampal neuronal damage in the CA1 area induced by PTZ kindling. **b** Histological examination by Nissl staining showing that paeonol blocked hippocampal neuronal damage in the CA1 area induced by PTZ kindling. n = 3. $^{**}P < 0.01$ versus NC; $^{\#\#}P < 0.01$ versus EP and $^{\#}P < 0.05$ versus EP

of cleaved caspase-3 divided by the optical density of β-actin.

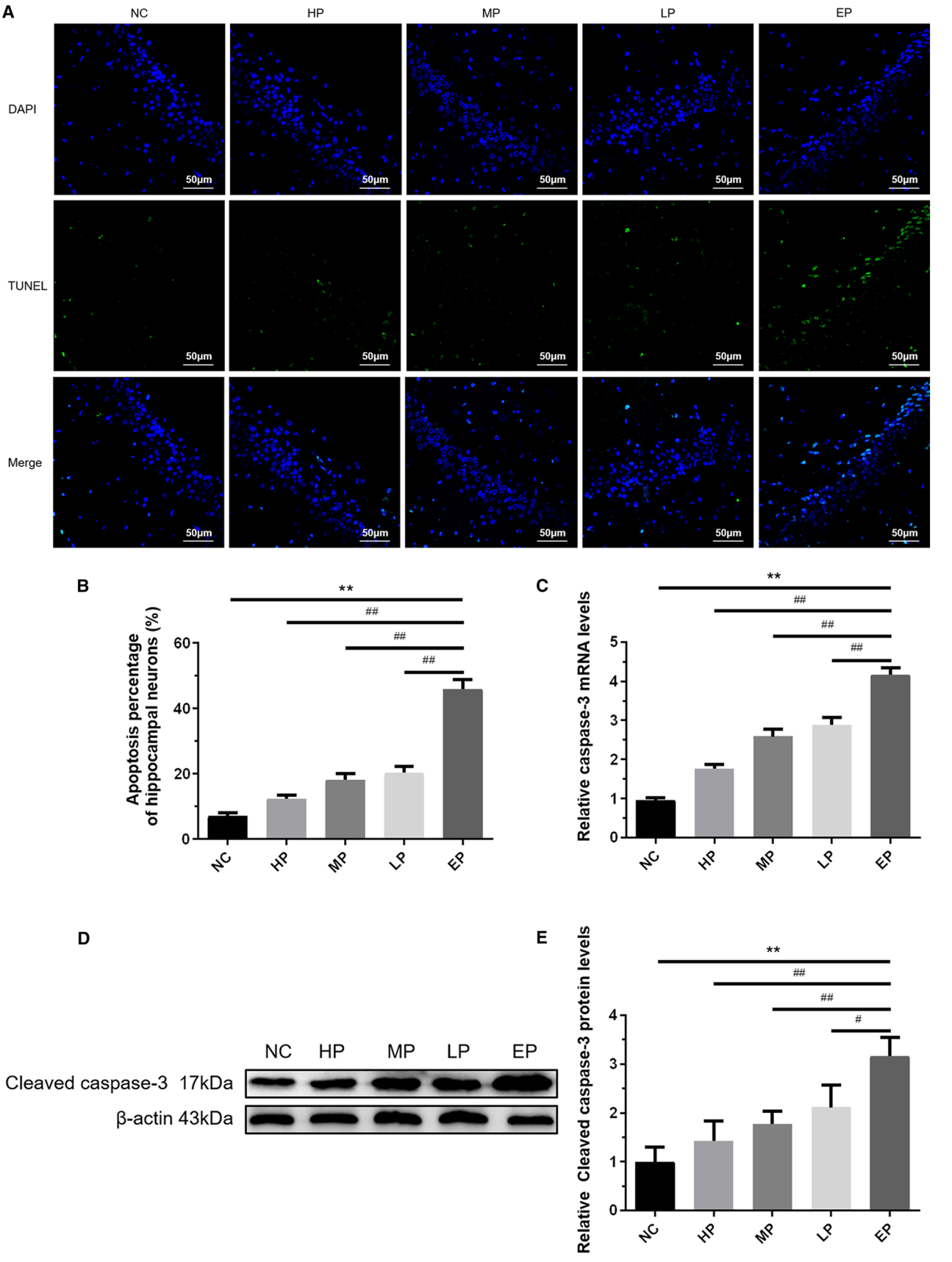
Statistical Analysis

Data were expressed as the mean ± SD. Comparisons were assessed with Tukey's post hoc test and one-way analysis of variance (ANOVA) using SPSS 17.0 (Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Paeonol Exerts an Anticonvulsant Profile in the PTZ Kindling Model of Epilepsy

