Chemical composition and anticonvulsant activities of herb pair of Gastrodia elata Blume-Acorus tatarinowii Schott decoction on experimentally induced seizures in mice

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Abstract

Epilepsy is a serious public health problem in the world. At present, over 30% of affected patients remain refractory to currently available treatment. Medicinal plants as pharmaceuticals and healthcare treatments have been frequently used in the management of epilepsy in China for many centuries. *Gastrodia elata-Acous tatarinowii* (GEAT), as a classic and most commonly used herb pair in traditional Chinese medicine (TCM), has been employed to control seizures for thousands of years. However, the animal experiment data on its anticonvulsant effect is limited in the literature. Thus, this study aimed to reveal the therapeutic actions of GEAT decoction against seizures in mice. UHPLC-MS/MS was performed to analyze the chemical components of GEAT decoction. The mice were given GEAT decoction for 7 days, and MES, PTZ, and 3-MP injection was given 30 min after the last administration. Video monitoring was performed for comparisons. In addition, the PTZ-induced kindling models were conducted to investigate the seizure severity, anxiety and cognitive profile, inflammation, and oxidative stress parameters in mice. The results showed that GEAT decoction dose-dependently protected mice against MES, 3-MP, and PTZ-induced acute seizures. Furthermore, GEAT decoction significantly ameliorated seizure severity, decreased the accumulation of inflammatory mediators TNF-α, IL-1β, and IL-6, mitigated oxidative stress, as well as alleviated anxious-like behavior and cognitive deficits in PTZ-kindled mice. These results suggest that GEAT decoction possesses certain anticonvulsant properties, which might be clinically useful as phytotherapy alone or as an adjunct therapy for the prevention and treatment of seizures and epilepsy.

1. Introduction

Epilepsy is a common and complex neurological disease, affecting more than 70 million people around the world. Epilepsy has become a major concern in developing countries which affects approximately 80% of people (Khattak et al. 2021). Statistics show that epilepsy affects an estimated 12 million people in India (Uthara, Basheer and Anil 2017). In China, it is estimated that there are more than 12 million patients with epilepsy, of which about 8 million patients with active epilepsy are getting the drug, and about 40, 0000 new epilepsy patients are added every year (China Association against Epilepsy 2015; Kaur et al. 2015; Johnson 2019). The prevalence and incidence of epilepsy are slightly higher in males than that in females (Beghi 2020). In addition, its incidence has a bimodal distribution with the highest risk in children and the elderly (Thijs et al. 2019). With the aging of the population increasing dramatically, the prevalence of epilepsy diseases is increasing rapidly in many countries in the future. Focal seizures are more common than generalized seizures both in children and in the elderly (Beghi 2020). Compared with elderly patients with epilepsy, the pathogenesis of epilepsy in childhood is more complex and diverse. There is a considerable part of childhood with epilepsy progressing to intractable epilepsy. Extensive research has demonstrated that intractable epilepsy is caused by numerous precipitating factors and can produce a great impact on the cognitive mental, psychological, and social functions of childhood with epilepsy (Hwang et al. 2019). Seizures might be the prominent feature in inherited metabolic disease (Hundallah and Tabarki 2021). Due to the poor understanding of the pathogenesis and the lack of significant therapeutic regimens, a difficult problem exists, therefore, for more effective therapies or alternative approaches to effective treatment-intractable epilepsy management (Löscher and Klein 2020). Therefore, the research on epilepsy and its treatment have extremely important practical significance and urgency.

At present, the major choice for the treatment of epilepsy in the clinic still relies on drugs (He et al. 2018). Despite the existing clinical antiepileptic drugs (AEDs) producing satisfactory seizure control for about 2/3 of epileptic patients, these available AEDs fail to control epileptic activity in about 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, current available AEDs only target symptoms but cannot prevent the underlying natural epileptogenesis and prognosis of epilepsy. With the worldwide and long-term use of these AEDs, their adverse effects have gradually emerged (Golyala and Kwan 2017; Silva et al. 2019; Li, Sun and Wang 2020). For example, some common side effects including dizziness, headache, drowsiness, and attention deficit disorder, as well as cardiovascular abnormalities, hematotoxicity and heart damage, endocrine disorders, and suicide risk, etc. have been emphasized (Li, Sun and Wang 2020). Besides, large doses of antiepileptic drugs using may have harmful effects on intellectual development or language function (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 the 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).

TCM has a long history in the treatment of epilepsy, which was recorded in the classical masterpieces *Inner Canon of Huangdi* (, in Chinese) 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 of seizures in children (Bai et al. 2019). From the classic and traditional medicine point of view, the representative herbal or ethnic medicine widely used for treating seizures and epilepsy in TCM mainly included *G. elata*, *A. 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 the most prominent effect used most frequently in the treatment of intractable epilepsy in children (Bao, Huang and Wang 2012). In addition, the 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* makes expectoration easy and relieves mental stress. In our previous studies, the α-asaronol from *A. tatarinowii* decreased the severity of seizures in mice models of epilepsy, showing a broad spectrum of anticonvulsant activity (He et al. 2018; Jin et al. 2020). Considering the compatibility mechanisms of formulas in TCM, the current study aimed to evaluate the anticonvulsant activities of GEAT decoction against seizures using electric and chemical substances-induced epilepsy models in mice. Furthermore, the regulatory effect of GEAT decoction on seizure severity, cognitive function, inflammation, and oxidative stress in PTZ-kindling mice was also assessed to support the anticonvulsant properties attributed to the two interactions herbs in traditional clinical practice.

2. Materials And Methods

2.1. 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. Afterward, combined the filter liquor and then concentrated using a rotary evaporator

(model: Heidolph Hei-VAP). The concentrated solution was transferred to a glass bottle and then reserved at 4°C in the ice box.

2.2. UHPLC-MS/MS analysis of GEAT decoction

UHPLC-MS/MS (Thermo Fisher Scientific, USA) equipped with an electrospray ionization (ESI) source was applied for the qualitative analysis of phytochemical compounds from GEAT decoction.

2.2.1 Chromatographic condition

Chromatography was performed on a Zorbax Eclipse C_{18} (1.8 μm×2.1 mm×100 mm). The mobile phase A consisted of 0.1% formic acid, and the mobile phase B was acetonitrile. Analysis accomplished by using a gradient elution of 5% B at 0–2 min, 5–30% B at 2–6 min, 30% B at 6–7 min, 30–78% B at 7–12 min, 78% B at 12–14 min, 78–95% B at 14–17 min, 95% B at 17–20 min, 95–5% B at 20–21 min, and 5% B at 21–25 min. The flow rate was 0.3 mL/min. The column temperature was set at 30°C. The injection volume of the sample was 2 μL.

2.2.2 Mass spectrometry condition

The Ion mode was set to positive and negative mode. MS conditions were: Spray voltages: 3.5 kV and – 3.5 kV; Capillary temperature: 330°C; Sheath gas: 45 arbs; Aux gas: 15 arb and probe heater temperature: 325°C. Scan mode was full ms. Scanning mode: full scan (Full Scan, m/z 100 ~ 1500) and data-dependent mass spectrometry (dd-MS2, TopN = 10); resolution: 120,000 (MS1) & 60,000 (MS2). Collision Mode: High Energy Collision Dissociation (HCD). Compound Discoverer 3.3 was used for data analysis.

2.3. Animals

SPF adult male 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 environment (23 ± 2 ℃; 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 were carried out by the NIH guidelines. All experimental protocols were approved by the Animal Care Committee of Zunyi Medical University (Zhuhai, China) (ZYLS-[2020] No. 2–081).

2.4. Drugs and reagents

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

2.5. Treatment processes

2.5.1. Acute seizures test

The mice were divided randomly into five groups, with 12 mice in each group. The model control mice received 0.9% sodium chloride (NaCl) containing 0.5% Poloxamer. The mice of the positive control group received CBZ (a most commonly used antiepileptic drug), at a dose of 50 mg/kg. The mice of the treated groups received three different doses of GEAT decoction at 50, 100, and 200 mg/kg, respectively. The different doses of GEAT decoction, normal saline, and positive drugs were treated to mice in a double-blind way, and the mice were orally administrated doses of NaCl, CBZ, or GEAT decoction once a day for consecutive 7 days.

2.5.2. Chronic seizures test

The mice were divided randomly into 6 groups, 12 mice in each group. The different doses of GEAT decoction, normal saline, and positive drugs treated in mice in a double-blind way, and the mice were orally administrated test doses of NaCl, CBZ, or GEAT decoction once a day for consecutive 28 days. The mice in the control group received 0.9% sodium chloride (NaCl) containing 0.5% Poloxamer. Except for the mice in the normal control group, all the mice in the other group were administered PTZ in a dose of 35 mg/kg for 14 days on alternate days. The mice in the control group received normal saline injections.

