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The anti-depression effect of Xylaria nigripes in patients with epilepsy: A multicenter randomized double-blind study

Wei-Feng Peng [a], Xin Wang [a,b,*], Zhen Hong [c], Guo-Xing Zhu [c], Bing-Mei Li [d], Ze Li [e], Mei-Ping Ding [f], Zhi Geng [g], Zheng Jin [h], Ling Miao [i], Li-Wen Wu [j], Shao-Kang Zhan [k]

[a] Department of Neurology, Zhongshan Hospital, Fudan University, Shanghai, China
[b] The State Key Laboratory of Medical Neurobiology, The Institutes of Brain Science and the Collaborative Innovation Center for Brain Science, Fudan University, Shanghai, China
[c] Department of Neurology, Huashan Hospital, Fudan University, Shanghai, China
[d] Epilepsy Center of the Second Affiliated Hospital of Guangzhou Medical University, Guangdong Province, China
[e] Department of Neurology, The First Municipal Hospital of Guangzhou, Guangdong Province, China
[f] Department of Neurology, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang Province, China
[g] Department of Neurology, The Sixth People's Hospital of Shanghai Jiaotong University, Shanghai, China
[h] Department of Neurology, The Fifth People's Hospital, Fudan University, Shanghai, China
[i] Department of Neurology, Renji Hospital, Shanghai Jiaotong University, Shanghai, China
[j] Department of Neurology, Peking Union Medical College Hospital, Beijing, China
[k] Institute of Statistics and Public Health, Shanghai Medical College, Fudan University, China

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ABSTRACT

Purpose: The comorbidity of depression in patients with epilepsy is common and treatment is still controversial. This pilot study was aimed at evaluating the efficacy and safety of Xylaria nigripes for treating depressive symptoms in patients with epilepsy during 12 weeks of treatment.
Methods: A multicenter, double-blind, placebo-controlled, randomized superiority study was performed. A total of 104 patients with epilepsy who fulfilled the study criteria were randomized 1:1 to receive Xylaria nigripes (the Wu Ling group) or placebo (the placebo group) treatment in the 12-week period of study. The participants were visited on weeks 0, 2, 4, 8, and 12 of the treatment course.
Results: Eighty-one patients finished all of the visits. The primary efficacy endpoint in this study was the total effective rate for depression, which was significantly greater in the Wu Ling group (51.3%, $n = 39$) than in the placebo group (35.7%, $n = 42$, 0.51–0.36 = 0.15, 95% CI −0.06 to 0.37, $U = 2.83$, $P = 0.002$) after 12 weeks of treatment. No differences in seizure frequency or changes in severity were found between the Wu Ling and the placebo groups. In addition, the quality of life and seizure worry subscale scores in patients with epilepsy were also improved more notably in the Wu Ling group than in the placebo group ($P < 0.05$). Most of the adverse effects (AEs) in this study were mild and had no differences between the Wu Ling and the placebo groups.
Conclusion: Xylaria nigripes could alleviate depressive symptoms within 12 weeks treatment and was well tolerated in patients with epilepsy.

1. Introduction

Depression is the most frequently occurring psychiatric comorbidity in patients with epilepsy, with an average prevalence of 20–30% [1,2]. Depression has significant negative impacts on quality of life in patients with epilepsy [3], and is associated with a poor response to pharmacological and surgical treatments of epileptic disorders [4,5].

However, depressive symptoms in epilepsy have not been fully recognized by neurologists. The treatment for comorbid depression in patients with epilepsy is also challenging. Some reports of pharmaceutical and psychotherapeutic approaches for depressive symptoms in patients with epilepsy are open-labeled [6–9]. The safety of selective serotonin reuptake inhibitor (SSRI) antidepressants in patients with epilepsy is still controversial. Although some

* Corresponding author at: Department of Neurology, Zhongshan Hospital, The State Key Laboratory of Medical Neurobiology, The Institutes of Brain Science and the Collaborative Innovation Center for Brain Science, Fudan University, No. 180, Fenglin Road, Shanghai 200032, China.
Tel.: +86 021 64041990x8022; fax: +86 21 65649416.
E-mail address: wang.xin@zs-hospital.sh.cn (X. Wang).

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investigations showed that SSRIs had little effects on seizure frequency [10,11], others indicated that SSRIs could exacerbate seizures by lowering the seizure threshold or by interacting with antiepileptic drugs (AEDs) [12,13], especially when taken in overdose [14,15]. There have been no guidelines, expert consensus or recommendations for the treatment of comorbidity of depression in patients with epilepsy until now. Randomized clinical trials and new therapies are urgently needed.

Xylaria nigripes (also referred to as Wu Ling Shen) is a traditional Chinese medicine which belongs to the Xylariaceae family of fungi. It grows several feet underground in the fungus combs of the Odontotermes termite species during the spring and summer seasons, and contains many bioactive molecules such as glycosides, steroids and amino acids [16,17]. Studies demonstrated Xylaria nigripes had antidepressant and sleep-regulating effects, and was used to treat depression and insomnia in clinical practice [18,19]. In a multicenter randomized double-blind parallel study, Xylaria nigripes (under the brand name 'Wu Ling Capsule') had a similar effect to Deanxit to alleviate depressive, anxiety and insomia symptoms in patients with depression [20]. In animal studies, Xylaria nigripes was found to prolong the latency of convulsive seizure and delay the kindling process in penty1enetetrazol induced rat epilepsy models [21]. The pharmacological mechanisms of Xylaria nigripes appeared to be complicated. As indicated by Ma et al. [22], Xylaria nigripes promoted the activity of glutamate decarboxylase and the combination of γ-aminobutyric acid (GABA) with GABA receptors. It also had anti-oxidative and anti-inflammatory effects [23–25]. In this study, therefore, we used Xylaria nigripes as an anti-depressive therapy in patients with comorbidity of epilepsy and depression to evaluate its effects on depression and safety for seizures.

