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Effect of γ-Aminobutyric Acid on Patients with Refractory Epilepsy

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Abstract This study used mice as experimental materials to study the effects of γ-aminobutyric acid (GABA), rose and tryptophan on epilepsy. In addition, the combination of GABA, rose and tryptophan was studied to alleviate epilepsy. The optimal dosage of GABA, tryptophan and rose were determined to be 100 mg/kg, 160 mg/kg and 1.6 g/kg, respectively, according to the behavior of mice and oxidative stress indexes in serum. Screening the combination of GABA-compatible functional substances that can improve epilepsy, the optimal concentrations of GABA+ rose, GABA+ tryptophan, GABA+ rose + tryptophan were determined to be 10 mg/kg+1.4 g/kg, 10 mg/kg+170 mg/kg, 10 mg/kg+1.4 g/kg+170 mg/kg. Three kinds of whole nutrition food with the ability to relieve epilepsy in mice were obtained: GABA+ tryptophan (formula food 1), GABA+ rose (formula food 2), and tryptophan (formula food 3). Results showed that the three products had no significant effect on the body weight of mice, and the incubation period of epileptic seizure of mice was prolonged by 1 min. Meanwhile, the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in serum increased, while the content of malondialdehyde (MDA) decreased. Combined with the new nutrition knowledge and advanced food processing technology, three kinds of whole nutrition medical food were prepared. This food has a good effect of relieving epilepsy, but also can meet the normal nutrient needs.

Keywords: *GABA, epilepsy, medical food*

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1. Introduction

The pathogenesis of epilepsy is closely related to neurotransmitters. Oversynchronous firing of brain neurons is the main mechanism of epilepsy. Normally there is a balance between excitatory and inhibitory neurotransmitters. When excitatory neurotransmitters are increased or inhibitory neurotransmitters are reduced, neuronal stability is broken and seizure-like discharge occurs. At present, it has been found that among the amino acid neurotransmitters related to epilepsy, glutamate (Glu), aspartic acid (Asp) and so on have a promoting effect on epilepsy, which is called excitatory amino acids. γ-aminobutyric acid (GABA), glycine (GLY) and other epileptic seizure inhibitory effect, known as inhibitory amino acids. Among the above amino acid neurotransmitters, the most important are Glu, GABA and their receptors.

As the first neurotransmitter to be studied, GABA is closely related to the occurrence of epilepsy. So far, there are three types of GABA receptors, including GABA-A, GABA-B and GABA-C, among which GABA-A receptor is the most common and important inhibitory transmitter receptor in the brain. GABA-A is most closely related to epilepsy and is mainly distributed in the substantia nigra and globus pallidus in the brain. Studies have shown that GABA and its receptor functions are involved in the pathogenesis of epilepsy [1,2]. When GABA levels are reduced or absent, seizures increase or persist. On the contrary, when the level of GABA increases, it can stop the seizure.

The results showed that the expression level of nitric oxide synthase (NOS), the content of MDA and the activity of lactate dehydrogenase (LDH) in the hippocampus of hereditary epileptic rats (TRM) were all increased, while the activity of SOD was not significantly changed. Therefore, oxidative stress may be one of the pathogenesis of epilepsy. Epileptic seizures will produce oxidative stress, and continuous neuronal firing and seizures will lead to a series of physiological changes, resulting in neuronal damage and even death [3].

Modern pharmacological studies have shown that rose has the functions of eliminating free radicals, antioxidant activity, anti-thrombotic, anti-inflammatory, antibacterial, anti-cancer, immunomodulatory, lowering blood lipid and

preventing heart disease [4]. L-Trp enters the body through the diet on the one hand as the raw material for the synthesis of tissue protein, on the other hand, it is catabolized. Tryptophan (Trp) is catalyzed by tryptophan hydroxylase (TPH) and tryptophan decarboxylase (TDC) to produce 5-hydroxytryptamine (5-HT). 5-HT, also known as serotonin, is widely found in the cerebral cortex and synapses. As the most profound inhibitory neurotransmitter in the central nervous system, 5-HT has the most extensive influence on epilepsy [5]. There are seven subtypes of 5-HT1 to 5HT7, of which 5-HT1, 5-HT2, 5-HT3, 5-HT4 and 5-HT7 receptors are closely related to the occurrence of epilepsy. 5-HT3 receptor is a ligand-gated ion channel, which can inhibit epileptic seizures by changing the permeability of Na^+, K^+ and Ca^{2+} to cause depolarization of neurons [6].