2.6. MES test

MES tests were carried out according to the previously described method (He et al. 2018; Krall et al. 1978). Thirty mice were randomly divided into five groups and administered with the double-blind method as mentioned earlier. 0.5, 1, 2, and 4 h after the last administration, mice were stimulated with a 0.25 s, 64 Hz, 50 mA stimulus by ear-clip electrodes using an electronic generator (Rodent Shocker). Mice were considered “protected” when full hind-limb tonic extension (HLTE) was absent from them (He et al. 2018; Goerl et al. 2021). The number of mice protected from HLTE induced by electrical stimulation was recorded after the last drug was administered 0.5, 1, 2, and 4 h.

2.7. PTZ-induced seizures test

2.7.1. Acute PTZ-induced mouse 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 200 mg/kg), for 7 days. One hour after the last dose, 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 were considered “protected” when there is the absence of a single 5-sec episode of clonic spasms (Krall et al., 1978). Latent time for the onset, the number of animals of generalized tonic-clonic seizures (GTCS), clonic seizures (CS) as well as mortality were recorded for 20 min after PTZ injection. In addition, seizure severity was evaluated primarily based on the Racine scale with minor modifications. Briefly, stage 0: no response; 1: facial and ears twitching; stage 2: hyperactivity, vibrissae twitching, and myoclonic jerks; stage 3: unilateral forelimb clonus; stage 4: clonic convulsions with preservation of righting reflex; stage 5: generalized GTCS loss of postural control (Zhang et al. 2019).

2.7.2. Chronic PTZ-induced kindling mice 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 sub convulsive dose of PTZ (25 mg/kg); CBZ + PTZ group, in which each mouse was 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 was daily treated with a corresponding dose of GEAT decoction 30 min before PTZ injection. All groups were treated for 28 days. The Racine Scale was used to record and assess the seizure severity of mice within 20 min after each PTZ injection (Zhang et al. 2019). 24 h after completion of the kindling test, the behavioral assessment models were carried out to evaluate the ameliorative effects of GEAT decoction on anxiety, and cognitive function in the kindled mice. After completion of the behavioral testing, 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 was removed and the hippocampal was collected and immediately stored at − 20 ºC. The pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 levels were tested using enzyme-linked immunosorbent assay (ELISA, Elabscience Biotechnology Co., Ltd) with the sensitivity of 18.75 pg/mL, 4.688 pg/mL, and 9.375 pg/mL, respectively. In addition, the biomarkers of oxidative stress including SOD, MDA, GSH, and CAT content in the hippocampus was also detected using corresponding assays (Nanjing Jiancheng Reagent Co., Ltd). The performance of the biochemical tests strictly followed the instructions of each assay. Besides, all of the mice in the study underwent a battery of behavioral tests, in the following order: high plus maze (29th day after the induction of status seizure) and open field test (30th day after the induction of status seizure).

2.8. 3-MP-induced seizures test

3-MP-induced seizures tests were carried out according to previously described methods (He et al. 2018; Bai et al. 2019). Mice grouping and treatment in this test were similar to that of the 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. The latency to myoclonic jerks was noted along with the occurrence of generalized tonic seizures and CS. The mice's death was also monitored. The observation period was 20 min for an individual mouse.

2.9. Elevated plus maze test

The elevated plus maze (EPM) test 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. Based on a standard type pre-experiment, the high plus maze test was performed on 29 th day when PTZ was administered 24 h later 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 spent and residence time of mice entering the open arm within 10 min was recorded by software monitored during the test.

2.10. 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. Based on a standard-type pre-experiment, the OFT was performed on the 30th day when PTZ was administered 48 h later to mice in the 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 time spent on mice entries into the central zone within 5 min.

2.11. Statistical analysis

Data in this study were presented as mean ± SEM. One-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test was performed to analyze the data. Kruskal-Wallis ANOVA was applied for Racine score. The Chi-square test was used for counting data. Values of $P < 0.05$ were considered statistically significant. The statistical analyses were conducted using GraphPad Prism 7 software.

3. Results

3.1. Chemical analysis of the GEAT decoction

The UHPLC-MS/MS technology was carried out for the preliminary analysis of GEAT decoction. The total ion chromatogram (TIC) was extracted as demonstrated in Fig. 1. A total of 174 components were identified from GEAT decoction using a broad targeted metabolomics approach based on UHPLC-MS/MS. Among them, the main structural types are benzene and substituted derivatives, carboxylic acids and derivatives, cinnamic acids and derivatives, furanoid lignans, prenol lipids, phenol esters, pyridines and derivatives, Fatty Acyls, etc. A total of 20 components were more than 1% relative, as shown in Table 1.

Table 1
Chemical composition of GEAT decoction

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
1	DL-Arginine	C6 H14 N4 O2	7200-25-1	174.11154	0.759	0.738
2	Bis(2,2-dihydroxyethyl) hydrogen phosphate	C4 H11 O8 P	NA	218.01901	0.763	0.461
3	Diphenyl sulfoxide (DA9185000)	C12 H10 O S	945-51-7	202.04511	0.765	1.400
4	Sucrose	C12 H22 O11	57-50-1	342.11564	0.771	0.710
5	Mannitol	C6 H14 O6	87-78-5	182.07816	0.773	0.064
6	1-[(3-Carboxypropyl)amino]-1-deoxy-beta-D-fructofuranose	C10 H19 N O7	10003-63-1	265.11574	0.777	2.984
7	2-C-methylerythritol 4-phosphate	C5 H13 O7 P	206440-72-4	216.03951	0.777	5.190
8	Boc-Glu-OH	C10 H17 N O6	2419-94-5	247.10524	0.779	0.932
9	L-Glutamic acid	C5 H9 N O4	56-86-0	147.05296	0.78	0.320
10	Muramic acid	C9 H17 N O7	1114-41-6	251.10015	0.78	0.358
11	L-Glutamic acid	C5 H9 N O4	56-86-0	147.05253	0.781	0.165
12	Sucrose	C12 H22 O11	57-50-1	342.11609	0.781	1.578
13	Benserazide	C10 H15 N3 O5	322-35-0	257.1024	0.788	0.478
14	azidamfenicol	C11 H13 N5 O5	13838-08-9	295.09025	0.789	0.331
15	3-(Sulfooxy)butanoic acid	C4 H8 O6 S	82542-96-9	184.0037	0.792	0.456
16	Betaine	C5 H11 N O2	107-43-7	117.07907	0.793	0.755

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
17	2-(Ethylsulfanyl)ethyl 7-(4-methoxyphenyl)-2-methyl-4-(6-nitro-1,3-benzodioxol-5-yl)-5-oxo-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate	C29 H30 N2 O8 S	NA	566.17002	0.793	0.193
18	Diethylpyrocarbonate	C6 H10 O5	1609-47-8	162.0526	0.794	0.227
19	[(Carbamoylamino)methyl]carbamate	C3 H6 N3 O3	NA	132.04124	0.795	0.279
20	3,5-Dinitro-N-(4H-1,2,4-triazol-4-yl)benzamide	C9 H6 N6 O5	NA	278.04005	0.796	1.085
21	(2S)-2-{[{[(2R,3S,4R)-5-(4-Amino-2-oxo-1(2H)-pyrimidinyl)-3,4-dihydroxytetrahydro-2-furanyl]methoxy}(hydroxy)phosphoryl]amino}propanoic acid	C12 H19 N4 O9 P	NA	394.0871	0.797	2.067
22	2-Pyrrolidinecarboxylic acid	C5 H9 N O2	147-85-3	115.06346	0.804	0.366
23	Trigonelline HCl	C7 H7 N O2	6138-41-6	137.04757	0.806	0.048
24	Zoxazolamine	C7 H5 Cl N2 O	61-80-3	168.00861	0.807	0.168
25	2-Sulfosuccinic acid	C4 H6 O7 S	5138-18-1	197.9826	0.81	0.224
26	[1-Cyano-2-(1H-pyrrol-2-yl)vinyl]phosphonic acid	C7 H7 N2 O3 P	NA	198.01947	0.81	0.297
27	p-Hydroxybenzaldehyde	C7 H6 O2	123-08-0	122.03688	0.814	0.014
28	MFCD02326088	C20 H16 N2 O2 S2	NA	380.06359	0.815	1.456
29	4-Methoxyaniline	C7 H9 N O	104-94-9	123.0683	0.826	1.027
30	(+/-)-2-Hydroxyglutaric acid	C5 H8 O5	2889-31-8	148.03683	0.831	0.165
31	Adenine	C5 H5 N5	73-24-5	135.05443	0.832	0.355
32	N-(2-AMINOETHYL)ETHYLENEUREA	C5 H11 N3 O	6281-42-1	129.09017	0.835	0.366
33	(2R,4R)-4-Amino-1-(3,5-dinitrobenzyl)-2,4-pyrrolidinedicarboxylic acid	C13 H14 N4 O8	NA	354.07963	0.835	0.752