We first investigated the anticonvulsant effect of paeonol. Seizure stage scores, durations and onset latencies (time period between PTZ administration and the onset of a seizure event) were recorded. As demonstrated in Fig. 1a, the


A
NC
HP
MP
LP
EP
DAPI
TUNEL
Merge
50μm
B
Apoptosis percentage of hippocampal neurons (%)
C
Relative caspase-3 mRNA levels
D
Cleaved caspase-3 17kDa
β-actin 43kDa
E
Relative Cleaved caspase-3 protein levels
**
##
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◂**Fig. 3** Effects of paeonol on hippocampal apoptosis in PTZ-kindled rats. **a**, **b** TUNEL staining (200 × magnification) showing that paeonol blocked hippocampal neuronal apoptosis in the CA1 area induced by PTZ kindling. **c** Statistical results showing that paeonol inhibited the PTZ-induced increase in caspase-3 mRNA expression in a dose-dependent fashion. **d**, **e** Representative immunoreactive bands and statistical results showing that paeonol dose dependently prevented the PTZ-induced increase in the expression of cleaved caspase-3. Data are expressed as the mean ± SEM. n = 3. **P < 0.01 versus NC; #P < 0.05 versus EP; and ##P < 0.01 versus EP

seizure score of rats in the EP group after 28 PTZ injections reached 4.67 ± 0.52. However, 20, 40, and 60 mg/kg paeonol treatments attenuated the seizure stage to 3.83 ± 0.41, 2.83 ± 0.41, and 2.17 ± 0.41, respectively. This indicates that paeonol disrupted the PTZ kindling process. In addition, paeonol caused the latency to generalized seizures to significantly increase and decreased the duration of seizures in a dose-dependent fashion (Fig. 1b, c), suggesting that paeonol has anticonvulsant effects in PTZ-kindled rats.

Paeonol Blocks Hippocampal Neuronal Damage in PTZ-Kindled Rats

In epilepsy, the hippocampus has always been a focus of attention since seizures causes hippocampal neurogenesis abnormalities [44–46]. We therefore made an investigation on the effects of paeonol on PTZ kindling-induced hippocampal neuronal damage. Histological examination was first done using H/E staining and Nissl staining. As demonstrated in Fig. 2a, most neurons in the EP group were damaged. Moreover, the number of damaged hippocampal neurons in rats decreased gradually as the concentration of paeonol increased. Among the five groups, the hippocampal neurons of the NC group were the least damaged and almost completely intact (the white arrows in Fig. 2a indicate damaged neurons) [38]. As demonstrated in Fig. 2b, the EP group had the fewest number of neurons and the thinnest cell layer. As the concentration of paeonol increases, there was a gradual increase in the number of hippocampal neurons in rats. These demonstrate that paeonol may be capable of preventing PTZ-induced hippocampal neuronal damage [36]. Then evaluation of neuronal apoptosis was done using TUNEL staining. Compared with the NC group, apoptotic neurons in the hippocampal CA1 area were markedly increased in the EP group. Paeonol dose-dependently prevented PTZ kindling-induced neuronal apoptosis induced by (Fig. 3a, b). Finally, detection of apoptosis-related factor caspase-3 was done in the hippocampus by western blot and qRT-PCR. Compared to the NC group, caspase-3 expression was markedly increased in the EP group. PTZ kindling-induced effects could be attenuated by paeonol in a dose-dependent fashion (Fig. 3c–e). All these data demonstrate the capacity of paeonol in blocking hippocampal neuronal damage and apoptosis from PTZ kindling.