At present, the treatment of epilepsy includes drug therapy, surgical treatment and so on. Medication can control seizures, but there are side effects when taken over a long period of time. Surgical treatment of epilepsy requires that the source of epilepsy is clearly located, the focus is single and limited, and the seizure cannot be controlled by drugs. But the surgical treatment conditions are harsh and risky. Medical food can both alleviate disease and sustain growth. A ketogenic diet (KD) is a diet that is high in fat and low in carbohydrates, supplemented with appropriate amounts of protein, vitamins and minerals. As early as 1920s, KD therapy has been applied in the treatment of epilepsy [7]. KD has been used in epilepsy for nearly a hundred years, mainly for the treatment of refractory epilepsy [8], with significant therapeutic effects [9,10]. Studies showed that KD therapy reduced the seizure frequency of 40% ~ 67% children with epilepsy by 50% [11].

A ketogenic diet consists of a diet high in fat, moderate in protein, and very low in carbohydrates, which encourages the body to use fat instead of glucose to make adenosine triphosphate (ATP). In the traditional ketogenic diet, the mass ratio of fat to protein plus carbohydrate is 4:1, and the energy ratio produced is about 8% protein, 2% carbohydrate and 90% fat [12,13]. In order to normalize protein and carbohydrate intake and increase patient compliance, KD has evolved from a classical ketogenic diet (LKD) to a ketogenic diet rich in medium chain triglycerides (MCT), modified Atkins diet (MAD), and a low glycemic index therapeutic ketogenic diet (LGIT). Clinical studies have found that KD has a protective effect on the nervous system. Under the condition of "sugar free", the body increases the synthesis of γ-aminobutyric acid in the brain, which limits the production of reactive oxygen species and improves the anti-injury ability of neurons [14].

This research combines the new nutrition knowledge and advanced food processing technology to design and develop the clinical symptomatic and effective anti-epilepsy special type of total nutrition medical food, which has obvious effects and has independent intellectual property rights. The product contains a huge market prospect and development potential, will have a broad market prospect, both scientific research and economic significance, more health and social benefits.

2. Materials and Methods

2.1. Method of Administration and Sample Collection

One hundred and twenty mice (male of Kunming species) were randomly divided into blank group, PTZ-1, PTZ-2, PTZ-3, PTZ-4 and PTZ-5, and weighed.

Mice were fed for a week, and PTZ was injected intraperitoneally in the blank group, with the dose of 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, respectively. After intraperitoneal injection for 30 min, the epileptic seizure grade and mortality of mice were recorded. After the observation, the eyeballs were removed and blood was collected. The SOD, CAT, GSH-Px activity and MDA content in serum of mice were detected by the kit. At the same time, the brain was removed, dried with filter paper, weighed, coated with aluminum and platinum, numbered, and stored in a -70°C refrigerator for later use.

Seizure grade in mice was evaluated by Racine [15] as follows. Grade 0, no response. Grade I, wet dog-like tremor, facial muscle twitching, clonus, tremor (such as blinking, whisker beating, rhythmic chewing, etc.). Grade II, I grade attack with rhythmic nodding. Grade III, paroxysmal seizures with forelimb clonus, but no hind limb upright movement. Grade IV, with hind limb upright motion, accompanied by oblique bilateral fall or standing with forelimb clonus. Grade V, imbalance, toppling, limb twitching, clonus, generalized rigidity, falling, rolling and loss of postural control.

