

Evaluation of the Anticonvulsant Activities of Gastrodia Elata Bl.- Acorus Tatarinowii Decoction on Experimentally Induced Seizures in Mice

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Abstract

Epilepsy is a serious public health problem in the world. At present, the effect of drug treatment of epilepsy is not satisfactory. Medicinal plants as pharmaceuticals and for healthcare treatments in the management of epilepsy in China for many centuries. Especially, *Gastrodia elata* Bl.-*Acorus tatarinowii*, as a classic and important herb pairs in folk medicine, has been used in folk medicine to control seizures. However, the animal experiment data of its anticonvulsant effect is limited in the literature. The objective of this study was to mainly analyze the anticonvulsant activity of *Gastrodia elata*-*Acorus tatarinowii* (GEAT) decoction in maximal electroshock (MES), pentylenetetrazole (PTZ) and trimercaptopropionic acid (3-MP) induced seizures in mice, providing scientific basis for the treatment of convulsive disorders in traditional medicine. In addition, the improvement effect were examined on seizure severity, anxiety, cognitive dysfunction, inflammation and oxidative stress in PTZ kindled mice. The results showed that GEAT decoction dose-dependently protected mice against MES, 3-MP and PTZ induced acute seizures. Meanwhile, GEAT decoction ameliorated seizure severity, decreased the accumulation of inflammatory mediators TNF- α , IL-1 β , and IL-6, mitigated oxidative stress, as well as alleviated anxious-like behavior and cognitive deficits in PTZ-kindled mice. Our data evidenced that the anticonvulsant properties attributed to GEAT decoction as adjunctive therapy for epileptic patients in folk medicine.

Introduction

Epilepsy is a common and complex neurological disease, affecting more than 65 million people around the world. There are more than 12 million epilepsy patients in China, of which about 8 million are active epilepsy patients, and about 40,000 new epilepsy patients are added every year (Kaur et al. 2015; Johnson, 2019). Epilepsy has become the second largest neurological disease in China, which is second only to headache, especially most of epileptics are children and elderly patients (China Association against Epilepsy, 2015). In addition, at this stage, China has entered population-aging society and accompanied by three-child policy fully implemented, so that the number of epileptics in China will increase for a considerable period of time in the future. Compared with elderly patients with epilepsy, the pathogenesis of epilepsy in childhood are more complex and diverse. More importantly, a considerable part of them belong to intractable epilepsy, which produces a great impact on the cognitive mental, psychological and social functions of childhood with epilepsy (Hwang et al. 2019). Due to the poorly understanding of the pathogenesis to the lack of significant therapeutic regimens, the treatment of epilepsy, especially intractable epilepsy, with low cure rate, lack of treatment methods and scarce drugs has become a difficult problem in the medical field (Löscher and Klein, 2020). Therefore, the research on epilepsy and its treatment has extremely important practical significance and urgency.

At present, the major choice for the treatment of epilepsy in the clinic is still mainly drugs (He et al. 2018). However, the existing clinical antiepileptic drugs (AEDs) are only about 2/3 of epileptic patients achieve a satisfactory seizure control, and the treatment effect is poor or ineffective for the other 1/3 of epileptic patients (He et al. 2018; Kondrat-Wróbel and Łuszczki, 2018; Bai et al. 2019). Currently available antiepileptic drugs can also not prevent the development of epilepsy drug resistance, which is considered

to be a challenge in epilepsy treatment. In addition, although chemical drugs are widely used because of their convenient use, rapid action, high clinical efficacy and good availability, they have side effects due to the need for long-term use, which will damage your health (Golyala and Kwan, 2017; Silva et al. 2019). What might be a solution to the problems facing drug resistance and side effects, those traditional Chinese medicine or botanical drugs that have been used for a long time have gradually drawn attention of drug developers and researchers in recent years (Lin and Hsieh, 2021; Khattak et al., 2021). For example, the natural components cannabidiol extracted from *Cannabis sativa* L. has been approved by FDA for the treatment of Lennox Gastaut syndrome and Dravet syndrome in children with refractory epilepsy (Mitelpunkt et al. 2019).

Traditional Chinese medicine has a long history in the treatment of epilepsy, which was recorded in the classical masterpieces *Inner Canon of Huangdi* (《黄帝内经》) as early as 2200 years ago. In particular, these records revealed the national characteristics and unique advantages of traditional Chinese herbs in the treatment and control seizure in children (Bai et al. 2019). According to traditional medicine accounts, the representative herbal or ethnic medicine widely used in epilepsy treatment in traditional Chinese medicine include *Gastrodia elata*, *Acorus tatarinowii*, *Arisaema heterophyllum* Blume and *Polygala tenuifolia* (Xiao et al. 2015; Zhao et al. 2018; Bai et al. 2019). It has been reported that *G. elata* and *A. tatarinowii* with most prominent effect were used most frequently in the treatment of intractable epilepsy in children (Bao, Huang and Wang, 2012). In addition, Dingxian pill recorded in *Yi Xue Xin Yu* and Dianxian Kang capsule approved by CFDA as well as other commonly used drugs for the treatment of epilepsy mainly contain these two herbs. In these prescriptions, *G. elata* has the function of expelling wind and relieving convulsion, and *A. tatarinowii* make expectoration easy and relieving mental stress. In our previous studies the α -asaronol from *A. tatarinowii* decreased the severity of seizures in mice model of epilepsy, showing a broad spectrum of anticonvulsant activity (He et al. 2018; Jin et al. 2020). Considering the compatibility mechanisms of formulas in traditional Chinese medicine, the current study aimed to evaluate the anticonvulsant and anxiolytic activities of GEAT decoction against seizures using electric or chemical substances induced epilepsy models in mice to support the anticonvulsant properties attributed to the two interactions herbs in traditional clinical practice.