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
34	N2-Succinyl-L-glutamic acid 5-semialdehyde	C9 H13 N O6	NA	231.07412	0.836	0.838
35	(2S)-3-Methyl-2-({[(3S,4S,5R)-2,3,4-trihydroxy-5-(hydroxymethyl)tetrahydro-2-furanyl]methyl}amino)butanoic acid (non-preferred name)	C11 H21 N O7	NA	279.13133	0.838	0.266
36	2-Chloro-5-[1,2,4]triazol-4-yl-benzoic acid	C9 H6 Cl N3 O2	842977-25-7	223.01442	0.839	0.844
37	MFCD00135810	C11 H17 N O8	24967-27-9	291.09493	0.84	0.654
38	L-Tyrosine	C9 H11 N O3	60-18-4	181.07377	0.841	0.069
39	MFCD00091314	C17 H15 Cl F3 N O4	2803-57-8	389.06264	0.848	0.247
40	Phenylmethylsulfonyl fluoride	C7 H7 F O2 S	329-98-6	174.01549	0.854	0.161
41	4,5-Dihydro-2-thiophenylboronic acid	C4 H7 B O2 S	NA	130.02549	0.855	0.311
42	Citric acid	C6 H8 O7	77-92-9	192.02617	0.856	3.831
43	L-Valine	C5 H11 N O2	72-18-4	117.0791	1.127	0.060
44	Cytosine	C4 H5 N3 O	71-30-7	111.04355	1.13	0.015
45	Nicotinamide	C6 H6 N2 O	98-92-0	122.04753	1.131	0.157
46	MFCD00135810	C11 H17 N O8	24967-27-9	291.09533	1.139	0.526
47	L-Pyroglutamic acid	C5 H7 N O3	98-79-3	129.04258	1.147	0.817
48	Uridine	C9 H12 N2 O6	58-96-8	244.06924	1.156	0.057
49	Adenosine	C10 H13 N5 O4	58-61-7	267.09642	1.194	0.327
50	p-Coumaric acid	C9 H8 O3	501-98-4	164.04724	1.208	0.265

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
51	D-(−)-Salicin	C13 H18 O7	NA	286.10522	1.236	1.151
52	Isoguanosine	C10 H13 N5 O5	1818-71-9	283.09173	1.292	0.015
53	Guanine	C5 H5 N5 O	73-40-5	151.04933	1.294	0.033
54	Guanosine	C10 H13 N5 O5	118-00-3	283.09126	1.295	0.018
55	DIETHYL (BOC-AMINO)MALONATE	C12 H21 N O6	102831-44-7	275.13652	1.306	0.311
56	1,2-di-O-methyl-4-[(2R)-2,4-dihydrobutyramido]-4,6-dideoxy-alpha-D-mannopyranoside	C12 H23 N O7	NA	293.14693	1.308	0.447
57	2-[(4-hydroxy-6-methylpyrimidin-2-yl)sulfanyl]-N-(2-nitrophenyl)acetamide	C13 H12 N4 O4 S	NA	320.05648	1.311	0.200
58	L-Leucine	C6 H13 N O2	61-90-5	131.09462	1.351	0.321
59	Orcinol gentiobioside	C19 H28 O12	164991-86-0	448.15812	1.436	0.143
60	N,N'-[(6-Phenyl-2,4-pyrimidinediyl)di-4,1-phenylene]bis(3-nitrobenzamide)	C36 H24 N6 O6	NA	636.17256	1.437	0.256
61	p-Cresylsulfate	C7 H8 O4 S	3233-58-7	188.01365	1.439	9.800
62	1-(3-Methoxyphenyl)-3-{4-[(2-nitrophenyl)sulfonyl]-1-piperazinyl}-2,5-pyrrolidinedione	C21 H22 N4 O7 S	NA	474.11925	1.525	0.634
63	Benzaldehyde	C7 H6 O	100-52-7	106.04206	1.558	2.473
64	Orcinol glucoside	C13 H18 O7	21082-33-7	286.10521	1.563	9.907
65	MFCD00024401	C12 H18 N4 O4	NA	282.13231	1.619	0.285
66	4,4'-Dinitroazobenzene	C12 H8 N4 O4	3646-57-9	272.05324	2.005	0.287
67	4-(2-Hydroxyethyl)phenyl hydrogen sulfate	C8 H10 O5 S	NA	218.02437	2.071	0.165

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
68	lactide	C6 H8 O4	95-96-5	144.04215	2.141	0.154
69	Phenylacetylene	C8 H6	536-74-3	102.04707	2.159	0.369
70	Cinnamic acid	C9 H8 O2	102-94-3	148.05233	2.162	0.456
71	5-Hydroxymethylfurfural	C6 H6 O3	67-47-0	126.03178	2.488	2.584
72	MFCD00110732	C7 H8 O5	2029-49-4	172.03633	2.518	0.201
73	Calcium pantothenate	C9 H17 N O5	137-08-6	219.11021	2.776	0.022
74	4-Methyl-6,7-dihydroxycoumarin	C10 H8 O4	529-84-0	192.04222	3.182	0.011
75	Piscidic Acid	C11 H12 O7	469-65-8	256.05816	3.274	3.536
76	5-Methyl-2-(methylsulfonyl)-3-thiophenecarboxylic acid	C7 H8 O4 S2	NA	219.98596	4.152	0.198
77	L-Tyrosine	C9 H11 N O3	60-18-4	181.07307	4.412	0.009
78	L-Tryptophan	C11 H12 N2 O2	73-22-3	204.08981	4.464	0.039
79	L-Tryptophan	C11 H12 N2 O2	73-22-3	204.08929	4.484	0.009
80	Parishin E	C19 H24 O13	952068-57-4	460.12178	4.539	0.733
81	O ~ 6~-Benzylguanine	C12 H11 N5 O	19916-73-5	241.09609	4.826	0.253
82	Protocatechualdehyde	C7 H6 O3	139-85-5	138.03171	4.965	0.004
83	Perillartine	C10 H15 N O	30950-27-7	165.11547	5.211	0.003
84	Cianidanol	C15 H14 O6	154-23-4	290.07875	5.293	0.021
85	(+)-Catechin hydrate	C15 H14 O6	225937-10-0	290.07915	5.301	0.058
86	Perillene	C10 H14 O	539-52-6	150.10451	5.311	0.005

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
87	L-5-Hydroxytryptophan	C11 H12 N2 O3	4350-09-8	220.08432	5.369	0.004
88	2-Hydroxy-4-(methylsulfonyl)isophthalic acid	C9 H8 O7 S	NA	259.99895	5.424	0.381
89	Caffeic acid	C9 H8 O4	331-39-5	180.0419	5.437	0.130
90	5-Hydroxy-1-tetralone	C10 H10 O2	28315-93-7	162.06805	5.478	0.086
91	2-(3-CARBOXYPROPIONYL)-6-HYDROXY-CYCLOHEXA-2,4-DIENE CARBOXYLIC ACID	C11 H12 O6	NA	240.06311	5.627	0.334
92	Parishin C	C32 H40 O19	174972-80-6	728.21705	5.681	0.336
93	L-Phenylalanine	C9 H11 N O2	63-91-2	165.07892	5.765	0.065
94	Methyldopa	C10 H13 N O4	555-30-6	211.08387	5.768	0.182
95	3-[2-(1,3-Benzothiazol-2-yl)hydrazino]-2H-indol-2-one	C15 H10 N4 O S	NA	294.05626	5.787	0.274
96	Dehydroandrographolide	C20 H28 O4	134418-28-3	332.19847	5.872	0.002
97	Ethyl ferulate	C12 H14 O4	4046-02-0	222.0891	5.908	0.006
98	Benzoic acid	C7 H6 O2	65-85-0	122.03689	6.052	0.126
99	Gastrodin	C13 H18 O7	62499-27-8	286.10527	6.231	0.008
100	Parishin A	C45 H56 O25	62499-28-9	996.3117	6.266	0.020
101	7-Methoxycoumarin	C10 H8 O3	531-59-9	176.04723	6.501	0.007
102	AF4878000	C11 H12 O3	94-02-0	192.0784	6.506	0.758
103	2-Hydroxy-4-methoxybenzaldehyde	C8 H8 O3	673-22-3	152.04724	6.559	0.031
104	Coumarin	C9 H6 O2	91-64-5	146.03665	6.731	0.005