2.2. Determination of Amino Acid Neurotransmitters in Brain by HPLC

The brain tissue was taken out from the refrigerator, and after it was restored to room temperature, 5 mL of 2% trichloroacetic acid was added and placed into a glass homogenizer, and homogenized in an ice bath. The homogenate was collected in a 1.5 mL centrifuge tube and centrifuged at a low temperature of 12000 r/min (4°C) for 30 min. Take 5 μl of supernatant, dilute it with 5 μl of triple distilled water, add 40 μl of OPA derived reagent, swirl it for 30 s, stand for 1 min, take 20 μl of sample.

Analysis conditions: Phenomenex Synergi Hydro-RP C_{18} column (150 mm×4.6 mm, 4 μm) and protective core (4 mm×2.0 mm) were used. Mobile phase A was sodium acetate buffer (10 mmol/L, pH was adjusted to 5.5 by glacial acetic acid), and B was acetonitrile. The gradient elution modes (T, B%) were : (0 min, 8%), (3 min, 8%), (15 min, 40%), (18 min, 8%), (20 min, 8%), and the total elution time was 20 min. The flow rate was 1 mL/min, the column temperature was 30°C, and the excitation wavelength and emission wavelength of the fluorescence detector were 340 nm and 455 nm.

2.3. Determination of Antioxidant Activity

The antioxidant activity of rose water extract was

determined by DPPH free scavenging assay. DPPH-methanol solution with a mass concentration of 0.1 mg/mL was prepared and placed in a dark place for use. Preparation of standard curve: Trolox was prepared with methanol of 25 μg/mL, 20 μg/mL, 15 μg/mL, 10 μg/mL, 5 μg/mL, 0 μg/mL to be tested. Add 80 μl to 96-well plate, and then add 120 μl of the prepared DPPH-methanol solution. The 96-well plate was placed in a constant temperature culture oscillator at 25°C without light and reacted for 30 min. The absorbance of the plate was measured at 517 nm using a microplate analyzer.

Add rose water extract 80 μl to the 96-well plate, then add 120 μl DPPH-methanol solution. The 96-well plate was placed in a constant temperature culture oscillator at 25°C without light. After reaction for 30 min, the absorbance was measured at 517 nm with a microplate analyzer. The absorbance was denoised as AI. In the blank group, the same amount of methanol solution was used to replace the samples to be tested, and the other same groups were denoted as AC. DPPH-methanol solution was replaced with the same amount of methanol solution in the control group, and the other groups were denoted as AJ. The clearance rate was calculated according to Equation (1), and the sample concentration corresponding to the clearance rate between 20% and 80% was selected. The DPPH free radical scavenging ability was calculated according to the scalar curvature.

$$\text{Clearance rate} = (1 - \frac{Ai - Aj}{Ac}) \times 100\% \quad (1)$$

2.4. Detection Index of Animal Model

When epilepsy occurs, Glu rises and GABA levels fall in the brain, and Glu: GABA rises. The current methods for testing epileptic mice include testing the GABA content in the mouse brain and electroencephalogram (EEG) testing. The EEG test is cumbersome and time-consuming as an indicator of epilepsy in mice. The Glu and GABA content in the mouse brain as an indicator for the detection of brain extraction is difficult to operate and can only be utilized once in experimental animals. Freitas [16] et al. confirmed through experiments in rats that oxidative stress plays an important role in the development of epilepsy and that the duration of epilepsy is positively correlated with oxidative damage. The antioxidant enzymes, including CAT, SOD and GSH-Px, are used in experimental animals to mitigate the damage caused by oxidative stress, and the most commonly used marker of lipid peroxidation damage is MDA. Therefore, the enzymatic activity of antioxidant enzymes and the level of malondialdehyde in the peripheral blood of experimental animals can be used as indicators for the detection of epilepsy in experimental animals. It is convenient for large-volume testing.

2.5. Statistical Analysis

The measured data were expressed as mean ± standard deviation (x±SD). SPSS software was used for statistical statistics, and t test was performed.

3. Results and Discussion

3.1. Determination of Detection Indexes for Animal Models

3.1.1. Behavioral Observations

An epileptic mouse model was constructed by intraperitoneal injection of PTZ. The seizure class of mice increased with increasing dose of PTZ, and the mortality rate of mice increased accordingly. The seizure class behavior of mice is shown in Figure 1. The seizure class and mortality rate of mice treated with different doses of PTZ are shown in Table 1.