Materials And Methods

Preparation of GEAT Decoction

GEAT decoction in our study was composed of *G. elata* ("Tianma" in Chinese) and *A. tatarinowii* ("Shichangpu" in Chinese). Herbs were purchased from Beijing Tongrentang pharmaceutical chain Co., Ltd. Briefly, *G. elata* (30 g) and *A. tatarinowii* (15 g) were soaked in 500 mL of distilled water under normal temperature for 60 min before being boiled for 0.5 h. Filter and collect the filter liquor, and then add 250 mL of distilled water to the residue and continue to boil for 25 min. Afterwards, combined the filter liquor and then concentrated using a rotary evaporator (model: Heidolph Hei-VAP). The concentrated solution was transfer to a glass bottle, and then reserved at 4 °C in ice box.

Animals

SPF adult Kunming mice (Scxk (Guangdong) 2020-0051) weighing between 24 and 28 g were obtained from the BesTest Bio-Tech Co., Ltd. They were housed in the regulated environmental (23±2 °C; 50±10% humidity, 12 h light/dark cycle) with free access to pellet food and water. All experiments complied following the guidance of management regulations of Guangdong Medical Laboratory Animal Center (Guangdong, China), and carried out in accordance with the NIH guidelines.

All experimental protocols were approved by the Animal Care Committee of Zunyi Medical University (Zhuhai, China) (ZYLS-[2020] No. 2-081).

Drugs and reagents

Pentylentetrazol (PTZ) was purchased from Alfa Aesar, Shanghai, China. Lot: 10180463; Trimercaptopropionic acid (3-MP, Lot: LD50Q10), and reference drug carbamazepine (CBZ, Lot: LLA0P07) was purchased from J&K Scientific Ltd., Beijing, China. Both GEAT decoction and CBZ were dissolved in saline containing 0.5% Poloxamer.

Treatment processes

The mice were divided randomly into 6 groups, 6 mice in each group. The normal control and model control mice received 0.9 % sodium chloride (NaCl) containing 0.5% Poloxamer. The mice of positive control group received CBZ (a most commonly used antiepileptic drugs), at a dose of 50 mg/kg. The mice of treated groups received three different doses of GEAT decoction at 50, 100 and 200 mg/kg, respectively. The different dose of GEAT decoction, normal saline and positive drugs treated to mice in a double-blind way, and the mice were administrated daily doses of NaCl, CBZ or GEAT decoction once by way of stomach for 14 days.

MES test

MES test were carried out according to previously described method (He et al. 2018). Mice were stimulated with a 0.25 s, 64 Hz, 50 mA stimulus by ear-clip electrodes using an electronic generator (Rodent Shocker). Mice was considered "protected" when there were no mice deaths and full hindlimb extension was absent from the MES-stimulated seizures (He et al. 2018; Goerl et al. 2021). Before the drug administration, all mice were preliminarily screened under the electrical stimulation conditions set in the experiment in order to ensure that each experimental mouse has a positive reaction (i.e. full tonic extension) to the MES test. Then, these qualified mice were randomly divided into six groups and administered with double-blind method as per item 2.4 shown. The number of protected mice after electrical stimulation was recorded after drug administered 0.5, 1, 2 and 4 h.

PTZ-induced seizures

PTZ-induced acute seizure model

Mice from each group treated the drug doses described in the experimental groups (0.9 % NaCl, CBZ 50 mg/kg, GEAT decoction 50 mg/kg, GEAT decoction 100 mg/kg, or GEAT decoction 100 mg/kg), for 14 days. After the last dose of the drugs, 85 mg/kg of freshly prepared solution of PTZ was administered subcutaneously to all the mice. Then, the tested mice were placed immediately in a transparent plastic square box for observation for 20 min. Mice was considered “protected” when the duration of generalized tonic seizure was less than 5 s. Latent time for the onset, the number of animals of tonic and clonic seizures as well as the mortality were recorded for 20 min after PTZ injection.

PTZ-induced chronic seizure model

The mice were randomly divided into six groups: normal group, in which each mouse was daily oral administration of NaCl; Model group (NaCl+PTZ), in which each mouse was daily oral administration of NaCl 30 min before administered a subconvulsive dose of PTZ (25 mg/kg); CBZ+PTZ group, in which each mouse were daily treated with CBZ (50 mg/kg) 30 min before PTZ injection; GEAT decoction (50, 100 and 200 mg/kg)+PTZ group, in which each mouse were daily treated with corresponding dose of GEAT decoction 30 min before PTZ injection. All groups were treated for 14 days. The Racine Scale was used to recorded and assess seizure severity of mice within 20 min after PTZ injection. After the last drug administration, except for the normal control group, the mice were observed for 20 min and then all mice were immediately executed. Blood from the heart was collected and centrifuged at 1000 g for 5 min, and collected plasma for standby. The brain tissue were removed and hippocampal was collected and immediately stored at -20 °C. The pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 levels was tested using enzyme-linked immunosorbent assay (ELISA). In addition, the biomarkers of oxidative stress including SOD, MDA, GSH and CAT content in hippocampus was also detected using corresponding assays (Nanjing Jiancheng Reagent Co., Ltd). The performance of the biochemical tests was strictly follow the instructions of the each assay. Besides, all of the mice in the study underwent a battery of behavioral tests, in the following order: high plus maze (10 days after the induction of status seizure) and open field test (10 days after the induction of status seizure).

3-MP-induced seizures

3-MP-induced seizures test were carried out according to previously described methods (He et al. 2018; Bai et al. 2019). Mice grouping and treatment of mice in this test were similar to that of PTZ-induced acute seizure test. 30 min after the last treatment, 60 mg/kg of freshly prepared solution of 3-MP was administered subcutaneously to all the mice. Latent time for the onset, the number of animals of tonic and clonic seizures as well as the mortality were recorded for 20 min after 3-MP injection.