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
105	3,5-Dimethoxy-4-hydroxybenzaldehyde	C9 H10 O4	134-96-3	182.05779	6.753	0.026
106	3,5-Dimethoxy-4-hydroxybenzaldehyde	C9 H10 O4	134-96-3	182.0571	6.764	0.006
107	2,6-Dimethyl-4,10-dioxatricyclo[5.2.1.0 ~ 2,6~]decane-3,5-dione	C10 H12 O4	80558-50-5	196.07344	6.88	0.208
108	Ferulic Acid	C10 H10 O4	1135-24-6	194.05714	6.885	0.013
109	Z-Ser(Bzl)-OH	C18 H19 N O5	20806-43-3	329.12591	6.924	0.317
110	Gallic acid trimethyl ether	C10 H12 O5	118-41-2	212.06838	7.007	0.048
111	Isoeugenol acetate	C12 H14 O3	93-29-8	206.09418	7.01	0.015
112	Veratric acid	C9 H10 O4	93-07-2	182.05794	7.061	0.011
113	Cyclo(D-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl)	C36 H66 N6 O6	NA	678.50361	7.096	0.259
114	Azelaic acid	C9 H16 O4	123-99-9	188.10405	7.46	0.327
115	Arteannuin	C15 H20 O3	50906-56-4	248.14102	7.505	0.019
116	Methyl 4-hydroxy-3-methoxycinnamate	C11 H12 O4	2309-07-1	208.07343	7.572	0.015
117	Ferulaldehyde	C10 H10 O3	20649-42-7	178.06293	7.611	0.008
118	Citropten	C11 H10 O4	487-06-9	206.05783	7.812	0.006
119	o-Veratraldehyde	C9 H10 O3	86-51-1	166.0629	7.929	0.319
120	Curcumol	C15 H24 O2	4871-97-0	236.17737	8.041	0.038
121	Ligustilide	C12 H14 O2	4431-01-0	190.09931	8.494	0.040
122	Abscisic acid	C15 H20 O4	14375-45-2	264.13603	8.551	0.008
123	Abscisic acid	C15 H20 O4	14375-45-2	264.13579	8.566	0.020

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
124	Atractylenolide II	C15 H20 O2	73069-14-4	232.14614	8.779	0.055
125	Nardosinone	C15 H22 O3	23720-80-1	250.15663	8.779	0.045
126	Arglabin	C15 H18 O3	84692-91-1	246.12531	8.854	0.012
127	UC2976000	C12 H16 O4	106797-53-9	224.10455	8.939	0.248
128	β-Asarone	C12 H16 O3	5273-86-9	208.10979	9.016	0.054
129	Resveratrol	C14 H12 O3	501-36-0	228.07843	9.113	0.003
130	Dihydroresveratrol	C14 H14 O3	58436-28-5	230.09401	9.178	0.010
131	Artemisinin	C15 H22 O5	63968-64-9	282.14627	9.182	0.055
132	Berberrubine	C19 H15 N O4	15401-69-1	321.09984	9.213	0.007
133	Dihydroartemisinin	C15 H24 O5	71939-50-9	284.16231	9.236	0.069
134	2-Adamantanone	C10 H14 O	700-58-3	150.10437	9.303	0.003
135	Naringenin	C15 H12 O5	480-41-1	272.06813	9.696	0.003
136	(3aR,4R,5R,6aS)-5-Hydroxy-4-[(1E,3S)-3-hydroxy-1-octen-1-yl]hexahydro-2H-cyclopenta[b]furan-2-one	C15 H24 O4	26054-67-1	268.16698	9.698	0.371
137	2,4-Di(1-pyrrolidinyl)quinazoline	C16 H20 N4	NA	268.16746	9.698	0.787
138	7-Methoxy-4-methylcoumarin	C11 H10 O3	2555-28-4	190.06276	9.802	0.090
139	Ethyl ferulate	C12 H14 O4	4046-02-0	222.0887	9.986	0.010
140	Benzyl sulfone	C14 H14 O2 S	620-32-6	246.07123	10.235	0.165
141	Fraxinellone	C14 H16 O3	28808-62-0	232.10964	10.238	0.008

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
142	2-[4-(Cyclopentylcarbamothioyl)-1-piperazinyl]-N-(4-methoxyphenyl)acetamide	C19 H28 N4 O2 S	NA	376.19194	10.397	0.315
143	Columbin	C20 H22 O6	546-97-4	358.14119	10.411	0.008
144	Senkyunolide A	C12 H16 O2	63038-10-8	192.11493	10.722	0.014
145	Isoalantolactone	C15 H20 O2	470-17-7	232.1461	10.843	0.385
146	Pinoresinol dimethyl ether	C22 H26 O6	29106-36-3	386.17226	10.978	0.010
147	Parthenolide	C15 H20 O3	20554-84-1	248.14086	11.026	0.018
148	Epimagnolin B	C23 H28 O7	1134188-26-3	416.18287	11.12	0.040
149	Atractylenolide I	C15 H18 O2	73069-13-3	230.1304	11.31	0.049
150	Eudesmin	C22 H26 O6	526-06-7	386.17211	11.322	0.020
151	6-Gingerol	C17 H26 O4	23513-14-6	294.18307	11.325	0.021
152	2-[(2R,4aR,8R,8aR)-8-Hydroxy-4a,8-dimethyldecahydro-2-naphthalenyl]acrylic acid	C15 H24 O3	4586-68-9	252.17212	11.337	1.888
153	Hexyl cinnamaldehyde	C15 H20 O	101-86-0	216.15111	11.341	0.488
154	Ethyl cinnamate	C11 H12 O2	4192-77-2	176.08348	11.655	2.182
155	Curdione	C15 H24 O2	13657-68-6	236.17729	11.757	0.448
156	Indane	C9 H10	496-11-7	118.07836	11.852	0.352
157	MFCD00027233	C11 H14	16002-93-0	146.10942	11.853	1.121
158	DO0750000	C15 H24 O2	88-26-6	236.17727	11.955	0.547
159	Artemisinic acid	C15 H22 O2	80286-58-4	234.16167	12.238	0.073
160	MFCD00021091	C14 H18	1079-71-6	186.14066	12.614	0.387

Number	Name	Formula	CAS number	Calc. MW	RT (min)	*Relative Content (%)*
161	Veraguensin	C22 H28 O5	19950-55-1	372.19312	12.759	0.025
162	α-Linolenic acid	C18 H30 O2	463-40-1	278.2242	13.101	0.144
163	α-Cyperone	C15 H22 O	473-08-5	218.16691	13.142	0.089
164	Curcumenol	C15 H22 O2	19431-84-6	234.16168	13.271	0.043
165	α-Asarone	C12 H16 O3	2883-98-9	208.10973	13.378	0.009
166	(E,E)-alpha-Farnesene	C15 H24	502-61-4	204.18768	13.83	0.495
167	Curcumene	C15 H22	644-30-4	202.17204	13.972	0.816
168	Alnustone	C19 H18 O	33457-62-4	262.13539	14.063	0.003
169	Germacrone	C15 H22 O	6902-91-6	218.1668	14.48	2.172
170	Camphor	C10 H16 O	76-22-2	152.12004	15.134	0.024
171	Dodecyl sulfate	C12 H26 O4 S	151-41-7	266.15507	15.694	0.621
172	α-Linolenic acid	C18 H30 O2	463-40-1	278.22438	15.919	0.292
173	16-Hydroxyhexadecanoic acid	C16 H32 O3	506-13-8	272.235	16.008	0.203
174	Linoleic acid	C18 H32 O2	60-33-3	280.24013	17.202	1.040

3.2. Effect of GEAT decoction on MES-induced seizures

The evaluation of the effects of GEAT decoction on the MES test in mice was shown in Table 2. As can be seen from Table 1, oralally administration of GEAT decoction for 14 days dose and time-dependently protected mice from hind-limb tonic extension (HLTE) in comparison with the control group. Specifically, 1 h after the last drug administration, GEAT decoction at a dose of 50, 100, and 200 mg/kg protected mice from HLTE to 33.3, 66.6, and 83.3% respectively, while 50 mg/kg CBZ used as a reference drug also showed 83.3% protection in MES model of seizures as compared to the control group. The projected number of animals reduced in 4 h after the last drug administration, which may be related to the metabolism and excretion of active ingredients.

Table 2
GEAT decoction administered orally to mice for 7 days enhanced the percent protection from HLTE in MES model (n = 6).

Group	Dose (mg/kg)	MES (n_1/n_2)[a]			
		0.5 h[b]	1 h[b]	2 h[b]	4 h[b]
Saline	-	0/12	0/12	0/12	0/12
CBZ	50	10/12	10/12	6/12	4/12
GEAT decoction	50	2/12	5/12	3/12	0/12
	100	6/12	8/12	6/12	3/12
	200	10/12	10/12	8/12	6/12

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

3.3. Effect of GEAT decoction on 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 mean seizure thresholds of 243.5 and 254.5 s, respectively (Table 2). In addition, GEAT decoction at 100 and 200 mg/kg offered 50.0, 66.7, and 83.3, 66.7% protection against PTZ-induced GTCS and mortality, while CBZ at 50 mg/kg produced a slightly smaller proportion of protective activity as GEAT decoction at 200 mg/kg (Table 3). In contrast, the GEAT decoction in all experimental groups did not completely inhibit clonic seizures. However, compared to the saline-treated control group, seizure scores in GEAT decoction decreased in a dose-dependent manner, whereas mice in the model group showed significantly higher seizure scores after administration of 85 mg/kg of PTZ, showing a good antiepileptic effect. Similar, 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 retardation in the seizure scores for all the treatment days.