As seen in Figure 1 and Table 1, the grade of seizures in mice increased as the injected dose of PTZ increased. When the injected dose of PTZ reached 70 mg/kg, the mice started to show mortality. the higher the injected dose of PTZ, the higher the mortality rate.

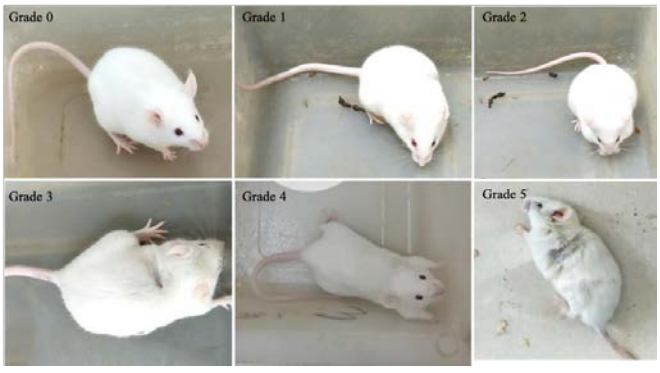

Figure 1. Seizure hierarchy behavior diagram of model mice

Table 1. Grade of epilepsy and mortality in mice

Subgroup	Dose (mg/kg)	Grade 0	Grade1	Grade2	Grade3	Grade4	Grade5	Mortality (%)
Control	0	100%	0	0	0	0	0	0
PTZ-1	50	25%	50%	25%	0	0	0	0
PTZ-2	60	10%	25%	50%	15%	0	0	0
PTZ-3	70	0	0	10%	10%	15%	65%	10%
PTZ-4	80	0	0	0	0	0	100%	20%
PTZ-5	90	0	0	0	0	0	100%	100%

3.1.2. Results of Biochemical Indexes in Mice

The results of oxidative stress indexes in the blood of mice treated with different doses of PTZ are shown in Figure 2. The contents of GABA, Glu and Glu: GABA ratio in the brain are shown in Figure 3.

As shown in Figure 2, the enzyme activities of GSH-Px, CAT, and SOD in mice serum decreased with the increase of seizure grade. When the intraperitoneal dose of PTZ reached 90 mg/kg, the enzyme activities of GSH-Px, CAT and SOD were the lowest, with enzyme activities of 46.45±2.70 U/mL, 48.76±12.76 U/mL and 1.88±0.53 U/mL, respectively. 47.46%, 37.81% and 49.33% were reduced compared with the blank group. As the grade of epileptic grand mal increased, the MDA content in the serum of mice increased. The highest MDA level (20.12±2.51 nmol/mL) was observed when the PTZ intraperitoneal dose reached 90 mg/kg, which was 30.23% higher compared with the blank group.