Elevated plus maze

The elevated plus maze (EPM) is a simple method to assess anxiety-like behaviors in mice by estimating contradictory and conflicting behavior between the exploring characteristics of animals to new/different environments and the fear of hanging open arms forms (Guillén-Ruiz et al. 2021). The maze (Shanghai xinruan Information Technology Co., Ltd, XR-XG201) consists of a plus-shaped platform 50 cm above the

floor with two open (35 cm long × 5 cm wide) arms, a central square (5 cm long × 10 wide), and two closed (35 cm long × 5 cm wide × 15 cm height) arms. In this study, the high plus maze test was performed at 10 days when PTZ was administered 2 h to mice in PTZ-induced chronic seizure model. Each mouse was placed in the central area of the maze and monitored for 10 min, and the times and residence time of mice entering the open arm within 10 min were recorded by software monitored during the test.

Open field test

The open field test (OFT) was mainly and commonly used to observe the locomotor activity, exploratory behavior and neuropsychiatric changes of experimental animals in new and different environments (Flores-Fuentes et al. 2021). The opening box inner with the floor divided into 9 equal quadrants (Shanghai, XR-XZ301) is 50 cm in diameter and 40 cm in height. In this study, the OFT was performed at 10 days when PTZ was administered 2 h to mice in PTZ-induced chronic seizure model. The mice were placed in the opening box inner, and the video analysis system was used to analyze the total distance and movement time of mice in the central area within 5 min.

Statistical analysis

Data in this study were presented as mean ± SDE. One-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test was performed to analyze the data, while Chi square test was used for counting data. Values of $p < 0.05$ were considered statistically significant. The statistical analyses were conducted using Prism 5 software.

Results

MES test

The evaluation of the effects of GEAT decoction on MES test in mice was shown in Table 1. As can be seen from Table 1, the orally administration of GEAT decoction displayed significantly protected effect against convulsion caused by electrical stimulation in a dose-dependently manner at 50, 100, 200 mg/kg, which offered a maximum protections against generalized seizures (tonic hind limb extension) in 1h after the last drug administration. Specifically, GEAT decoction at 50, 100 and 200 mg/kg provided 33.3, 66.6, 83.3 % protections against seizures in MES test, respectively. The protected number of animals reduced in 4 h after the last drug administration, which may be related to the metabolism and excretion of active ingredients.

PTZ-induced seizures

In PTZ induced acute seizure model, compared to the model group, GEAT decoction exhibited a significant delay in the latency of seizures at the tested dose of 100 and 200 mg/kg with a mean onset latency of 248.8 and 259.5 s, respectively (Fig.1A). In addition, GEAT decoction at 100 and 200 mg/kg offered 50.0, 75.0, and 83.3, 75.0% protection against PTZ-induced tonic seizure and mortality, while CBZ

at 50 mg/kg produced same proportion of protective activity as GEAT decoction at 200 mg/kg (Fig.1B). In contrast, the GEAT decoction in all experimental groups did not develop clonic seizures. In PTZ induced chronic seizure model, as shown in Fig.2, injection of PTZ to mice resulted in degrees of seizure severity and resulted in more complex seizures, while treatment with GEAT decoction dose-dependently produced a retardation in the seizure scores for all the treatment days.

3-MP-induced seizures

As shown in Fig. 1C, regard to the latency to tonic seizures, an obvious decrease in in the NaCl group was observed. Oral administration of GEAT decoction resulted in a different degrees of extension on onset latency. Especially, GEAT decoction at 200 mg/kg significantly increased onset latency (278.5 ± 30.7 s) of seizures compared to that of model group (200.4 ± 11.9 s). Besides, pretreatment GEAT decoction at 50, 100 and 200 mg/kg resulted in a 33.3, 67.7, and 67.7% protection respectively against tonic seizures and 50.0, 67.7, 83.3% protection respectively against death in 3-MP-induced seizures test. No obvious protection was observed in all GEAT decoction treatment groups on the clonic seizure of 3 MP-induced convulsion (Fig.1D). Taken together, GEAT decoction reduced the severity of convulsive activity and also prevent seizures.

Effects of GEAT decoction on Pro-inflammatory cytokines

As shown in Fig.3, results showed elevated pro-inflammatory cytokines including IL-6, IL-1 β and TNF- α levels of in the model group as compared to normal group both in the hippocampus and serum of PTZ-induced mice. In detail, in the hippocampus, administration of GEAT decoction at the dose of 50, 100 and 200 mg/kg produced a reduction of IL-6, IL-1 β and TNF- α levels in different degrees (Fig.1 A, B, C). Especially, compared with model (saline, PTZ existence) group, treatment with GEAT decoction dramatically reversed the effect of PTZ on IL-6, IL-1 β and TNF- α levels at the dose of 200 mg/kg ($p < 0.01$). Whereas, regarding IL-6 and TNF- α , treated GEAT decoction (100 mg/kg) and CBZ (50 mg/kg) showed lower levels than that of mice in model (saline, PTZ existence) group, but both of them did not demonstrate a protective effect regarding IL-1 β when compared to the model group. In addition, a significant difference in IL-6, IL-1 β and TNF- α levels between GEAT decoction 50 mg/kg groups and the PTZ group were not observed in our study. In the serum tests (Fig. 3 D, E, F), GEAT decoction at 50, 100 and 200 mg/kg produced a significant reduction in IL-1 β level compared to the model (saline, PTZ existence) group. Changes in IL-6 and TNF- α levels are basically consistent with those in hippocampus. For the CBZ, no significant difference was observed among IL-6, IL-1 β and TNF- α levels.