The primary objective of this study was to evaluate the efficacy of Xylaria nigripes on depressive symptoms in patients with epilepsy during 12 weeks of treatment. The secondary objectives included two aspects: (1) assessing the influences of Xylaria nigripes on seizure frequency, seizure severity, sleep quality and quality of life for patients with epilepsy; (2) evaluating the safety of Xylaria nigripes in patients with epilepsy.

2. Methods

2.1. Study design

This study (temporarily referred to as WL-2010) was a multicenter, double-blind, randomized, placebo-controlled, superiority clinical trial. The patients in this study were randomized 1:1 to receive Xylaria nigripes (the Wu Ling group) or a placebo (the Placebo group) for 12 weeks.

For the primary endpoint, the total effective rate for depressive symptoms in patients with epilepsy was compared between the Wu Ling and the Placebo groups at the end of 12 weeks' treatment. The sample size was determined to be about 100 cases for each group by the statistical calculation formula, using at least 10% difference of the total effective rate for depression between the Wu Ling and the Placebo groups. Randomization was performed centrally by the Clinical Research Organization (CRO). The random numbers were generated using the PROC PLAN process of the SAS 9.2 software. Randomization lists were assigned to every study center. Investigators and participants were all blinded to the assignment. The Wu Ling Capsule and the placebo could not be differentiated from appearance.

All of the measurement scales in this study, including the Hamilton Depression Rating Scale (HAMD), the National Hospital Seizure Severity Scale (NHS3), the Pittsburgh Sleep Quality Index (PSQI), and the Quality of Life in Epilepsy Inventory (QOLIE-31) were evaluated in each center by one or two neurological doctors who had been trained in advance and whose evaluations were found to be in good agreement.

The protocol of the trial was approved by the local independent ethics committees of all the participating hospitals. All the patients recruited had to agree with and sign a written informed consent prior to entering the study. This study had been registered at the ClinicalTrial.gov system of the U.S. National Institutes of Health and the Identifier was NCT01125241.

2.2. Patients

The patients were recruited from the epileptic clinics of nine hospitals located in Shanghai, Hangzhou, Guangzhou and Beijing. Patients were enrolled if they consented to participate in the study and met the following inclusion criteria: (1) older than or equal to 18 years of age (no gender limitation); (2) a diagnosis of epilepsy based on clinical data and EEG recording, and having had regular treatment with AEDs for at least 6 months, without recent adjustments to medication, in order to avoid the confounding effects of AEDs adjustment; (3) at least 24 h from the last seizure before accepting psychological evaluation to avoid the effect of postictal state; (4) having a HAMD score of greater than or equal to 17; (5) not taking any antidepressants or antipsychotics in the previous 2 weeks.

The exclusion criteria included the following: (1) having a history of psychiatric symptoms other than depression; (2) having a history of suicidal thoughts or suicidal behaviors; (3) having severe cognitive impairment, chronic organic failures or malignant tumors; (4) liver function tests indicating alanine aminotransferase (ALT) or aspartate aminotransferase (AST) higher than 1.5 times the normal reference value, or a white blood count of less than 2500/μl, or a neutrophils count of less than 1000/μl; (5) being pregnant or lactating.

The patients were fully informed of the benefits and risks of entering this study. They had the right to drop out the study at any time if they felt no improvement of depression or experienced exacerbation of seizures. They would be recommended to visit a psychiatrist if they had suicidal thoughts or had very severe depressive symptoms. If they improved markedly and wanted to continue taking the medicine, they would receive another extra free supply of the Wu Ling Capsule for 3 months after ending the study. This extra period of taking the Wu Ling Capsule was also monitored.

2.3. Study endpoints

The efficacy of Xylaria nigripes on depression in patients with epilepsy was evaluated by the HAMD reductive rate. As firstly recommended by Snaith et al. [26], the HAMD scale has become a common tool to assess severity of depressive symptoms and the efficacy of treatment [27]. The HAMD reductive rate is calculated as: (score before treatment − score after treatment)/score before treatment × 100%. There are four levels of efficacy based on the HAMD reductive rate: ≥75% means clinical recovery, 50–74% means significantly improved, 25–49% means slightly improved, and <25% means ineffectiveness. The total effective rate for depression is calculated as: (clinical recovery rate + significantly improved rate)/*n*. The primary efficacy endpoint was to evaluate the total effective rate for depression in patients with epilepsy. The secondary efficacy endpoints included changes in seizure frequency, seizure severity (the NHS3 score), sleep quality (the PSQI score) and quality of life (the QOLIE score). Adverse events (AEs), laboratory data and vital signs were analyzed as safety variables.

2.4. Study procedures

The Wu Ling Capsule and the placebo were both produced by Zhejiang Jolly Pharmaceutical Limited Corporation (Hangzhou,

Zhejiang Province, China). After randomization, the participants were assigned to a parallel group and administered with the Wu Ling Capsule or the placebo orally at a dose of three tablets (each containing 0.33 g of the drug or placebo powder) three times daily for 12 weeks.