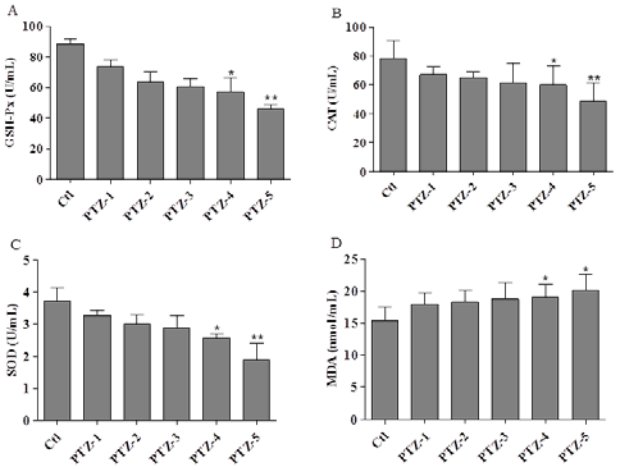

Figure 2. Indicators of oxidative stress in the blood of mice

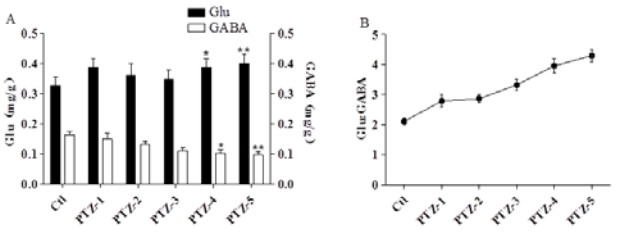

Figure 3. GABA and Glu content in mouse brain and Glu: GABA

As shown in Figure 3, PTZ-4 had a significant difference ($p < 0.05$) and PTZ-5 had a highly significant difference ($p < 0.01$) compared to the blank group. It can be concluded that intraperitoneal injection of PTZ-4 or PTZ-5 can be used as epilepsy model constructs. Glu: GABA ratio increased with the increase of epilepsy grade.

The changes of Glu: GABA in Figure 3 are consistent with the changes of oxidative stress indicators in Figure 2. The Glu and GABA content in the brain of experimental animals as assay indicators for brain extraction is difficult to operate, and experimental animals can be utilized only once. Therefore, the antioxidant enzyme activity and malondialdehyde content in the blood of experimental animals can be used as indicators for the detection of epilepsy in experimental animals, which is convenient for high-volume testing.

3.2. Functional Substance Preparation

The laboratory obtained high GABA-producing Lactobacillus shortum by mutagenesis breeding. The GABA transformation solution was obtained by transformation at pH 4.38, temperature 30°C, inoculum level 20.07 g/L and monosodium glutamate addition 69.49 g/L. The GABA was obtained after concentration, filtration, crystallization and recrystallization by decolorization at pH 5.09, temperature 30°C and activated carbon addition of 1.35%. The high purity GABA was prepared with a product purity of 99.5%, which meets the requirement of food grade GABA production.

The optimal dosages of GABA, tryptophan and rosea were determined to be 100 mg/kg, 160 mg/kg and 1.6 g/kg, respectively, by behavioral and serum oxidative stress indicators in mice. Based on the above findings, three medical formulations containing GABA+tryptophan (formulation 1), GABA+rose (formulation 2), and tryptophan (formulation 3) were formulated for the relief of epilepsy.

3.3. Functional Research of Functional Substances

3.3.1. Experimental Method

Fifty mice (Kunming breed males) were randomly divided into five groups. They were divided into formulated food 1, formulated food 2, formulated food 3, blank group, and model group according to the feeding of formulated food.

After mice were fed for one week, epilepsy models were constructed in all groups except the blank group. PTZ 45 mg/kg was injected intraperitoneally for 4 weeks, and the epilepsy model was observed for 30 min after each injection to determine the success of epilepsy model construction (appearance of epileptic behavior).

Epileptic mice were gavaged according to the grouping. The blank and model groups were fed chow, and the remaining subgroups were fed differently according to formulated food. After two weeks of feeding, PTZ 45 mg/kg was injected intraperitoneally again. 30 min were observed and the mice were recorded for seizures. Blood was removed from the eyes of the mice immediately after the observation and stored at 4°C with the addition of anticoagulant. The content of MDA and the enzyme activity of CAT, GSH-Px and SOD in the blood of mice were measured using the kit.

Table 2. Formulation composition and content (100 mL)

Component	Content	Component	Content
Energy (kcal)	100	GABA(mg)	3.15
Protein (g)	4.75	Rosette (g)	0.441
Fat (g)	7.75	Tryptophan (mg)	53.55
Carbohydrates (g)	3	Soy phospholipids (g)	4
Mineral premix (g)	3	Vitamin premix(g)	0.15

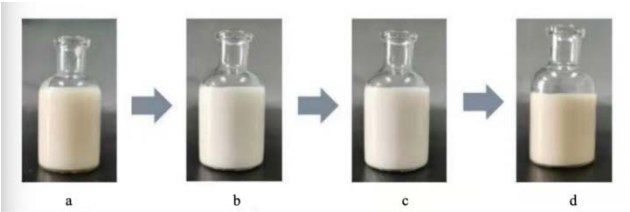

Figure 4. Medical formulation process (a: Preliminary mixing. b: High speed shear, 3500 rpm, 20 min. c: 100 bar, Homogenization four times. d: 121°C, 10 min）

3.3.2. Medical Food Performance Measurement

Three medical formulations for epilepsy relief were subjected to centrifugal stability and dilution 1000 times for particle size, span (PDI) and zeta-potential, and the results are shown in Figure 5.