Effects of GEAT decoction on Oxidative Stress Parameters

In terms of quantification of oxidative stress parameters, in hippocampus, all treatments presented higher activity of SOD when compared to the model (saline, PTZ existence) group. Particularly, pre-treatment GEAT decoction at 200 mg/kg significantly elevated SOD activity in the hippocampus compared to the model group ($p < 0.01$) (Fig. 4A). Regard to the CAT, a significant decrease in the model group was observed when compared to the normal group ($p < 0.01$). Administration of GEAT

decoction at the dose of 200 mg/kg produced a better elevation effect of CAT activity in hippocampal of mice in comparison with model group ($p < 0.01$) (Fig. 4B). In addition, results showed an enhanced production of MDA as well as a reduced production of GAH in hippocampal in PTZ induced mice. Interestingly, GEAT decoction treated reversed the changes in varying degrees (Fig. 4C, and D). However, the levels of these oxidative stress parameters in animals treated with CBZ was not significant changed in comparison with model group.

Elevated plus maze

It has been proposed that depression and anxiety symptoms are frequent occurrence in epilepsy, therefore anxiety-like behavior was evaluated in this study. As shown in Fig.5 A and B, the time in the open arms of EPM and the percentage of entries of mice into the open arms were evaluated. The results indicated that PTZ-induced seizures mice displayed evidently anxiety-like behavior compared with mice in normal group (Fig. 5A, B). Luckily, pre-treated with GEAT decoction reduced anxiety-like behavior of mice in the EPM test in varying degrees. In particular, the percent time spent on the open arms and the percentage of entries into the open arms showed a prominently increased when pre-treated with GEAT decoction at the dose of 200 mg/kg compared with the model group. The behavior trace of GEAT decoction on subcutaneous PTZ-induced mice in EPM are shown in Fig. 6A.

Open field test

Likewise, the time spent and distance in the central areas were used as an anxiety-like indicator determined in OFT. As shown in Fig.5 C, the time in the central area for mice in normal group was 34.0 ± 2.7 s and 15.3 ± 3.6 s for mice in model group. For GEAT decoction at dose of 50, 100 and 200 mg/kg, the time spent in the central area was 20.0 ± 3.9 , 25.8 ± 3.3 , and 34.3 ± 8.9 s, respectively. As shown in Fig. 5D, GEAT decoction at dose of 100 and 200 mg/kg significantly increased the total walking distance in the central areas compared that of mice in model group. However, these indicators in CBZ and GEAT decoction (50 mg/kg) did not have significant differences in comparison with model group in the same period of treatment, as shown in Fig. 5C and D. The behavior trace of GEAT decoction on subcutaneous PTZ-induced mice in OPT are shown in Fig. 6B.

Discussion

Epilepsy induced by many reasons is a most common chronic brain disease, affecting about 65 million people worldwide (Johnson, 2019). Traditional Chinese medicine with a remarkable effect, few adverse reactions as well as rich clinical validation has been commonly used in China for a long history in treating epilepsy (Zhao et al. 2018). So far, 14 kinds of traditional Chinese medicine prescriptions or preparations for the treatment of various epilepsy, especially intractable epilepsy has included in the 2020 edition of Chinese Pharmacopoeia. According to statistics and analysis, the commonly used and clinically effective drug pairs "*G. elata*-*A. tatarinowii*" are the most representative clinical valuable drug pair in treatment of epilepsy and seizures in folk medicine in China (Bao, Huang and Wang, 2012; Zhao et al. 2018; Bai et al. 2019). There is no doubt that the effectiveness of the compatibility of this classic drug

pairs has been verified in clinical practice for a long time, but modern systematic pharmacological evaluation and mechanism research are relatively lacking. Therefore, in this study, several classical animal models of epilepsy was performed to evaluate antiepileptic effect and related mechanism of GEAT decoction. Additionally, the elevated plus-maze and open field tests were performed to examine the impact of GEAT decoction on anxiety-like behavior of PTZ-induced mice.

In this study, we firstly analyzed the anticonvulsant effects of GEAT decoction at different dosages on three different seizure models, the MES, 3-MP and PTZ tests. The results demonstrated that mice treated with GEAT decoction (50, 100, 200 mg/kg, po.) delayed an anticonvulsant activity in the MES, PTZ and 3-MP induced seizure models. Especially, GEAT decoction at 200 mg/kg delayed the onset latency and prevented the severity of PTZ-induced seizures, indicating its good anticonvulsant effect. In addition, similar dosages of GEAT decoction also performed well in MES and 3-MP seizure models. Therefore, this study provide proof of concept that GEAT decoction are pharmacologically active *in vivo* with a dose-dependent manner, which possessed a therapeutic potential to prevention and control seizures.

Evidence suggests that inflammation strengthen excitability of neuronal, and consequently prolongation of seizures and initiation cognitive dysfunctions, while alleviation of inflammation displayed anticonvulsant effects in intractable epilepsy (Kaur et al. 2015). Inflammatory mediators induced by cytokines may be not only a complication of epilepsy, but also an internal inducement of some epilepsy diseases. For example, a large number of inflammatory mediators, including IL-1 β , IL-6 and TNF- α were detected in the brain tissue of patients with intractable epilepsy (temporal lobe epilepsy and epilepsy caused by cortical dysplasia) (Bauer et al. 2017; Elgarhi et al. 2020; de Lima Rosa et al. 2021). In our study, we found that PTZ induced generalized seizures and elevated IL-1 β , IL-6 and TNF- α levels in kindled mice blood and brain. Gratifying, in this study the administration of GEAT decoction dependently reversed the increase of inflammatory cytokines IL-1 β , IL-6, and TNF- α levels in the serum and brain tissues of PTZ-induced seizures mice. Therefore, GEAT decoction may have potential value in the management of inflammatory diseases accompanied by epilepsy.