The participants were regularly visited at weeks 0, 2, 4, 8, and 12 of the treatment course. Demographic, seizure-related and medication data were collected before initiation of the treatment. The seizures were diagnosed and classified according to the International League Against Epilepsy (ILAE 1981) classification of epileptic seizures.

The severity of depression was assessed by using the 17-item Hamilton Depression Rating Scale (HAMD) in weeks 0, 2, 4, 8 and 12 of the treatment period. The Chinese version of HAMD has been tested and proved to have good validity and reliability [28].

The PSQI was used to evaluate the sleep quality of patients in weeks 0, 4, 8 and 12. The PSQI scale evaluates seven aspects of sleep quality including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disruption, use of sleeping medication, and daytime dysfunction. The total PSQI score is positively correlated with sleep disturbance and a score of $\geq$8 suggests bad sleep quality [29].

QOLIE-31 was assessed in weeks 0 and 12 of the treatment period. Seven subscales are included in QOLIE-31: overall quality of life, seizure worry, emotional well-being, energy/fatigue, cognitive function, medication effects and social function. A high QOLIE-31 score indicates a good quality of life [30].

Diaries to record seizures were given to every patient at the initiation of treatment. The *R* ratio is the ratio of change in seizure frequency to the sum of baseline and final seizure frequencies. The *R* ratio is calculated as: (final − baseline)/(final + baseline). The value of *R* lies between −1 to +1, and −0.33 equates to a 50% reduction in seizure frequency [31]. The severity of seizures in patients with epilepsy in the previous 3 months was evaluated by using NHS3 in weeks 0 and 12 of the treatment period [32].

Vital signs, including heart rate and blood pressure, and adverse events were monitored during the whole treatment period. Regular blood and urine tests, liver and renal function tests, electrocardiogram (ECG) and electroencephalogram (EEG) were carried out before the initiation and at the end of treatment. The abnormal rate of EEG was regarded as a parameter of evaluating seizure aggravation.

2.5. Statistical analyses

All statistical analyses were performed using SAS 9.2 software. The superiority trial employed statistical methods which were used to judge whether the improvement of depressive symptoms in the Wu Ling group was better than in the placebo group. Data analyzed in this study were from the per-protocol population. Quantitative variables are presented in the format "mean ± stan-standard deviation (SD)". Categorical variables are described by frequency and percentage. Hypothesis testing on comparisons between groups were carried out with an α-level of 0.05 and were two-sided. An independent *t*-test or Chi-square test was used when data conformed to a normal distribution; otherwise the Wilcoxon rank sum test was used. The comparisons of efficacy parameters between groups were conducted after adjusting for the effects of different study centers. A superiority test was performed to test the difference in total effective rate for depression between groups, using the following formula: $u = [(P_T - P_C) - \delta_0]/S_{PT\text{-}PC}$ (set $\pi_T - \pi_C > \delta = 0.10$). AEs are summarized for both groups and presented as the percentage of subjects having an event, which was compared between the Wu Ling and the Placebo groups using a Chi-square test.

3. Results

3.1. Description of patients

The recruitment period was from November 2010 to August 2013. The study ended before the target sample size was reached because the HAMD scores of many patients screened were less than 17 points, the process of recruiting was slow and the guarantee period of the study drug had expired.

By the end of the recruitment period, a total of 144 patients were screened, of which 104 patients fulfilled the study criteria and were included in this study. Forty patients were excluded for the following reasons: HAMD score <17 (30/40), elevated liver enzymes (2/40), not willing to enter study (5/40), and adverse effects of AEDs (3/40). The included patients were randomized to form a baseline before starting treatment. Four patients did not take the medicine after randomization and were excluded from the analysis. Therefore, the number of cases meeting per-protocol population and included in the analysis was 100. Ten patients dropped out in week 4, six patients in week 8, and one patient in week 12. The reasons for dropout were mostly withdrawal of consent (11 patients), and some were due to adverse events (4 patients) or loss of contact (1 patient). One patient dropped out because of an attack of SE, but she was verified to be in the placebo group after urgently unblinding (see Fig. 1).

As shown in Table 1, almost all of the demographic and seizure-related variables had no differences between the Wu Ling group ($n = 51$) and the Placebo group ($n = 49$) at baseline. The AEDs taken in this study were carbamazepine (CBZ), valproate (VPA), topiramate (TPM), oxcarbazepine (OXC), lamotrigine (LTG), levetiracetam (LEV), phenobarbital (PB), phenytoin (PHT), and clonazepam (CZP). There were no significant differences in the percentages of patients taking each kind of AED in the Wu Ling and the Placebo groups (see Table 1). The HAMD, PSQI and QOLIE-31 total scores and their subscale scores all showed no differences between the Wu Ling and the Placebo groups ($P > 0.05$). The total HAMD score ranging from 17 to 24 is regarded as an indicator of moderate depression which indicates severe depression when greater than 24. Patients with moderate depression dominated both the Wu Ling and the Placebo groups, at levels of 92.2% and 95.9%, respectively. The percentages of patients with bad sleep quality (PSQI score $\geq$ 8) were also similar in the Wu Ling and the Placebo groups, at 66.7% and 69.4%, respectively.