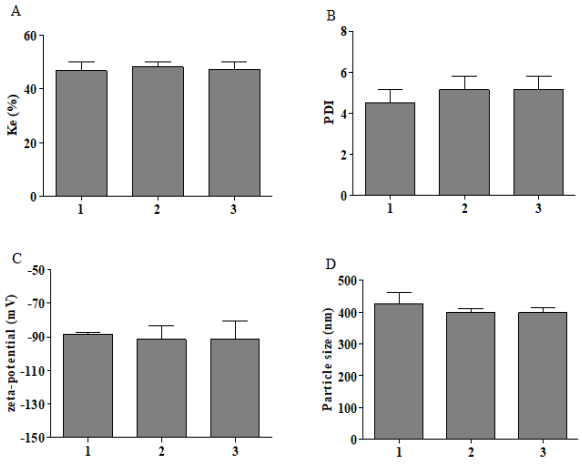

Figure 5. Diluted zeta-potential, particle size, span and centrifugal stability of formulated foods 1, 2 and 3

As shown in Figure 5, the particle sizes of the three formulations were 391.80 nm, 411.20 nm, and 382.90 nm, respectively; the span distances were 3.84, 5.18, and 4.46, respectively; the zeta-potentials were -89.54 mV, -92.63 mV, and -93.28 mV, respectively; and the centrifugal stabilities were 43.50%, 46.30%, and 44.3%, respectively. 46.30%, and 44.3%, respectively. From the data, it can be seen that the three emulsion formulations have good stability.

Three formulations of acute oral toxicity, stability and microbiological indicators test

A. Acute oral toxicity test

No abnormal reactions were observed in all mice after the original solution of formula food 1, 2 and 3 was given by gavage. No obvious symptoms of poisoning were observed for 14 consecutive days. The animals did not die, and no obvious abnormalities were seen in gross autopsy, and the results are shown in Table 3.

Mice were orally gavaged with formula food liquid amounting to 16 g/kg BW twice a day. The results showed that the maximum tolerated dose (MTD) of the sample was greater than 16 g/kg BW for both sexes. According to the acute toxicity classification standard, the sample is actually non-toxic grade.

B. Stability test

Formula food was accelerated for four times in three months under the condition of temperature 37±2°C and humidity 75±5%. The experimental results are shown in Table 4.

After four accelerated stability experiments in three months, according to the national health food registration review principles, the shelf life can be considered stable product quality within 24 months.

C. Microbiology experiments

According to GB 4789.2-2016 to determine the total number of bacteria in the formula, the test results are shown in Table 5.

Formulated foods 1-3 were sterilized, and no microbial colonies were seen in the formulated foods, which are sterile.

Table 3. Results of acute oral toxicity experiments in mice with formulated foods 1-3

	Sex	Dose(g/kg BW)	Number of animals	Body weight(g, $\bar{x}$ ±s)			Number of animal deaths	MTD (g/kg BW)
				Initial	On the seventh day	End		
Formula 1	♂	16	10	20.22±0.82	28.72±1.71	33.77±2.50	0	>16
	♀	16	10	20.11±0.84	25.39±1.99	27.88±1.92	0	>16
Formula 2	♂	16	10	20.11±0.82	29.74±2.17	34.40±2.53	0	>16
	♀	16	10	19.72±0.87	24.91±2.27	27.54±1.98	0	>16
Formula 3	♂	16	10	20.22±0.87	29.83±1.64	34.01±2.73	0	>16
	♀	16	10	19.72±0.69	25.82±2.90	27.95±3.26	0	>16