Studies have found that epilepsy is also closely related to oxidative stress and mitochondrial dysfunction (Chindo et al. 2021). The production of free radicals plays an important role in biological function regulation, cell structure damage and the pathogenesis of neurodegenerative diseases of the central nervous system. In particular, the increase in the synthesis and release of reactive oxygen species is closely related to the oxidation potential of the central nervous system (Frantz et al. 2021). Thus, the scavenging of hydroxyl radical, peroxy radical, and superoxide radical, as well as stimulating the synthesis of superoxide dismutase and glutathione peroxidase are very beneficial to the treatment of epilepsy. In the pathogenesis of chronic epilepsy, a large number of superoxide anions can be produced, and the endogenous antioxidant enzymes SOD, GSH, GSR and cat are rapidly consumed, resulting in the production of a large number of toxic lipid peroxide increased and induced the oxidative stress. In addition, in PTZ-induced kindling in mice, it was found that reactive oxygen species was activated, and its production agrees with decrease in antioxidant related enzymes (Frantz et al. 2017; Chindo et al. 2021). In this study, we found that treated with GEAT decoction reduced MDA levels in PTZ-kindled mouse

hippocampus, while increased CAT and SOD activities, as well as increased GSH levels when compared with that of PTZ-kindled mice. In other words, GEAT decoction improved the antioxidant capacity of brain tissue, reduced lipid peroxidation and peroxidation damage in mouse brain, thus corroborating the therapeutic benefits of GEAT decoction in the management of epilepsy.

It has been proposed that cognitive impairment, anxiety and depression are common accompaniment neurological of chronic epilepsy (Chindo et al. 2021). Patients with long term seizures can cause diversified degrees of brain injury and abnormal emotional during seizures (Sharma et al. 2021). More seriously, most cognitive impairment occurs after recurrent seizures or status epileptics, and the frequency, duration and severity of seizures are closely associated with the severity of cognitive impairment (Shuman et al. 2020). Thus, in our study, we explored the effects of GEAT decoction on anxiety and cognitive dysfunction in PTZ-induced mice using OFT and EPM tests. Data have shown that the time spent in the central areas of OFT and the time in the open arms of EPM were decreased in PTZ induced mice, which means a state of avoiding fear and anxiety behavior. Whereas, the GEAT decoction treatment provoked a significant increase of frequency entries and time spent in in the open arms in EPM tests. On the other hand, similar and positive effects were observed in PTZ and GEAT decoction treated mice in OFT compared with PTZ only. The results fully demonstrated that GEAT decoction evidently improved anxiety-like behavior and cognitive impairment in PTZ induced seizures in mice, which supported the traditional records that the couplet medicinals of *G. elata* and *A.tatarinowii* relieving convulsion and mental stress.

Conclusion

GEAT decoction showed an outstanding protected activities in MES, PTZ and 3-MP induced models of seizures. Especially, GEAT decoction have a promising activity in reducing inflammation and oxidative stress, as well as improving anxiety behavior in PTZ kindled mice, confirming that the potential efficacy of GEAT decoction in the prevention and treatment of epilepsy. Thus, GEAT decoction can be used to inhibit neuroinflammation, suppress oxidative damage and prevent cognitive deficits in chronic epilepsy mice. Further experimental and clinical studies could provide the deep insight into the best compatibility proportion, clinical effect and mechanistic pathway involved in the management of epileptic seizures by GEAT decoction.

Declarations

Author contributions

The manuscript was completed through contributions of all the listed authors. H-XR, Y-XF and S-Y designed and performed the experiments, Y-XF and S-Y analyzed the data. H-XR wrote and helped to modify the paper. All authors read and approved the final version of the manuscript.

Conflicts of interest statement

The authors declare that there is no conflict of interest.

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Provision of Data

Data will be provided upon a reasonable request

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Tables

Table 1. Anti-MES activity of GEAT decoction and CBZ administered orally to mice for 14 days.

| Group | Dose (mg/kg) | MES (n ₁ /n ₂) ^a | | | |
|----------------|--------------|--|------------------|------------------|------------------|
| | | 0.5 h ^b | 1 h ^b | 2 h ^b | 4 h ^b |
| NS | - | 0/6 | 0/6 | 0/6 | 0/6 |
| CBZ | 50 | 5/6 | 5/6 | 3/6 | 2/6 |
| GEAT decoction | 50 | 1/6 | 2/6 | 2/6 | 0/6 |
| | 100 | 3/6 | 4/6 | 3/6 | 1/6 |
| | 200 | 5/6 | 5/6 | 4/6 | 3/6 |

^a No. of mice protected/no. of mice tested. ^b Time after the last drug administration.