3.2. Efficacy of Wu Ling Capsule on depressive symptoms in patients with comorbidity of epilepsy and depression

The total effective rate for depression in weeks 2, 4, 8 and 12 was compared between the Wu Ling and the Placebo groups using superiority testing and adjusting for the effects of different centers. The results show that the total effective rate for depression in week 12 was significantly greater in the Wu Ling group (51.3%, $n = 39$) than in the Placebo group (35.7%, $n = 42$, 0.51–0.36 = 0.15, 95% CI −0.06 to 0.37, $U = 2.83$, $P = 0.002$), but there were no differences in week 2 (4.0% vs. 14.6%), 4 (23.3% vs. 22.2%) and 8 (35.9% vs. 34.9%) between the Wu Ling and the Placebo groups (see Fig. 2a). The total HAMD score at baseline showed no difference between the Wu Ling and the Placebo groups. The total HAMD scores and the differences in the HAMD scores before and after treatment (DHST) in weeks 2, 4, 8 and 12 were also compared between the Wu Ling and the Placebo groups. The results showed that in week 12 the total HAMD score (9.9 ± 5.2 vs. 12.1 ± 5.3, $Z = -2.088$, $P = 0.0367$) was significantly lower in the Wu Ling group than in the Placebo group, while the DHST (10.3 ± 4.69 vs. 6.9 ± 4.34, 10.3–6.9 = 3.4, 95% CI 1.38–5.38, $t = 3.37$, $P = 0.001$) was significantly greater in the Wu Ling group than in the Placebo group. There were no

Fig. 1. Flow chart of enrollment and follow-up of patients.

differences of total HAMD score and DHST in weeks 2, 4 and 8 between the Wu Ling and the Placebo groups (see Fig. 2b and c).

3.3. *Effects of Wu Ling Capsule on* R *ratio and NHS3 score in patients with comorbidity of epilepsy and depression*

The *R* ratio and NHS3 score which represented changes in seizure frequency and severity before and after 12 weeks' treatment were compared for the Wu Ling and the Placebo groups. As shown in Table 2, the *R* ratio, the NHS3 score at baseline, the NHS3 score at the end of treatment and the difference in NHS3 score before and after 12 weeks of treatment all showed no differences between the Wu Ling and the Placebo groups ($P > 0.05$, see Table 2).

3.4. *Effects of Wu Ling Capsule on sleep quality and quality of life in patients with comorbidity of epilepsy and depression*

The PSQI score at baseline and in weeks 4, 8 and 12 of the follow-up visits, and the difference in PSQI score before and after treatment were all compared for the Wu Ling and the Placebo groups. The results show that the PSQI score was slightly lower and the difference in the PSQI score before and after treatment was slightly greater in week 12 in the Wu Ling group than in the Placebo group (5.9 ± 3.5 vs. 7.6 ± 4.0, $Z = -1.904$, $P = 0.0568$ and 3.5 ± 4.12 vs. 1.9 ± 3.65, $t = 1.84$, $P = 0.070$ respectively), but these differences were not statistically significant. There were also no differences in PSQI score and in the change in PSQI score before and after treatment in weeks 4 and 8 follow-up visits, between the Wu Ling and the Placebo groups.

The total QOLIE-31 score and the seven subscales scores of the Wu Ling and the Placebo groups were also compared. There were no differences in the QOLIE-31 total score and the seven subscales scores between the Wu Ling and the Placebo groups at baseline. The differences in the total QOLIE-31 score (-6.9 ± 9.2 vs. -2.5 ± 7.0, $t = 2.37$, $P = 0.020$), the overall quality of life score (-6.5 ± 8.5 vs. -1.9 ± 6.3, $t = 2.75$, $P = 0.008$) and the seizure worry score (-6.9 ± 10.4 vs. -2.0 ± 9.6, $t = 2.15$, $P = 0.035$) before and after 12 weeks of treatment were all significantly greater in the Wu Ling group than in the Placebo group (see Fig. 3).

3.5. *Evaluation of tolerability*

3.5.1. *Adverse events*

There were 13 (13/51) patients in the Wu Ling group and 16 (16/49) patients in the Placebo group who reported AEs. There was no significant difference between the two groups in the

Table 1
Characteristics of patients with epilepsy in the Wu Ling and the placebo groups.

	Wu Ling group $n=51$	Placebo group $n=49$	*P* value
Age (year) mean ± SD	40.1 ± 13.4	45.2 ± 14.7	0.07
Male: female	19:32	25:24	0.17
Educational degree (≤9 years: >9 years)	20:31	27:22	0.11
Marital status (married:unmarried) (married/unmarried/divorced/widowed)	33:18	36:13	0.34
Height (cm) mean ± SD (full/part/retired/unemployed/student)	164.5 ± 8.1	164.5 ± 7.2	0.98
Weight (kg) mean ± SD	59.2 ± 10.6	61.9 ± 12.1	0.24
Duration of epilepsy (year) mean ± SD	19.0 ± 37.3	25.0 ± 51.0	0.28
Seizure types (GTCS/CPS/SPS/sGTC/AS/ATS)	17:26:3:16:1:1	16:23:5:12:1:0	0.79
Etiologies *n* (%)			
Brain injury	10 (19.6%)	8 (16.3%)	0.64
Intracranial infection	5 (9.8%)	1 (2.0%)	0.22
Birth asphyxia	4 (7.8%)	2 (4.1%)	0.69
Intracranial surgery	4 (7.8%)	11 (22.4%)	0.05
Unknown reasons	28 (54.9%)	27 (55.1%)	0.98
Seizure frequency in last 3 months mean ± SD	1.85 ± 2.37	2.33 ± 3.53	0.33
NHS3 score mean ± SD	11.6 ± 7.0	10.7 ± 7.0	0.53
History of SE (%)	7%	4%	0.17
AEDs used *n* (%)			
CBZ	20 (39.2%)	22 (56.4%)	0.56
VPA	28 (54.9%)	30 (61.2%)	0.52
TPM	13 (25.5%)	14 (28.6%)	0.77
OXC	3 (5.9%)	3 (6.1%)	0.96
LTG	9 (17.6%)	5 (10.2%)	0.28
LEV	5 (9.8%)	3 (6.1%)	0.50
PB	3 (5.9%)	2 (4.1%)	0.68
PHT	2 (3.9%)	0	0.16
CZP	6 (11.8%)	8 (16.3%)	0.51