Table 4. Stability test results of formula foods 1-3

		Protein (g/100g)	Fatty Acid (g/100g)	GABA (g/100g)	Tryptophan (g/100g)	Total Flavonoids (g/100g)
Formula 1	Zeroth months	2.68	5.57	4.06×10^{-4}	0.11	—
	The first month	2.64	5.76	4.05×10^{-4}	0.11	—
	The second month	2.68	5.62	3.95×10^{-4}	0.11	—
	The third month	2.64	5.84	4.52×10^{-4}	0.11	—
Formula 2	Zeroth months	2.93	5.96	4.28×10^{-4}	—	0.0028
	The first month	2.92	6.06	4.43×10^{-4}	—	0.0026
	The second month	2.96	5.86	4.29×10^{-4}	—	0.0028
	The third month	2.98	5.97	4.52×10^{-4}	—	0.0027
Formula 3	Zeroth months	2.77	5.37	—	0.11	—
	The first month	2.78	5.54	—	0.11	—
	The second month	2.74	5.70	—	0.11	—
	The third month	2.74	5.63	—	0.11	—

Table 5. Results of microbiological experiments on formulated foods 1-3

	Total number of colonies(CFU/Ml)
Formula 1	<1
Formula 2	<1
Formula 3	<1

Table 6. Body weight of mice at different stages ($\bar{x}$±SD, g)

Group	Number of animals	Before experiment (g)	After modeling (g)	Before Execution (g)
Formulation 1 (GABA + Tryptophan)	10	23.37±1.05	29.07±1.45	30.38±1.96
Formulation 2 (GABA + Tryptophan)	10	23.70±1.95	28.20±2.44	30.13±2.39
Formulation 3 (Tryptophan)	10	23.66±2.60	28.72±2.77	29.98±2.97
PTZ group	10	23.64±1.75	28.47±2.74	30.64±3.52
Blank group	10	25.17±1.97	28.61±3.22	30.48±3.77

Note: Compared to blank: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; Compared to the model group: $^{\Delta}p<0.05$, $^{\Delta\Delta}p<0.01$, $^{\Delta\Delta\Delta}p<0.001$

As can be seen from Tables 3-5, stability, toxicology and hygiene studies were conducted on the three formulas, and the products were non-toxic grade after acute oral toxicity experiments. After three months of four accelerated stability experiments, according to the national health food registration review principles, the shelf life can be considered stable product quality within 24 months.

3.3.3. Epilepsy Trial With Medical Food

3.3.3.1.Body Weight Change in Mice

The effects of the 3 formulations of food fed for 2 weeks on the body weight of mice are shown in Table 6.

After the administration, the body weight of the mice all increased, and there was no significant difference between the body weight of the mice in the administration group and the blank group, and no significant difference compared with the model group, which proved that the three medical foods can maintain the normal growth needs of mice.

3.3.3.2. Behavioral Results in Mice

The effects of the 3 formulas fed for 2 weeks on the duration of seizures in the model mice are shown in Figure 6 and Figures 7.

Compared with the model group, the time from intraperitoneal injection to seizure was prolonged by 1 min, and seizure symptoms were reduced and seizure duration was shortened.