Paeonol Exerts Antioxidant Activity in a PTZ Kindling Model of Epilepsy

Brain tissue is more susceptible to oxidative damage than other tissues [7], and paeonol has strong antioxidant properties [25, 26]. Therefore, we subsequently investigated whether paleonol exerts antioxidant activity in a PTZ kindling model of epilepsy. We first measured CAT, SOD, and T-AOC activities, as shown in Fig. 4a–c. Compared with the NC group, the CAT, SOD and T-AOC activities in the hippocampus were significantly lower in the EP group. The effects of PTZ kindling could be abolished by paeonol in a dose-dependent fashion. Then, the effect of paeonol on lipid peroxidation in the hippocampus was analyzed by examining the content of MDA in the rat hippocampus. As shown in Fig. 4d, compared to the NC group, the MDA content in the hippocampus of the EP group was increased significantly. Paeonol dose-dependently reduced the increase in MDA content induced by PTZ kindling. These results indicate that paeonol has an antioxidative effect in the PTZ-induced epilepsy rat models.

Discussion

Epilepsy is one of the most common and most severe brain disorders in the world. Excessive excitation of the CNS is considered as the main cause of epilepsy [47]. However, the pathogenesis of epilepsy is yet to be fully understood. Increasing evidence has shown that oxidative stress might be a consequence or cause of epilepsy [5]. Oxidative stress can increase the degree of neuronal hyperactivity during epileptic seizures and this can lead to increased susceptibility to epilepsy [7]. It has been reported that the traditional Chinese medicine ferulic acid, which has antioxidant activity, reduces seizures in rats by inhibiting peroxidation [48]. We hereby hypothesize that paeonol, which also has antioxidant properties, can also reduce seizures in rats by inhibiting peroxidation. In order for this hypothesis to be tested, a chronic epilepsy model by PTZ kindling was established. In various methods used for the construction of epilepsy models, PTZ kindling has been widely accepted as an experimental animal model for epileptogenesis [30, 49]. Our results showed that rats in the EP group were fully kindled after a sub-convulsive dose of PTZ (35 mg/kg, i.p.) was administered once every day for 28 days. Paeonol dose-dependently decreased the severity of seizures; furthermore, paeonol also markedly caused an increase in seizure latency and decreased the duration of generalized seizures (Fig. 1b, c). These results show that paeonol is anticonvulsant.