AEDs, antiepileptic drugs; GTCS, generalized tonic clonic seizure; CPS, complex partial seizure; SPS, simple partial seizure; sGTC, secondary generalized tonic clonic seizure; AS, absence seizure; ATS, atonic seizure; NHS3, National Hospital Seizure Severity Scale; SE, status epilepticus; CBZ, carbamazepine; VPA, valproate; TPM, topiramate; OXC, oxcarbazepine; LTG, lamotrigine; LEV, levetiracetam; PB, phenobarbital; PHT, phenytoin; CZP, clonazepam; SD, standard deviation.

number of AEs. One severe AE of SE was reported, but it was demonstrated that the patient was in the Placebo group and the AE was not related to the Wu Ling Capsule treatment. The symptoms of AEs can be categorized as follows by system organ class: (1) general disorders: fatigue; (2) gastrointestinal disorders: abdominal discomfort, elevated liver enzyme, constipation and increased saliva; (3) nervous system disorders: dreaminess, dizziness, feeling depressed; (4) respiratory, thoracic and mediastinal disorders: anterior thoracic discomfort, palpitation and ablepsy; (5) skin and subcutaneous tissue disorders: skin rash and sneezing. The most frequently reported AEs were fatigue (5%) and palpitations (4%). No significant differences in the above AEs were found between the Wu Ling and the Placebo groups (see Table 3).

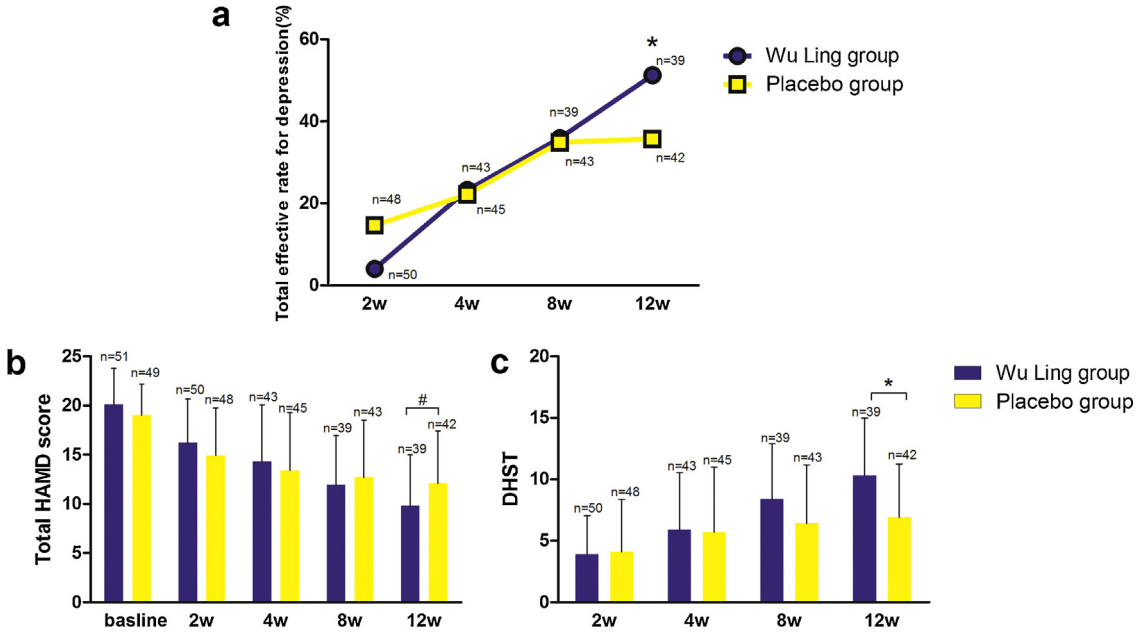

Fig. 2. (a) The total effective rate for depression in week 12 was significantly greater in the Wu Ling group than in the Placebo group, which had no difference between the two groups in weeks 2, 4 and 8, $^{*}P = 0.002$. (b, c) HAMD total score was significantly less while DHST was significantly greater in the Wu Ling group than in the Placebo group in week 12, $^{\#}P = 0.0367$, $^{*}P = 0.001$. HAMD, Hamilton Depression Rating Scale. DHST, the difference in HAMD score before and after treatment.

Table 2
Comparisons of R ratio and NHS3 score between the Wu Ling and the placebo groups.

Items mean ± SD (*n*)	Wu Ling group	Placebo group	*P* value
R ratio	−0.3 ± 0.8 (36)	−0.5 ± 0.7 (39)	0.23
NHS3 score at baseline	11.6 ± 7.0 (50)	10.7 ± 7.0 (48)	0.53
NHS3 score at the end of treatment	5.8 ± 5.4 (39)	5.4 ± 5.8 (42)	0.79
The difference in NHS3 score before and after 12 weeks of treatment	5.9 ± 8.0 (39)	5.1 ± 7.5 (42)	0.64

R ratio = (final seizure frequency − baseline seizure frequency)/(final seizure frequency + baseline seizure frequency). NHS3, the National Hospital Seizure Severity Scale; SD, standard deviation. *R* ratio represents changes in seizure frequency of the baseline and the end of 12 weeks' treatment. NHS3 score represents seizure severity.