Table 3
Effect of *GTAT* decoction *pretreatment* for 7 days on subcutaneous PTZ (85 mg/kg) induced acute seizures in mice (n = 12).

Group	Dose (mg/kg)	Latency time of the 1st seizures (s)	GTCS [a]	CS [b]	Death [c]	Seizure score
Saline	-	138.8 ± 9.01	12/12	12/12	10/12	5.00 ± 0.00
CBZ	50	227.8 ± 20.09**	4/12	12/12	6/12	3.83 ± 0.40*
GTAT decoction	50	182.4 ± 20.85	7/12	12/12	8/12	4.33 ± 0.42
	100	243.5 ± 16.26**	6/12	12/12	4/12	4.00 ± 0.45
	200	254.5 ± 23.54***	2/12	12/12	4/12	3.70 ± 0.33*

Note: Data are presented as Mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001 compared with model (PTZ + saline). [a] No. of mice occurrence of GTCS /No. of mice tested. [b] No. of mice occurrence of CS/No. of mice tested. [c] No. of mice death/No. of mice tested.

3.4. Effect of GEAT decoction on 3-MP-induced seizures

As shown in Table 4, with regard to the latency to tonic seizures, an obvious decrease in the NaCl group was observed. Oral administration of GEAT decoction resulted in different degrees of extension on onset latency. Especially, GEAT decoction at 200 mg/kg significantly inhibited and delayed the onset of myoclonic seizures with a seizure threshold of 280.2 ± 27.08 s compared to that of the model group (200.4 ± 11.96 s) ($P < 0.01$). Besides, pretreatment GEAT decoction at 50, 100, and 200 mg/kg resulted in 33.3, 67.7, and 67.7% protection respectively against tonic seizures and 50.0, 67.7, 83.3% protection of the mice from death in 3-MP-induced seizures test. No obvious protection was observed in all GEAT decoction and CBZ treatment groups on the clonic seizure induced by 3-MP (Table 4). Taken together, GEAT decoction reduced the severity of convulsive activity and also prevent tonic-clonic seizures in the 3-MP-induced drug-resistant seizures test.

Table 4
Effect of *GTAT* decoction *pretreatment* for 7 days on subcutaneous 3-MP (60 mg/kg) induced acute seizures in mice (n = 12).

Group	Dose (mg/kg)	Latency time of the 1st seizures (s)	Tonic seizures [a]	Clonic seizure [b]	Death [c]
Saline	-	200.4 ± 11.96	6/12	12/12	10/12
CBZ	50	276.7 ± 18.75**	2/12	12/12	0/12
GTAT decoction	50	217.0 ± 13.88	6/12	12/12	6/12
	100	252.7 ± 9.89*	4/12	12/12	4/12
	200	280.2 ± 27.08**	4/12	12/12	2/12

Note: Data are presented as Mean ± SEM; *P < 0.05, **P < 0.01 compared with model (PTZ + saline). [a] No. of mice occurrence of tonic seizures /No. of mice tested. [b] No. of mice occurrence of clonic seizure/No. of mice tested. [c] No. of mice death/No. of mice tested.

3.5. Effects of GEAT decoction on pro-inflammatory cytokines

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

3.6. Effects of GEAT decoction on oxidative stress parameters

PTZ-induced kindling markedly elevated oxidative stress in the mice. In terms of quantification of oxidative stress parameters in the hippocampus, all treatments presented the 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). About 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 the hippocampal of mice in comparison with the model group ($P < 0.01$) (Fig. 4B). In addition, results showed an enhanced production of MDA as well as a reduced production of GSH in hippocampal in PTZ induced mice. Interestingly, these changes were reversed upon GEAT decoction treatment in varying degrees (Fig. 4C and 4D). However, the levels of these oxidative stress parameters in animals treated with CBZ were not significant changed in comparison with the model group (P 0.05).

3.7. Effect of GEAT decoction on cognitive and behavioral functions in the EPM test

It has been proposed that depression and anxiety symptoms are a frequent occurrence in epilepsy, therefore anxiety-like behavior was evaluated in this study. As shown in Fig. 5A and 5B, the time spent in the open arms of the EPM and the percentage of entries of mice into the open arms were evaluated. The results indicated that PTZ-induced seizures mice displayed anxiety-like behavior compared with mice in the normal group (Fig. 5A and 5B). Luckily, pre-treated with GEAT decoction tended to increase the number of open-arm entries of mice in the EPM test in varying degrees, displaying lower levels of anxiety-like behavior in the EPM. In particular, the 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 kindling mice in EPM are shown in Fig. 6A.

3.8. Effect of GEAT decoction on cognitive and behavioral functions in OPT

Likewise, the time spent and distance in the central areas were used as an anxiety-like indicator determined in OFT. As shown in Fig. 5C, the time spent in the central area for mice in the normal group was 34.0 ± 2.7 s and 15.3 ± 3.6 s for mice in the model group. For GEAT decoction at doses 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 doses of 100 and 200 mg/kg significantly increased total distance of the mice moving in the central are compared that of mice in model group. However, changes in these indicators in CBZ and GEAT decoction (50 mg/kg) treatment did not show significant differences in comparison with the model group in the same period of treatment, as shown in Fig. 4C and D. The behavior trace of GEAT decoction on subcutaneous PTZ-induced kindling mice in OPT are shown in Fig. 6B.

4. Discussion

Epilepsy induced by many reasons is the most common chronic brain disease, affecting about 70 million people worldwide (Johnson 2019). Traditional Chinese herbal medicine has a long history of use for treating epilepsy.

Currently, herbal treatments for seizures has attracted lots of attention globally. In addition, the herbal treatment appears to be inexpensive, safe, easy to get, and effective in treating epilepsy (Zhao et al. 2018). So far, more than 14 kinds of TCM prescriptions or preparations for the treatment of various epilepsy, especially intractable epilepsy has included in the 2020 edition of Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee 2020). According to statistics and analysis, the commonly used and clinically effective drug pairs "*G. elata*-*A. tatarinowii*" are the most representative clinically valuable drug pair in the 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 these 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, three classical animal models of epilepsy were performed to evaluate the antiepileptic effect and related mechanism of GEAT decoction. Additionally, the EPM test and OPT were performed to examine the impact of GEAT decoction on the cognitive and behavioral functions of PTZ-kindling mice.

In this study, UHPLC-MS/MS was first utilized to identify the chemical compounds of GEAT decoction. In total, 174 compounds were identified from GEAT decoction, and 20 of them were more than 1% relative. Among them, researchers demonstrated that some potential compounds in GEAT decoction, such as α-asarone, gastrodin, and parishin C, etc., showed anticonvulsant efficacy by decreasing the seizures (He et al., 2018). Then, we evaluated the anticonvulsant effects of GEAT decoction at different dosages on three different acute 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 the onset of myoclonic seizures, inhibited generalized seizures 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 provides proof of concept that GEAT decoction are pharmacologically active *in vivo* with a dose-dependent manner, which possessed a therapeutic potential to prevent and control seizures. It is worth noting that 3-MP is an experimental model of drug-resistant seizures associated with P-glycoprotein (Pgp) overexpression (Pérez-Pérez et al. 2021), further studies are essential to determine if GEAT decoction is effective in more experimental models of drug-resistant epilepsy. Moreover, the repetitive administration of 3-MP induced seizure test should be established for determination the Pgp expression and/or function of the cortex and hippocampus in GEAT decoction-treated mice to explore the synergistic effect of GEAT decoction combination with currently available AEDs.

Evidence suggests that inflammation strengthens excitability of neuronal, and consequently prolongation of seizures and initiation of 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, high levels of inflammatory mediators, including IL-1β, IL-6, and TNF-α were detected in the brain tissue of patients with intractable epilepsy (temporal lobe 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-1β, 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-1β, IL-6, and TNF-α levels in the serum and brain tissues of PTZ-kindling mice. Therefore, GEAT decoction may have potential value in the management of inflammatory diseases accompanied by epilepsy.