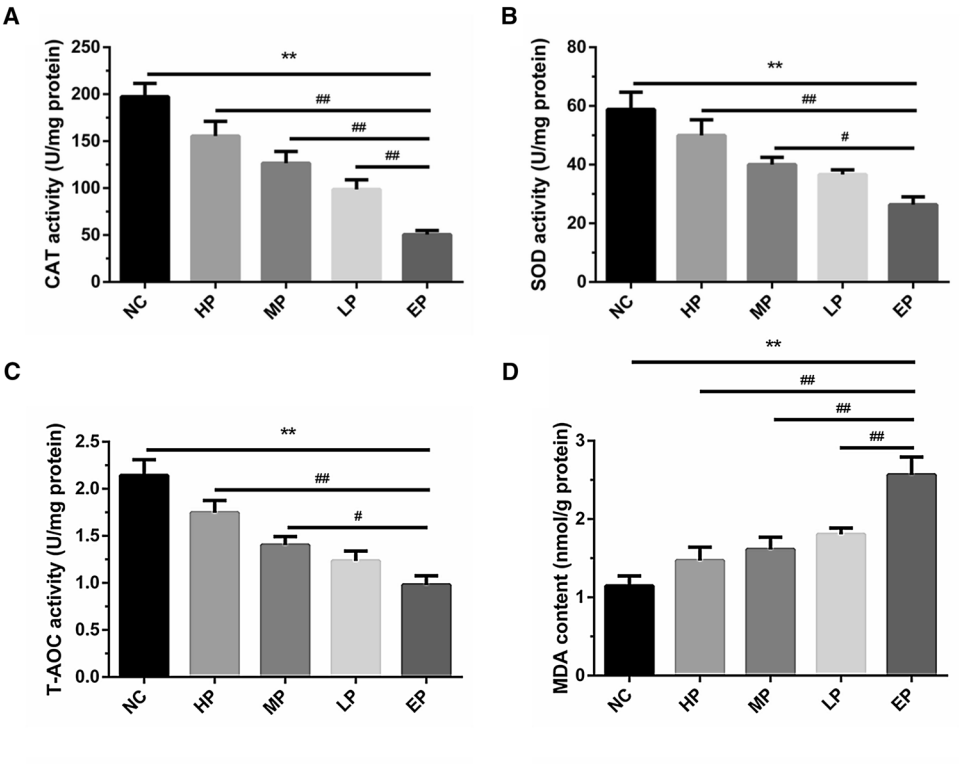

Fig. 4 Effects of paeonol on hippocampal oxidative stress in PTZ-kindled rats. **a** Paeonol increased CAT activity, **b** increased SOD activity, **c** increased T-AOC activity, and **d** reduced MDA content in the hippocampus of rats with epilepsy in a dose-dependent fashion. Data are expressed as the mean ± SEM. n = 3. **P < 0.01 versus NC; #P < 0.05 versus EP; and ##P < 0.01 versus EP

Seizures and the persistence of epilepsy can lead to neuronal damage [22]. The deletion in neurons can increase the severity of seizures [19], and more importantly, neuronal damage is directly related to the onset of epilepsy [20]. The hippocampus has always been a research hotspot for epilepsy, and Pitkänen et al. found that the hippocampi in the brains of epileptic patients eventually decrease in size [17]. Hence, after assessing the anticonvulsant effect of paeonol, we further made an investigation on the effect of paeonol on hippocampal neuronal damage in PTZ-kindled rats by H&E staining and Nissl staining. As shown in Fig. 2, most of the hippocampal neurons in the EP group were damaged, and the number of neurons in the EP group was only 87 ± 7.81/mm^2. However, in rats treated with 20, 40 and 60 mg/kg paeonol, their hippocampal neurons increased to 101 ± 9/mm^2, 127 ± 4.58/mm^2 and 136 ± 10.58/mm^2 respectively. These findings suggest that paeonol can prevent hippocampal neuronal damage induced by PTZ kindling. Apoptosis is known to be crucial for neuronal damage [21, 22]. The apoptotic pathway has become the main focus of most studies on epilepsy-induced cell death [50]. Data from this present study revealed that paeonol protects against PTZ-induced seizures and prevents the damage of hippocampal neurons. Therefore, we propose that paeonol might be protective against PTZ kindling-elicited hippocampal neuronal apoptosis. In order for this hypothesis to be tested, series of experiments were performed for the evaluation of the effect of paeonol on hippocampal neuronal apoptosis in PTZ-kindled rats. Our results revealed that paeonol inhibits neuronal apoptosis in a dose-dependent manner in the hippocampal CA1 areas of PTZ-kindled rats (Fig. 3a, b). Additionally, the expression of the pro-apoptosis factor cleaved caspase-3 was inhibited by paeonol (Fig. 3c–e). These results suggest that paeonol can prevent hippocampal damage elicited by PTZ kindling, exhibiting a neuroprotective effect.

A recent study showed that oxidative stress induced by epilepsy can trigger a chain reaction that causes an extensive damage to neurons in the brain, further aggravating epileptic seizures [16]. In this study, we clearly demonstrated that paeonol exerts neuroprotective and anti-seizure effects. This is because paeonol itself is a natural antioxidant that triggers the expression of some antioxidants such as SOD and CAT [25, 51]. Hence, we also studied the effect of paeonol on oxidative stress elicited by PTZ kindling in order to further study the neuroprotective effect of paeonol. Our results demonstrated that PTZ kindling also increased the MDA content and reduced the CAT, T-AOC and SOD activities in the hippocampus. Paeonol can dose-dependently inhibit the effects of PTZ (Fig. 4). These indicate that paeonol has an antioxidative effect in the PTZ-induced epilepsy rat model, and this effect may play a vital role in the neuroprotective effects of paeonol. However, the precise mechanism by which oxidative stress is regulated by paeonol remains unclear. Therefore, in future studies, further efforts will be made to clarify this issue.

Conclusion

We hereby conclude that paeonol exerts anticonvulsant and neuroprotective effects in PTZ-induced epileptic rats. These effects may be associated with the inhibition of oxidative stress processes.

Acknowledgements The authors of this manuscript are grateful to Jiamusi University in China for providing the facilities for this study.

Funding This research was funded by the Heilongjiang Provincial Department of Education Research Fund (2017-KYYWF-0583) and Heilongjiang University Student Innovation Project (201810222017).

Compliance with Ethical Standards

Conflict of interest We hereby declare no conflicts of interest.

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