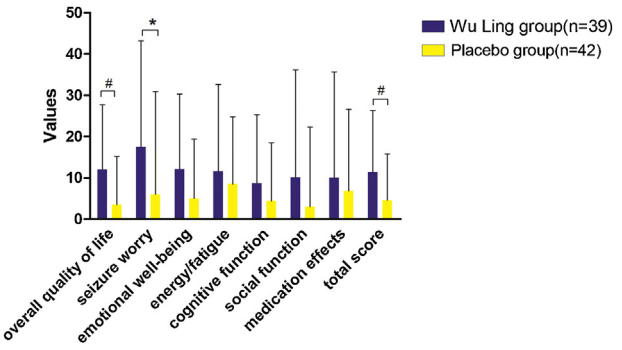

Fig. 3. The differences in total QOLIE-31 score, overall quality of life score and seizure worry score before and after 12 weeks of treatment were significantly greater in the Wu Ling group than in the Placebo group, $^{\#}P < 0.05$, $^{*}P < 0.01$. QOLIE-31, the Quality of Life in Epilepsy Inventory-31.

Table 3
Adverse events by system organ class.

Events *n* (%)	Wu Ling group (*n* = 51)	Placebo group (*n* = 49)	*P* value
The number of AE	13 (25.5%)	16 (32.7%)	0.49
Any severe AE	0 (0)	1 (6.3%)	0.31
Any AE related to study drug	6 (46.2%)	12 (75%)	0.11
Any AE leading to discontinuation of study drug	2 (15.4%)	3 (18.8%)	0.81
General disorders			
Fatigue	4 (30.8%)	1 (6.3%)	0.08
Gastrointestinal disorders			
Abdominal discomfort	2 (15.4%)	1 (6.3%)	0.42
Elevated liver enzyme	1 (7.7%)	0	0.26
Constipation	2 (15.4%)	1 (6.3%)	0.42
Increased saliva	1 (7.7%)	0	0.26
Nervous system disorders			
Dreaminess	0	2 (12.5%)	0.19
Dizziness	0	1 (6.3%)	0.36
Feeling depressed	0	2 (12.5%)	0.19
Status epilepticus	0	1 (6.3%)	0.36
Respiratory, thoracic and mediastinal disorders			
Anterior thoracic discomfort	1 (7.7%)	0	0.26
Palpitation	0	4 (25%)	0.05
Ablepsy	0	1 (6.3%)	0.36
Skin and subcutaneous tissue disorders			
Skin rash	1 (7.7%)	0	0.26
Sneezing	1 (7.7%)	1 (6.3%)	0.88

AE, adverse event.

3.5.2. Other safety measurements

No significant changes in body weight, heart rate or blood pressure were found in either the Wu Ling or the Placebo groups after treatment. One patient had an elevated liver enzyme after 4 weeks' treatment and was found to be infected with active hepatitis B. She dropped out of the study and recovered after treatment. Liver and renal functional variables (ALT, AST, gamma-glutamyltransferase, blood urea nitrogen and creatinine), and the abnormal rates of electrocardiogram (ECG) and electroencephalogram (EEG) tests at baseline and at the end of treatment were all compared between the Wu Ling and the Placebo groups, and the results showed no differences ($P > 0.05$).

4. Discussion

Although depression is a common comorbidity of epilepsy, the therapeutic methods are still limited and the evidence-based data are lacking. Antidepressants such as SSRIs have the risk of aggravating seizures, and several clinical trials are still open-label studies [8,10]. Therefore, new medical therapies and more randomized clinical trials are urgently needed. This study is one of the few double-blind randomized placebo-controlled parallel studies for patients with comorbidity of epilepsy and depression.

Xylaria nigripes had been used clinically to treat insomnia and depression, and was known to have anticonvulsive effects in animal studies, so it was utilized in this study for its possible benefits to alleviate depression and seizures simultaneously. This study indicated that Xylaria nigripes could significantly alleviate depressive symptoms in patients with comorbidity of epilepsy and depression compared with the placebo after at least 12 weeks' treatment. The result of our study was accordance with a previous clinical study in the general population with depression [20]. There might be two reasons for the results that Xylarian nigripes significantly alleviated depressive symptoms in week 12 but not in weeks 2, 4, 8. As indicated by Khan et al. [33], antidepressant treatment always had placebo response which affected the clinical trial outcome. The reason for no difference of total effective rate for depression between the Wu Ling and the placebo groups in weeks 2, 4, 8 might be due to its placebo response. But the total effective rate for depression had an increasing trend from week 2 to week 12, which indicated that Xylarian nigripes probably worked mildly and slowly. A previous animal study also found the phenomenon that Xylarian nigripes could have sedative effect only after 8 days continuous treatment with high dose [22]. The relatively high dropout rate in week 4 in this study might also due to the slow rate at which Xylaria nigripes took effect.

There were no significant changes in seizure frequency and severity after 12 weeks of Xylaria nigripes treatment compared with the placebo in this study. A previous animal study showed that Xylaria nigripes could delay the kindling process and ameliorate the learning deficits of rats with penty1enetetrazole-induced epilepsy [21]. However, to date (December 2014), there have been no reports about the effects of Xylaria nigripes on epileptic seizures in clinical studies. The results of WL-2010 indicated that Xylaria nigripes had no effect on epileptic seizures, and they indirectly demonstrated that it did not present any risk of aggravating seizures.