Figures

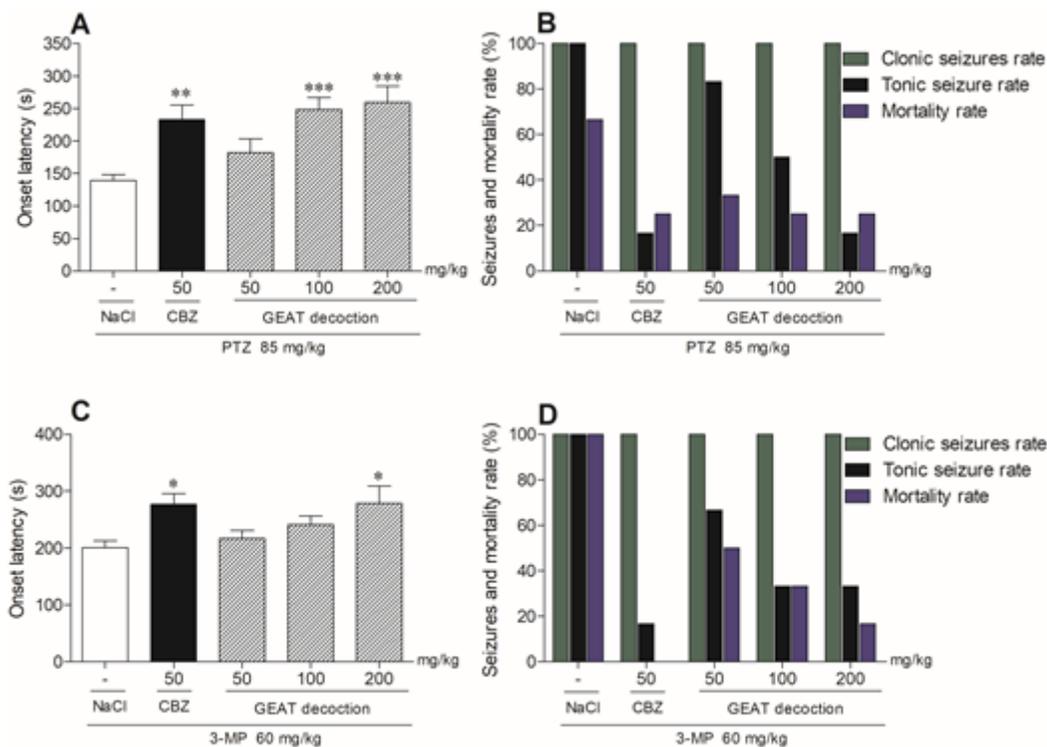


Figure 1

Effect of GEAT decoction and CBZ on subcutaneous PTZ and 3-MP induced seizures in mice. A: the onset latency in *sc*-PTZ-induced seizure; B: the clonic seizures, tonic seizures, and mortality rate in *sc*-PTZ induced seizure; C: the onset latency in *sc*-3-MP-induced seizure; and D: the clonic seizures, tonic seizures, and mortality rate in *sc*-3-MP-induced seizure. Data presented as mean \pm SEM, $n=6$ mouse per group. Statistical analyses were implemented using one-way ANOVA test. * $p<0.05$, ** $p<0.01$, *** $p<0.01$ compared with saline group.

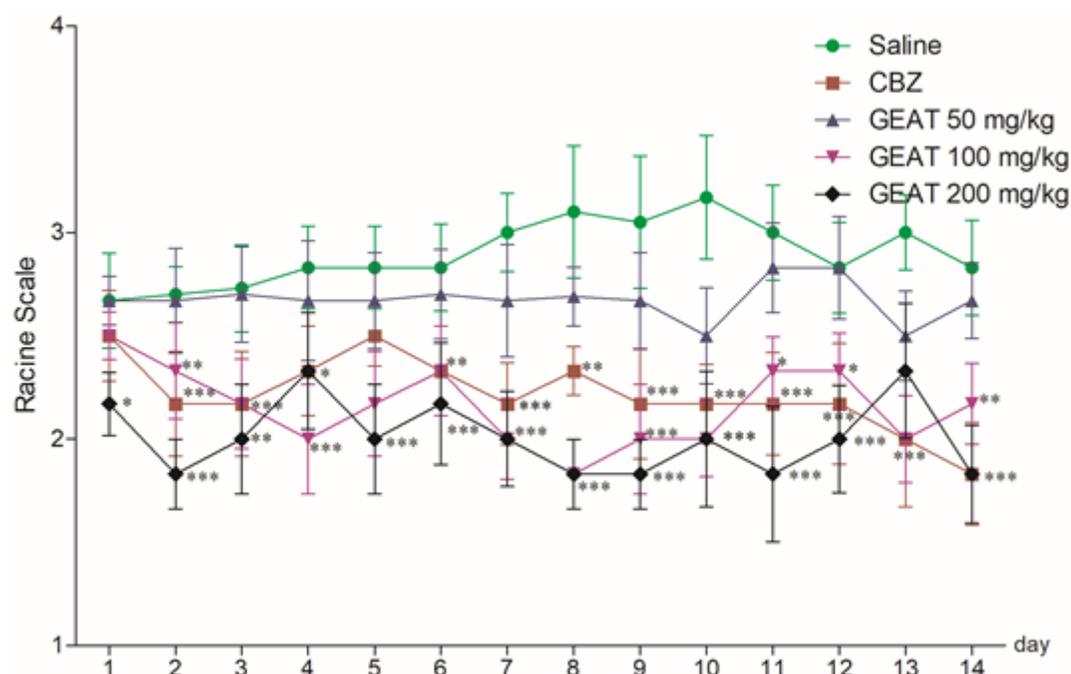


Figure 2

Effect of GEAT decoction and CBZ on subcutaneous PTZ-induced seizure in mice for 14 consecutive days. Racine Scale: 0, no response; 1, twitch of ears and face; 2, myoclonic seizures without rearing; 3, myoclonic seizures along with rearing; 4, rolling into one side with colic-tonic seizures; and 5, upside down along with generalized clonic-tonic seizures. Data expressed as Mean \pm SEM, n=6 mouse per group. Statistical analyses were implemented using one-way ANOVA test. * p <0.05, ** p <0.01, *** p <0.001 compared with saline group on the same day.