Studies have found that oxidative stress and mitochondrial dysfunction may be the causes and the results of genetic and acquired epilepsies (Chindo et al. 2021). Increased production of free radicals produces unwanted side or harmful effects on the structure and functions of neurons, changing or damaging the biological function regulation of the central nervous system. In particular, the increase in the synthesis and release of reactive oxygen species lead to great damage to the steady-state of the oxidation potential of the central nervous system (Frantz et al. 2021). Thus, removing excessive hydroxyl radical, peroxy radical, and superoxide radical, as well as elevating the activity of superoxide dismutase and glutathione peroxidase are very beneficial to ease symptoms or to control seizures. In the pathogenesis of chronic epilepsy, a large great number of superoxide anions free radicals were produced, while the endogenous antioxidant enzymes SOD, GSH, GSR, and CAT are rapidly consumed, resulting in the excessive production of toxic lipid peroxide that then led to oxidative stress and neuronal death. In addition, in PTZ-induced kindling in mice, it was found that reactive oxygen species were activated, and its production agrees with a decrease in antioxidant-related enzymes (Frantz et al. 2017; Chindo et al. 2021). In this study, we found that mice treated with GEAT decoction displayed a dose-dependent reduction in the production of MDA in PTZ-kindled mouse hippocampus, while showing an increase in activities of CAT and SOD activities, as well as exhibited an increase in the production of GSH when compared with that of PTZ-kindled epileptic mouse models. In other words, GEAT decoction improved the antioxidant capacity of brain tissue, and reduced lipid peroxidation and peroxidation damage in the 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 accompaniments neurological of chronic epilepsy (Chindo et al. 2021). Patients with long-term seizures can cause diversified degrees of brain injury and abnormal emotions 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). The EPM test and OPT are some of the most widely used tests to assess depression/anxiety and cognitive dysfunction in animals (Knight et al. 2021). Thus, in our study, we explored the effects of GEAT decoction on anxiety and cognitive dysfunction in the PTZ-kindled epileptic mouse model using OFT and EPM tests. Data have shown that the time spent in the central areas of OFT and in the open arms of EPM was decreased in PTZ-induced mice, which means a state of avoiding fear and anxiety behavior in these kindling mice. Whereas, the GEAT decoction treatment mice spent more time on the open arms of the EPM test and made more open arms entries than non-GEAT decoction-treated mice. Similarly, GEAT decoction also spent more time in the center zone of the OPT, made more center zone entries and traveled a greater distance in center zone than controls. The results preliminary demonstrated that GEAT decoction evidently improved anxiety-like behavior and cognitive impairment in PTZ-kindled epileptic mouse, which supported the traditional records that the couplet medicinal of *G. elata* and *A. tatarinowii* relieving convulsion and stress. However, no doubt that GEAT decoction capable to reduce anxiety and stress in this study, more in-depth studies on the alleviation of mental stress of GEAT decoction in various aspects are needed.

5. Conclusion

GEAT decoction showed outstanding protected activities in MES, PTZ, and 3-MP induced models of seizures. Especially, GEAT decoction has a promising activity in reducing inflammation and oxidative stress, as well as improving anxiety behavior in PTZ-kindled mice, confirming 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 deep insight into the best compatibility proportion, clinical effect, and mechanistic pathway involved in the management of epileptic seizures by GEAT decoction.

Declarations

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Competing Interests

The authors declare that there is no conflict of interest.

Author contributions

The manuscript was completed through the contributions of all the listed authors. H-XR, Y-Y, Y-XF, and S-Y designed and performed the experiments, and Y-Y 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.

Data Availability

Data will be provided upon a reasonable request.

Ethics approval

Animal studies were performed in strict accordance with the guidelines for the Care and Use of Laboratory Animals and granted by the Ethics Committee of Zunyi Medical University (Date: July 30, 2020/NO: ZYLS-[2020] No. 2-081).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Others

A preprint has previously been published [He et al. 2022] in “research square”, it has not been published by a journal.

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Figures

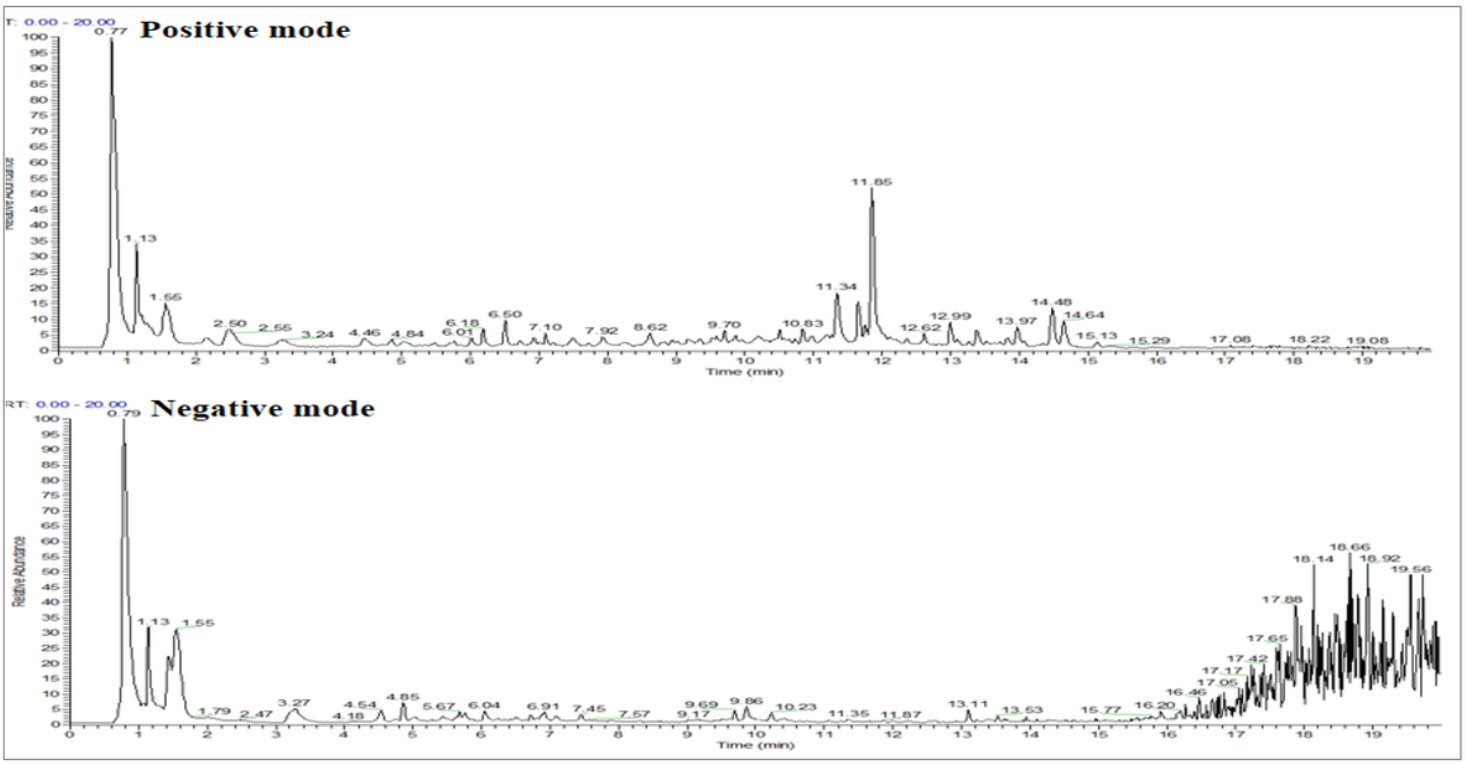

Figure 1

Mass spectrogram of GEAT decoction.