In addition, the total QOLIE-31 score, the overall quality of life score and the seizure worry score were all higher in the Wu Ling group than in the Placebo group in this study. We believed that the effects of Xylaria nigripes on quality of life and seizure worry might be secondary to the improvement of depressive symptoms in patients with epilepsy. After treatment, there was also a decreasing trend of the PSQI score in the Wu Ling group compared with the Placebo group, but it had no statistical significance in this study. Previous studies showed that Xylaria nigripes could induce

sleepiness and maintain sleep time in insomnia patients [19], but sleep disturbances such as insomnia and excessive daytime sleepiness were both very common in patients with epilepsy [34,35]. Daytime drowsiness had a particularly high prevalence due to the side effects of AEDs. We thought that the negative results of Xylaria nigripes on sleep quality in this study were probably attributable to its ineffectiveness on daytime drowsiness in patients with epilepsy. And there might also be some confounding effects of clonazepam on sleep, which was used to treat epileptic seizures in this study.

In this study, the total number of AE and percentage of AE possibly related to study drug were both slightly higher in the Placebo group than in the Wu Ling group. But the comparisons both had no statistical significance by chi-square test. We thought these AEs might be confounded with somatic symptoms of depression, which were hard to be distinguished by researchers. One patient had a severe AE of SE, but she had uncontrolled seizures 3–4 times every month at baseline, and was demonstrated to be in the Placebo group by urgently unblinding. No significant differences of blood tests variables and abnormal rates of ECG and EEG tests were found between the Wu Ling and the Placebo groups. Therefore, it suggested that Xylaria nigripes was safe and well tolerated by patients with epilepsy. In addition, as Xylaria nigripes is a traditional Chinese medicine with multiple bioactive products, its interaction with other drugs is not clear yet. There seemed no obvious interactions between Xylaria nigripes and the AEDs from clinical observations in this study.

One of the limitations of this study was that the sample size of patients with comorbidity of epilepsy and depression was relatively small. The most important reason for this might be related to the HAMD being less sensitive to patients with epilepsy. Some authors suggested that depressive symptoms in patients with epilepsy were always untypical. According to Wiegartz et al. [36], only 30% patients with epilepsy met the criteria of DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) for a current or lifetime-to-date major depressive disorder. A study by Gandy et al. indicated that HAMD had relatively poorer sensitivity (42%) compared with the Neurological Depressive Disorders Inventory for Epilepsy (NDDI-E) which had 84% sensitivity [37]. Another reason was that many patients with comorbidity of epilepsy and depression did not fully realize the negative influence of their depression on their quality of life and were not willing to accept treatment. In addition, stratification to balance the different antiepileptic medications in the Wu Ling and the Placebo groups was not performed because of the relatively small sample size of this study. However the percentages of all types of AEDs used at baseline were compared and no significant differences between the Wu Ling and the Placebo groups were found.

5. Conclusions

In conclusion, Xylaria nigripes could be an acceptable treatment to alleviate depressive symptoms in patients with epilepsy after at least 12 weeks of treatment. It could also improve some aspects of quality of life for patients with epilepsy without increasing the risk of exacerbating epileptic seizures, and be well tolerated. Overall, the WL-2010 study was still a preliminary small scale pilot study, but its results could help to evaluate feasibility, time, adverse events and effective size of a full-scale research project. A study with better screening tools, a larger sample size and a longer follow-up period would be expected in the future.

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Conflict of interest

None of the authors has any potential financial conflict of interest related to this study.