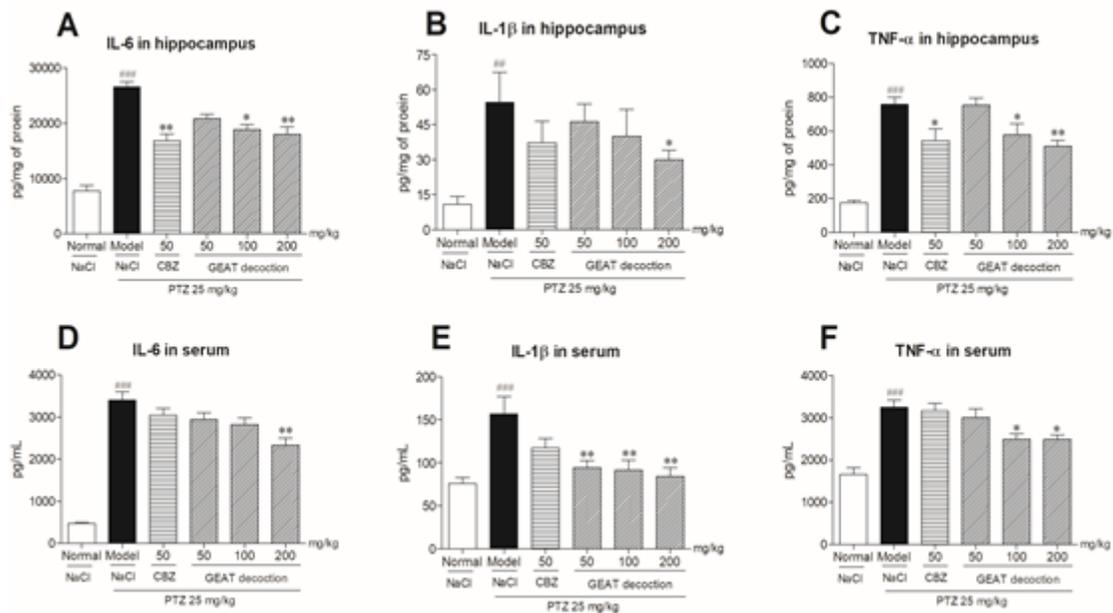


Figure 3

Effect of GEAT decoction and CBZ on pro-inflammatory cytokines in the hippocampus and serum of subcutaneous PTZ-induced mice. A, IL-6 in the hippocampus; B, IL-1 β in the hippocampus; C, TNF- α in the hippocampus; D, IL-6 in the serum; E, IL-1 β in the serum; F, TNF- α in the serum; Data presented as Mean \pm SEM, n=5 mouse per group. Statistical analyses were implemented using one-way ANOVA test. * p <0.05, ** p <0.01 compared with model (saline, PTZ existence) group. # p <0.05, ## p <0.01, ### p <0.001 compared with normal (saline, PTZ absence) group.

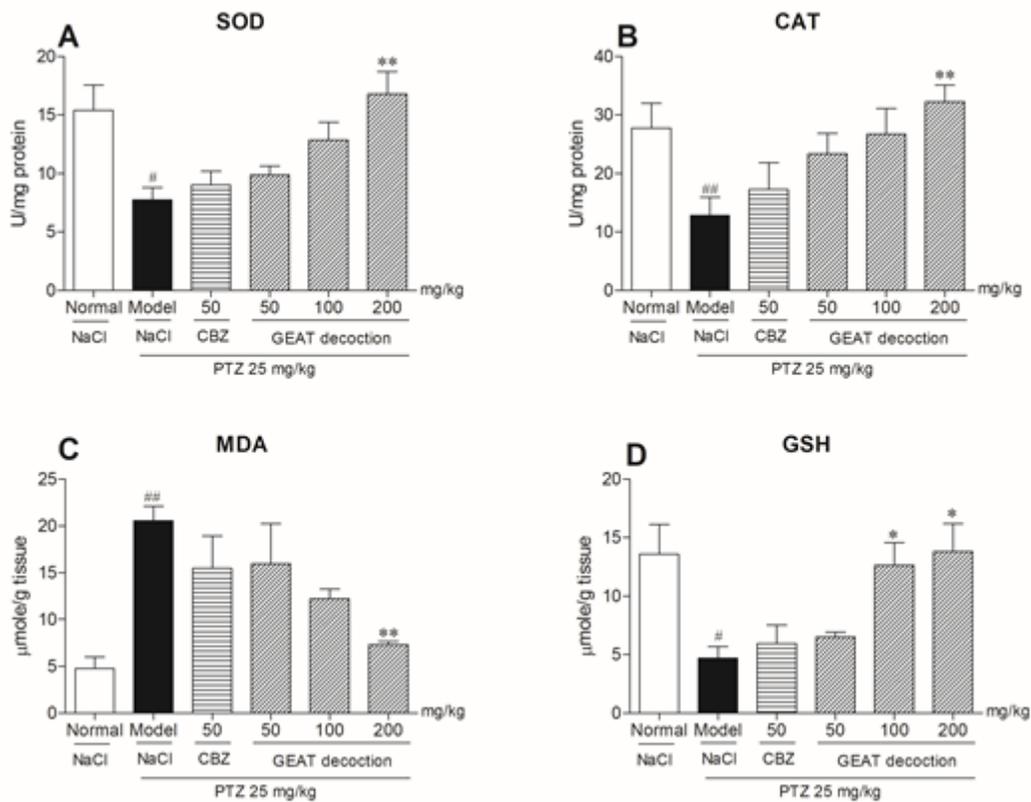


Figure 4

Effect of GEAT decoction and CBZ on levels of main oxidative stress markers in the hippocampus of subcutaneous PTZ-induced mice in 14 days. A, SOD activity; B, CAT activity; C, MDA levels; D, GSH levels. Data presented as Mean \pm SEM, n=5 mouse per group. Statistical analyses were implemented using one-way ANOVA test. * $p < 0.05$, ** $p < 0.01$ compared with model (saline, PTZ existence) group. # $p < 0.05$, ## $p < 0.01$, compared with normal (saline, PTZ absence) group.