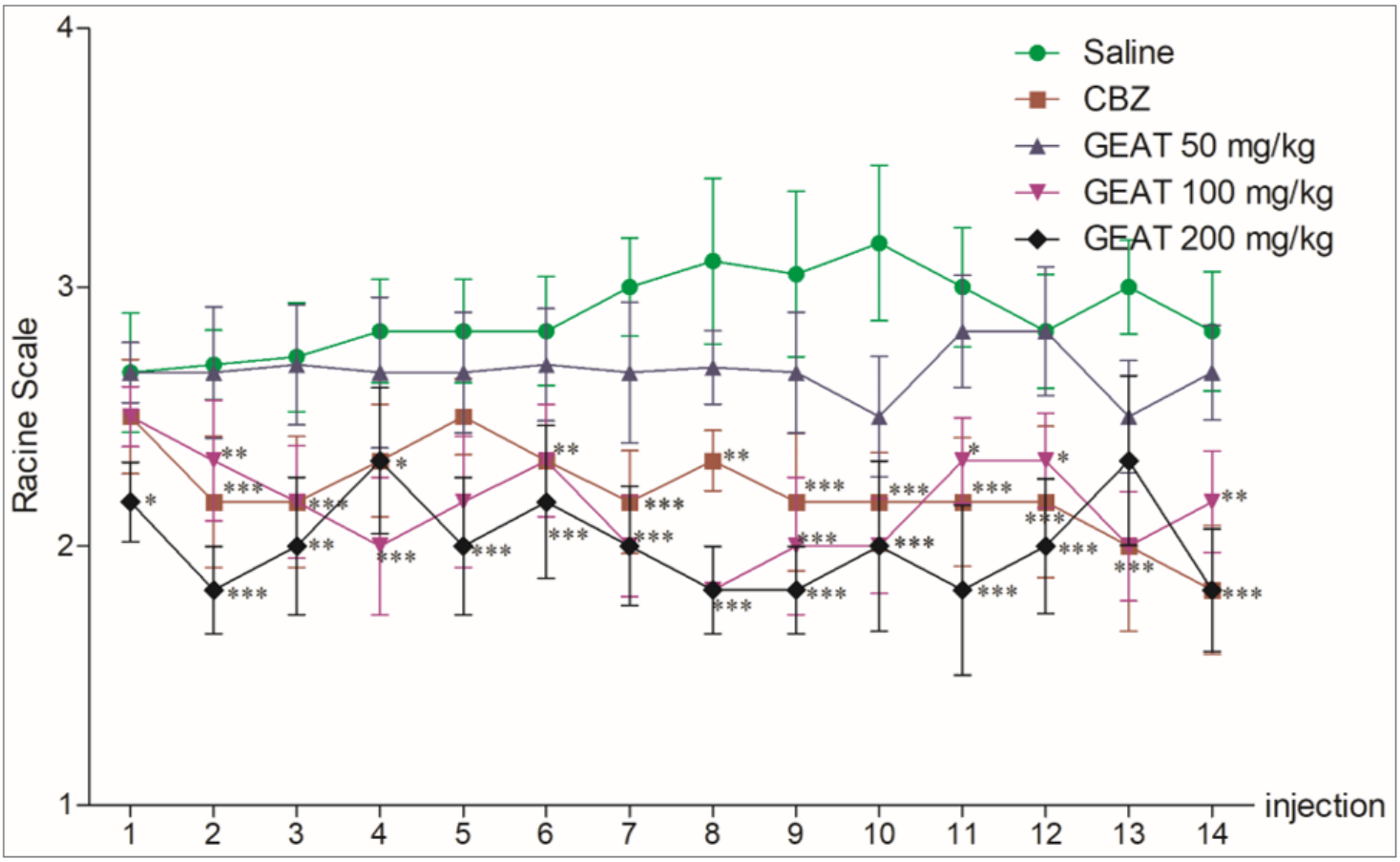

Figure 2

Effect of GEAT decoction and CBZ on subcutaneous PTZ-kindling seizure in mice for 14 injection every two days. The behavioral seizure and severity scale was observed and evaluated using the Racine scale as indicated earlier in PTZ-induced acute seizure test. Data expressed as Mean ± SEM, n=12 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.

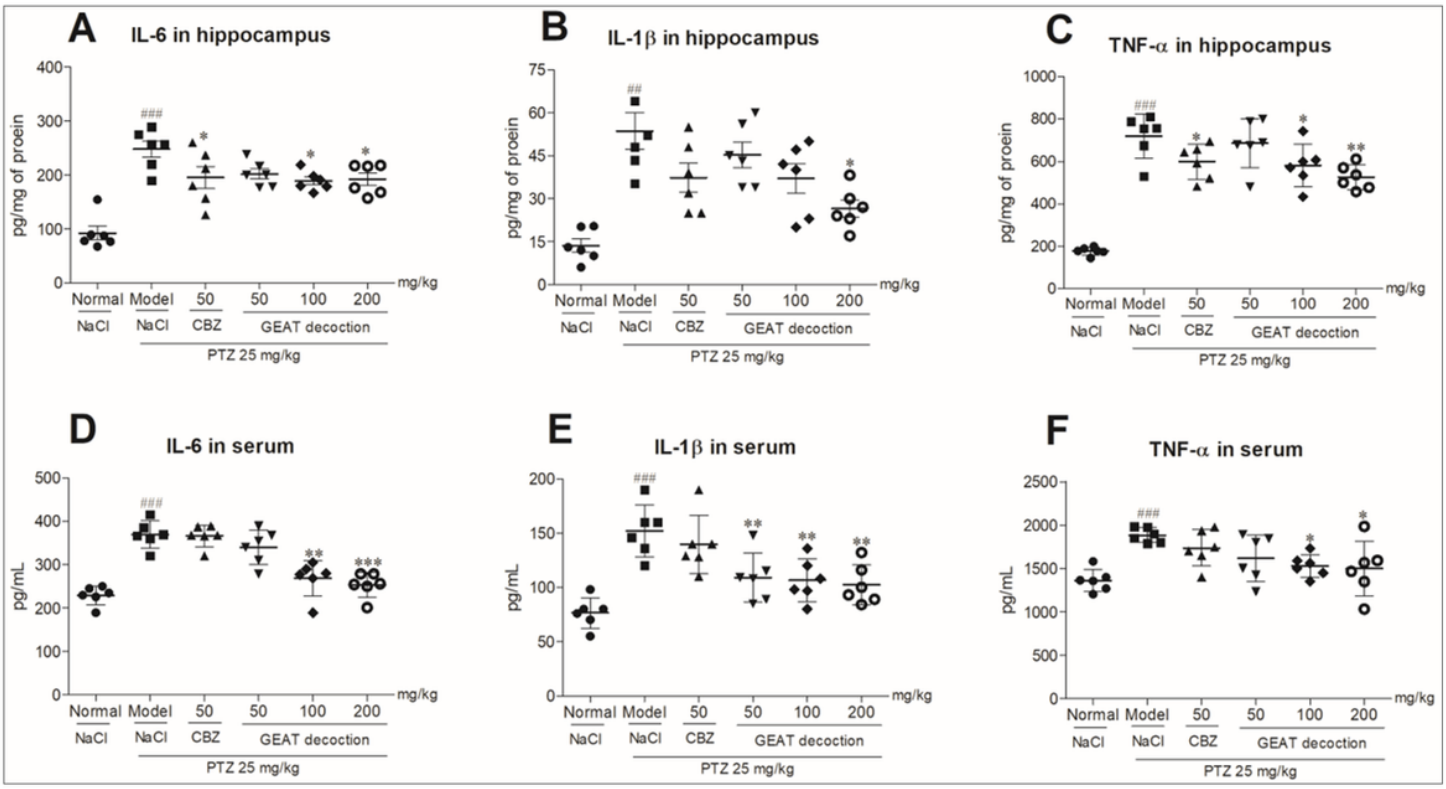

Figure 3

Effect of chronic administration GEAT decoction and CBZ on pro-inflammatory cytokines in the hippocampus and serum of mice in the PTZ-kindling model. A, IL-6 in the hippocampus; B, IL-1β in the hippocampus; C, TNF-α in the hippocampus; D, IL-6 in the serum; E, IL-1β in the serum; F, TNF-α in the serum; Data presented as Mean ± SEM. Statistical analyses were implemented using one-way ANOVA test. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.01$ compared with model (saline, PTZ existence) group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared with normal (saline, PTZ absence) group.