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References

[1] Gaitatzis A, Trimble MR, Sander JW. The psychiatric comorbidity of epilepsy. Acta Neurol Scand 2004;110:207–20.
[2] Fiest KM, Dykeman J, Patten SB, Wiebe S, Kaplan GG, Maxwell CJ, et al. Depression in epilepsy: a systematic review and meta-analysis. Neurology 2013;80:590–9.
[3] Boylan LS, Flint LA, Labovitz DL, Jackson SC, Starner K, Devinsky O. Depression but not seizure frequency predicts quality of life in treatment-resistant epilepsy. Neurology 2004;62:258–61.
[4] Petrovski S, Szoeke CE, Jones NC, Salzberg MR, Sheffield LJ, Huggins RM, et al. Neuropsychiatric symptomatology predicts seizure recurrence in newly treated patients. Neurology 2010;75:1015–21.
[5] Kanner AM, Byrne R, Chicharro A, Wuu J, Frey M. A lifetime psychiatric history predicts a worse seizure outcome following temporal lobectomy. Neurology 2009;72:793–9.
[6] Mehndiratta P, Sajatovic M. Treatments for patients with comorbid epilepsy and depression: a systematic literature review. Epilepsy Behav 2013;28:36–40.
[7] Au A, Chan F, Li K, Leung P, Li P, Chan J. Cognitive-behavioral group treatment program for adults with epilepsy in Hong Kong. Epilepsy Behav 2003;4:441–6.
[8] Specchio LM, Iudice A, Specchio N, La Neve A, Spinelli A, Galli R, et al. Citalopram as treatment of depression in patients with epilepsy. Clin Neuropharmacol 2004;27:133–6.
[9] Mazza M, Della MG, Di Nicola M, Martinotti G, Pozzi G, Janiri L, et al. Oxcarbazepine improves mood in patients with epilepsy. Epilepsy Behav 2007;10:397–401.
[10] Kanner AM, Kozak AM, Frey M. The use of sertraline in patients with epilepsy: is it safe. Epilepsy Behav 2000;1:100–5.
[11] Kuhn KU, Quednow BB, Thiel M, Falkai P, Maier W, Elger CE. Antidepressive treatment in patients with temporal lobe epilepsy and major depression: a prospective study with three different antidepressants. Epilepsy Behav 2003;4:674–9.
[12] Curran S, de Pauw K. Selecting an antidepressant for use in a patient with epilepsy. Safety considerations. Drug Saf 1998;18:125–33.
[13] Alper K, Schwartz KA, Kolts RL, Khan A. Seizure incidence in psychopharmacological clinical trials: an analysis of Food and Drug Administration (FDA) summary basis of approval reports. Biol Psychiatry 2007;62:345–54.
[14] Rosenstein DL, Nelson JC, Jacobs SC. Seizures associated with antidepressants: a review. J Clin Psychiatry 1993;54:289–99.
[15] Kanner AM. The treatment of depressive disorders in epilepsy: what all neurologists should know. Epilepsia 2013;54(Suppl. 1):3–12.
[16] Wang F, Han S, Hu S, Xue Y, Wang J, Xu H, et al. Two new secondary metabolites from *Xylaria* sp. cfcc 87468. Molecules 2014;19:1250–7.
[17] Rogers JD, Ju YM, Lehmann J. Some *Xylaria* species on termite nests. Mycologia 2005;97:914–23.
[18] Lu Y, Lu H, Qin D. The clinical effect of wuling capsule on wild depression. Chin Tradit Patent Med 2010;32:1083–4.
[19] Song X, He J, Zheng T, Ye R, Yuan Z. Research on treatment effects of Wuling capsule for sub-healthy state insomnia. Chin Arch Tradit Chin Med 2010;28:477–8.
[20] Shi LL, Zhao XH, Wang Y, Zhang J, Zhang HY, Wu K, et al. A randomized study on comparing effect and safety of wuling capsule and deanxit in patients with anxiety or depression status. Chin J Neurol 2009;42:776–9.
[21] Chen GF, Ren GL, Zhang LS, Hu XY. Effects of Wuling mycelia on pentylenetetrazole-induced epilepsy in rats. J Zhejiang Univ (Med Sci) 2012;41:647–52.
[22] Ma ZZ, Zuo PP, Chen WR, Tang LJ, Shen Q, Sun XH, et al. Studies on the sedative and sleeping effects of Wuling mycelia and its pharmacological mechanism. Chin Pharm J 1999;34:374–7.
[23] Lu XL, Yao XL, Liu Z, Zhang H, Li W, Li Z, et al. Protective effects of xyloketal B against MPP+-induced neurotoxicity in *Caenorhabditis elegans* and PC12 cells. Brain Res 2010;1332:110–9.
[24] Ko HJ, Song A, Lai MN, Ng LT. Antioxidant and antiradical activities of Wu Ling Shen in a cell free system. Am J Chin Med 2009;37:815–28.
[25] Ko HJ, Song A, Lai MN, Ng LT. Immunomodulatory properties of Xylaria nigripes in peritoneal macrophage cells of Balb/c mice. J Ethnopharmacol 2011;138:762–8.

[26] Snaith RP. Hamilton rating scale for depression. Br J Psychiatry 1977;131:431–2.
[27] Trajkovic G, Starcevic V, Latas M, Lestarevic M, Ille T, Bukumiric Z, et al. Reliability of the Hamilton Rating Scale for Depression: a meta-analysis over a period of 49 years. Psychiatry Res 2011;189:1–9.
[28] Zheng YP, Zhao JP, Phillips M, Liu JB, Cai MF, Sun SQ, et al. Validity and reliability of the Chinese Hamilton Depression Rating Scale. Br J Psychiatry 1988;152:660–4.
[29] Buysse DJ, Reynolds CR, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193–213.
[30] Liu XQ, Ren XL, Zhou GL, Chen PY. Reliabity and validity for quality of life in epilepsy-31 scale in adults wity epilepsy. Chin J Neuromed 2003;2:106–9.
[31] Kerr MP, Baker GA, Brodie MJ. A randomized, double-blind, placebo-controlled trial of topiramate in adults with epilepsy and intellectual disability: impact on seizures, severity, and quality of life. Epilepsy Behav 2005;7:472–80.
[32] O'Donoghue MF, Duncan JS, Sander JW. The National Hospital Seizure Severity Scale: a further development of the Chalfont Seizure Severity Scale. Epilepsia 1996;37:563–71.
[33] Khan A, Detke M, Khan SR, Mallinckrodt C. Placebo response and antidepressant clinical trial outcome. J Nerv Ment Dis 2003;191:211–8.
[34] van Golde EG, Gutter T, de Weerd AW. Sleep disturbances in people with epilepsy; prevalence, impact and treatment. Sleep Med Rev 2011;15:357–68.
[35] Giorelli AS, Passos P, Carnaval T, Gomes MM. Excessive daytime sleepiness and epilepsy: a systematic review. Epilepsy Res Treat 2013;2013:629469.
[36] Wiegartz P, Seidenberg M, Woodard A, Gidal B, Hermann B. Co-morbid psychiatric disorder in chronic epilepsy: recognition and etiology of depression. Neurology 1999;53:S3–8.
[37] Gandy M, Sharpe L, Perry KN, Miller L, Thayer Z, Boserio J, et al. Assessing the efficacy of 2 screening measures for depression in people with epilepsy. Neurology 2012;79:371–5.