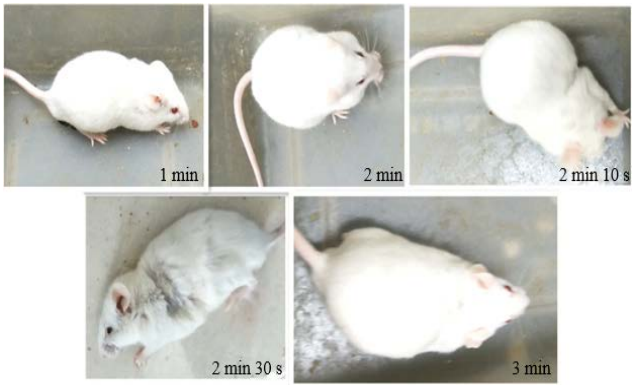

Figure 6. Seizure duration in epileptic mice

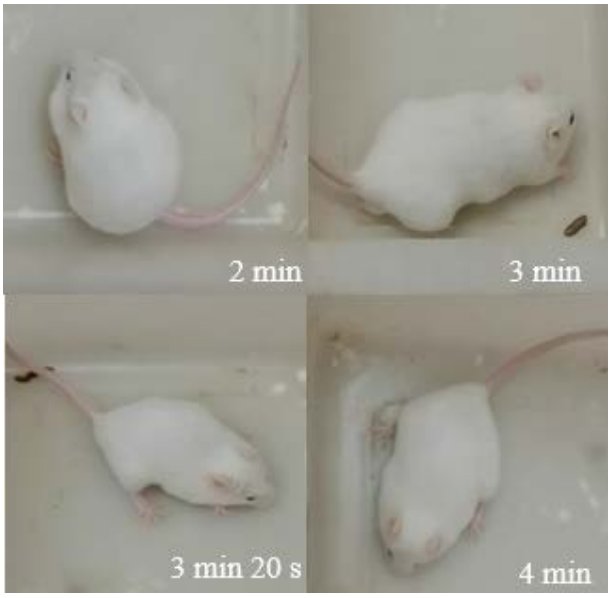

Figure 7. Seizure duration in mice with formulated foods

3.3.3.3. Effect of Medical Foods on Oxidative Stress Indicators in Blood

The effects of the 3 formulas fed for 2 weeks on the oxidative stress indicators in the blood of the model mice are shown in Figure 8.

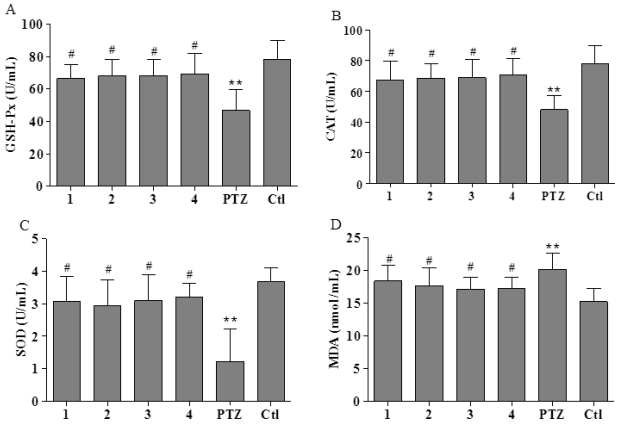

Figure 8. Effect of medical foods on oxidative stress indicators in blood

As shown in Figure 8, the levels of GSH-Px, CAT, SOD and MDA in the model group of mice were significantly different compared with the blank group ($P<0.05$). Thus, it is clear that the epilepsy model was successfully constructed.

Compared with the model group, the three medical foods showed significant differences ($P<0.05$) on GSH-Px, CAT, SOD enzyme activity and MDA content in mice serum. This indicates that the 3 medical foods have significant effects on relieving epilepsy.

4. Conclusion

Efficacy screening was performed according to the animal model of epilepsy to identify nutrients such as protein, carbohydrates, fatty acids, vitamins, minerals and other functional substances formulated with GABA that are suitable for epilepsy patients. The centrifugal stability was used as an index to determine the whole nutritional formula food for relieving epilepsy through single-factor experiments. The formulated food contained 4.75% protein, 7.75% fatty acids, 3% carbohydrates and appropriate amounts of vitamins and minerals. The optimal preparation conditions for the emulsions were determined as 3500 r/min, high speed shear for 20 min, homogenization at 100 MPa pressure for 4 times, and sterilization at 121°C for 10 min. Three medical formula food emulsions containing GABA+tryptophan (formula food 1), GABA+rose (formula food 2), and tryptophan (formula food 3) for the relief of epilepsy were obtained.

Stability, toxicology and sanitation studies were conducted on the three formulations, and the products were non-toxic grade after acute oral toxicity experiments. After three months of four accelerated stability experiments, according to the national health food registration review principles, the shelf life can be considered stable product quality within 24 months.

Efficacy experiments were conducted on the three products, and it was determined that the three products had no significant effect on the body weight of mice through the detection of behavioral and blood oxidative stress indicators. The seizure latency of the mice was prolonged by 1 min, while the enzyme activities of SOD, CAT and GSH-Px in the blood of the mice increased and the MDA content decreased.

Conflict of Interest

The authors declared that they have no conflicts of interest to this work.

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