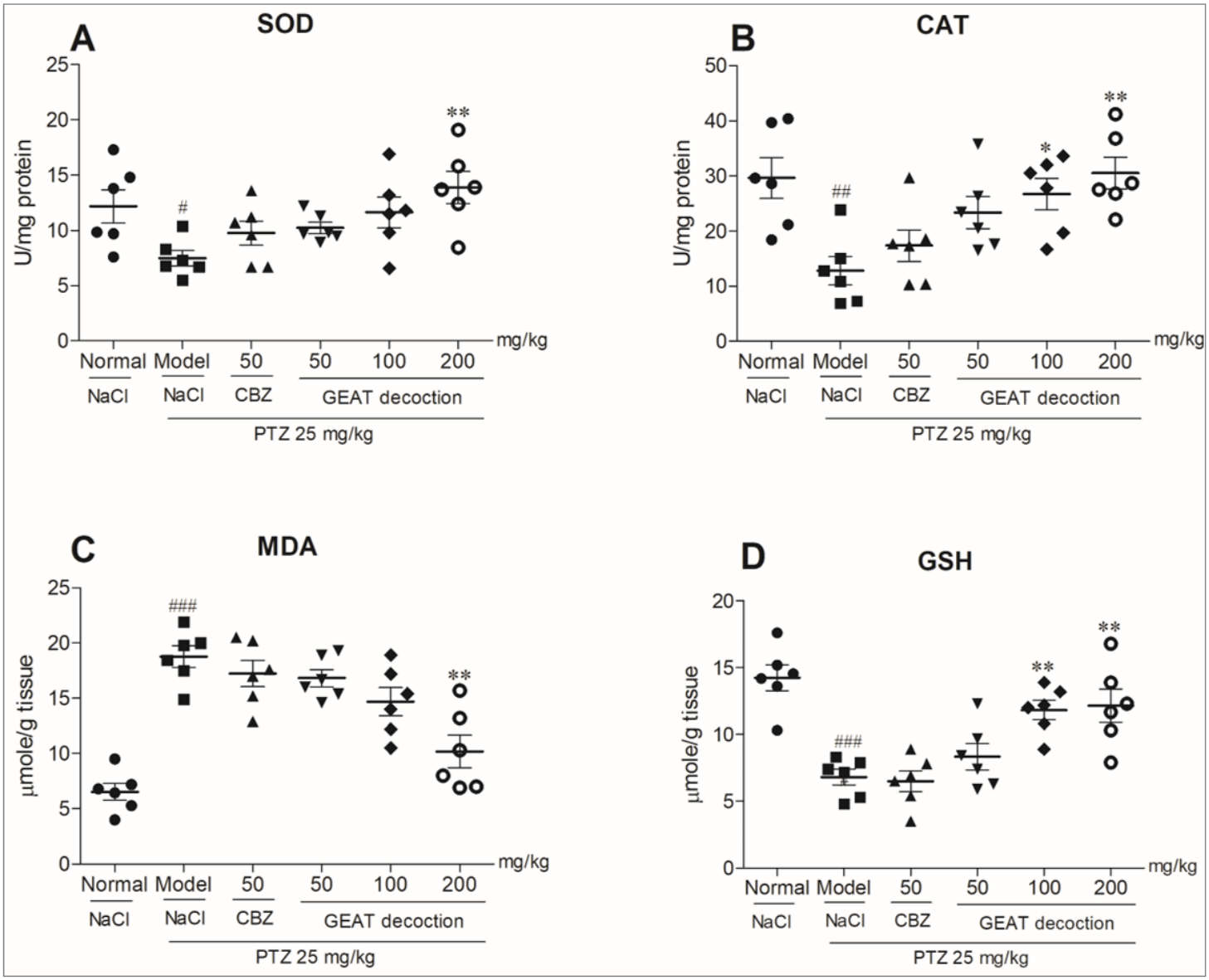

Figure 4

Effect of GEAT decoction and CBZ on levels of main oxidative stress markers in the hippocampus of subcutaneous PTZ-kindling mice. A, SOD activity; B, CAT activity; C, MDA levels; D, GSH levels. Data presented as Mean ± SEM. 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.

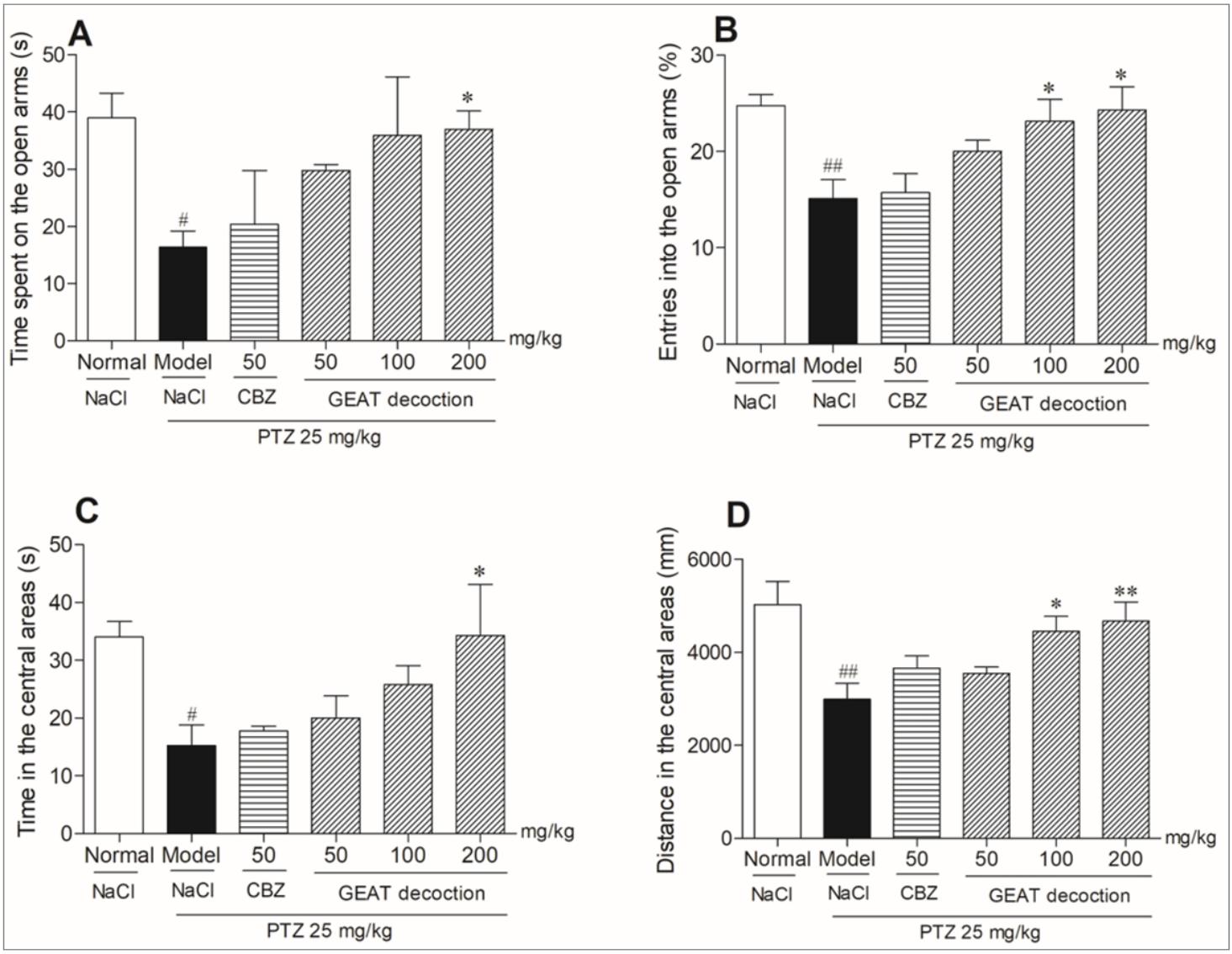

Figure 5

Effect of GEAT decoction and CBZ on subcutaneous PTZ-kindling mice in EPM test and OPT. A, the time spent in open arms in EPM test; B, the entries percentage of mice into the open arms in EPM test; C, the time spent in the central areas in OPT; D, distance travelled in the central areas in OPT. Statistical analyses were implemented using one-way ANOVA test and Chi square test. $^{*}P<0.05$, $^{**}P<0.01$ compared with model group (saline, PTZ existence). $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with normal group (saline, PTZ absence).

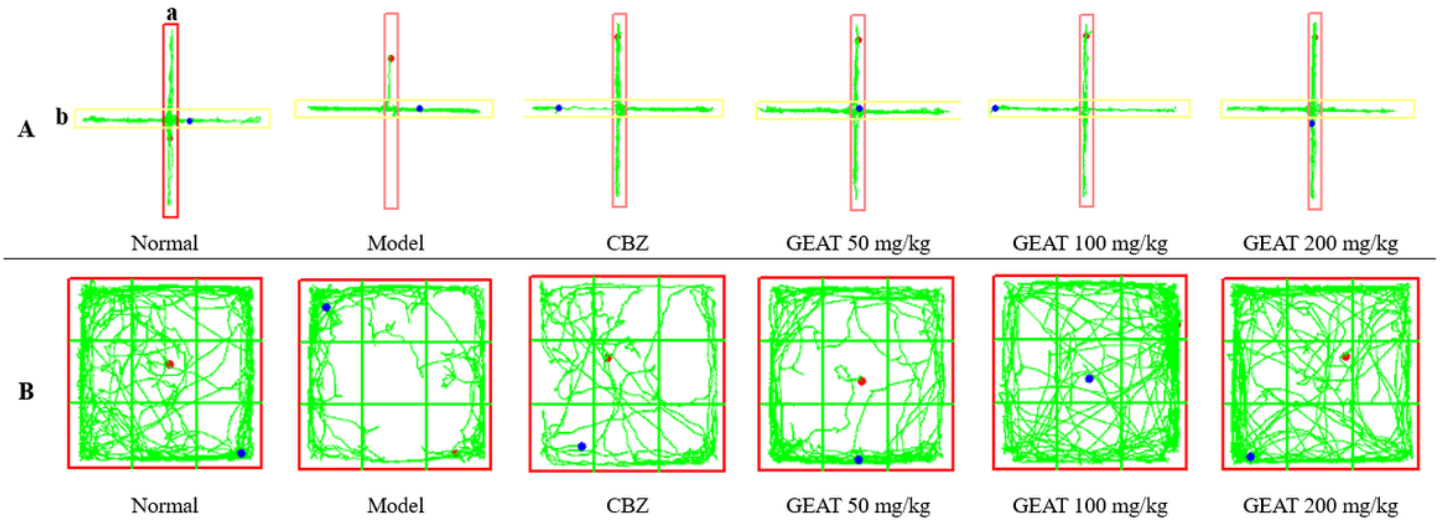

Figure 6

Behavior trace of GEAT decoction and CBZ on subcutaneous PTZ-induced mice in EPM test and OPT. A, EPM; B, OFT; a, open arms; b, closed arms.