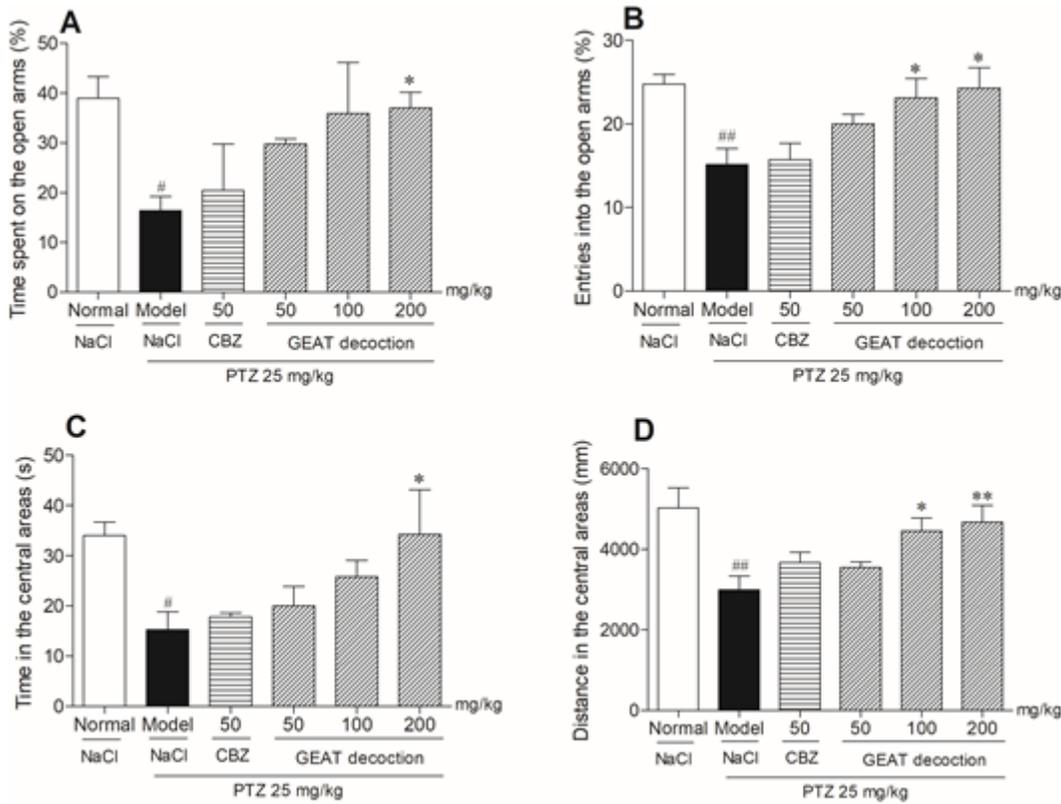


Figure 5

Effect of GEAT decoction and CBZ on subcutaneous PTZ-induced mice in elevated plus maze and open field tests. A, the percent time spent on the open arms in elevated plus maze test; B, the percentage of entries into the open arms in elevated plus maze test; C, time in the central areas in open field test; D, distance in the central areas in open field test. Statistical analyses were implemented using one-way ANOVA test and Chi square test. * $p < 0.05$, ** $p < 0.01$ compared with model (saline, PTZ existence) group. # $p < 0.05$, ## $p < 0.01$, compared with normal (saline, PTZ absence) group.

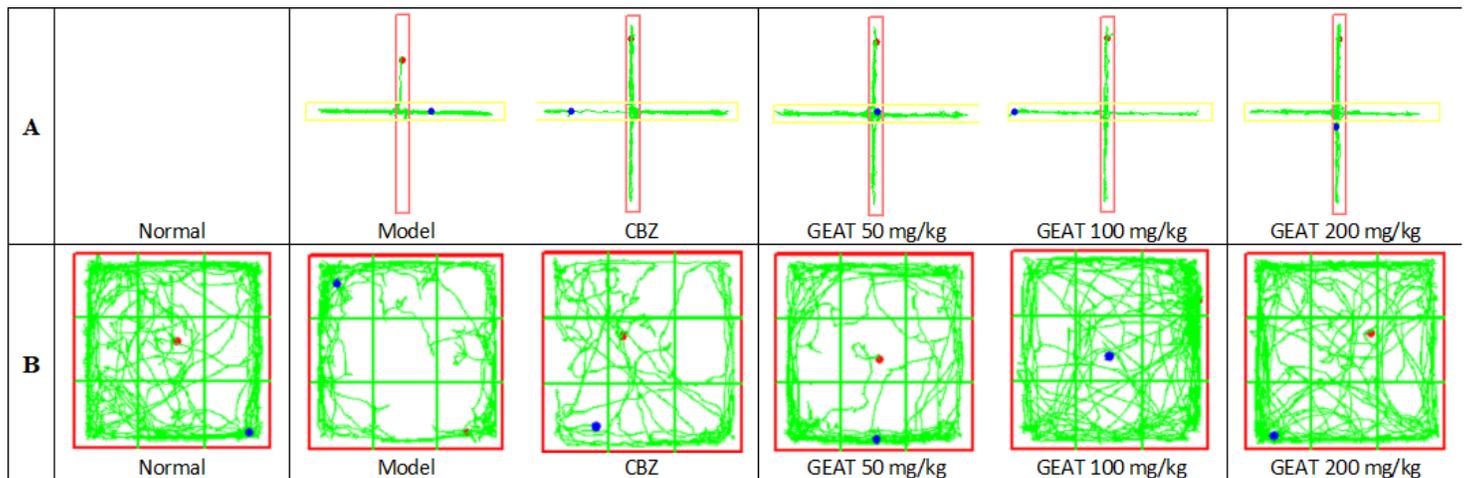


Figure 6

Behavior trace of GEAT decoction and CBZ on subcutaneous PTZ-induced mice in elevated plus maze and open field tests. A, EPM; B, OFT; a, open arms; b, closed arms.