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Effects of Folium Artemisiae Argyi on sleep quality and serum neurotransmitter levels in patients with glioma and comorbid depression

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CITATION

Feng J, He Q, Wang J. Effects of Folium Artemisiae Argyi on sleep quality and serum neurotransmitter levels in patients with glioma and comorbid depression. *Psycho-Oncologie*. 2026; 20(6): 5940. <https://doi.org/10.18282/po5940>

ARTICLE INFO

Received: 28 May 2026

Revised: 17 June 2026

Accepted: 07 July 2026

Available online: 20 July 2026

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Abstract: In this study, we analyzed the effect of Folium Artemisiae Argyi (FAA) on brain tumor-combined depression (BTD) patients. One hundred and four patients with BTD admitted to our hospital from January 2023 to January 2024 were selected for retrospective analysis. The grouping was based on the postoperative rehabilitation regimen recorded in the medical records: patients who received conventional rehabilitation alone were included in the control group, whereas those who additionally received FAA hair washing were included in the research group. Because treatment allocation was not randomized, potential selection bias could not be fully excluded. Self-rating anxiety/depression scale (SAS/SDS) and Pittsburgh sleep quality index (PSQI) were compared between the two groups before and after treatment, and serum nutritional markers and serum neurotransmitter levels were measured. Finally, a 1-year prognostic follow-up was performed. After treatment, SAS, SDS, PSQI, albumin, transferrin, prealbumin, total protein, 5-hydroxytryptamine, dopamine, and norepinephrine were more favorable in the research group than in the control group (all $P < 0.05$), whereas 5-hydroxyindole acetic acid and the proportion of high-risk NRS2002 classification were lower (both $P < 0.05$). The unadjusted 1-year overall survival rate was 92.31% in the research group and 76.92% in the control group (log-rank $P = 0.035$), with no loss to follow-up. FAA adjunctive hair washing was associated with improved mood, sleep quality, serum nutritional markers, and serum neurotransmitter profiles in patients with glioma and comorbid depression.

Keywords: brain tumor, Folium Artemisiae Argyi, insomnia, negative emotions, depression

1. Introduction

Brain tumors are neoplasms that originate from intracranial tissue or represent brain metastases, and they are commonly manifested by increased intracranial pressure and various neurological symptoms [1]. Clinical statistics indicate that brain tumors account for approximately 1.5% of all malignancies, with a global average incidence of about 7-10 per 100,000 and a rising prevalence in recent years [2]. As we all know, the brain is the center that controls all kinds of life activities in the human body. The occurrence of brain tumor poses great potential risks to normal human life activities; even with active treatment to save lives, there is irreversible nerve damage in patients [3]. Thus, sufficient importance should be attached to the diagnosis and treatment of brain tumor. At present, surgery remains the major approach for treating brain tumor [4]. However, craniotomy is associated with negative effects such as a series of nerve injuries, pain, and negative emotions [5], this has led to the fact that depression is very common in brain tumor patients. Statistics show that about more than 40% of brain tumor patients have different degrees of depression [6].

The occurrence of depression will greatly affect the patient's treatment compliance, and may prompt the patient to make behaviors such as abandonment of treatment, self-harm or even suicide, which will have a great negative impact on the treatment of brain tumors [7]. And how to improve the burden of depression on brain tumor patients has become a key link to improve the quality of patient recovery.

With the increasing attention paid to traditional Chinese medicine (TCM) rehabilitation treatment in clinical practice, exploring new postoperative rehabilitation treatment plans from the perspective of TCM has become a research hotspot. Folium Artemisiae Argyi (FAA) is an excellent hemostatic drug in TCM, which is the dried leaf of *Artemisia* from the Asteraceae family, with positive effects such as antibacterial, antiviral, antioxidant, antipyretic and sedative actions confirmed by clinical pharmacology [8]. Studies have shown that in the treatment of diseases such as Alzheimer's disease, FAA can not only better promote the cerebral hemodynamics of patients, but also enhance their neurological function and prognoses [9, 10]. Moreover, FAA is rich in proteins, amino acids, and other components, and is therefore usually used as an adjunctive therapeutic drug for various diseases to improve nutritional status and health [11]. These aids may not only improve sleep disorders in patients with brain tumor, but may also stimulate cerebral neurological healing and improve the nutritional status of the patient, but there is currently no research to verify its application effect in brain tumor-combined depression (BTD).

Therefore, this retrospective study focused on patients aged 60–74 years with postoperative glioma and comorbid depression, and evaluated whether FAA adjunctive hair washing was associated with changes in mood, sleep quality, nutritional status, and serum neurotransmitter levels.

2. Materials and methods

2.1 Patients

One hundred and four patients with BTD admitted to our hospital from January 2023 to January 2024 were selected for retrospective analysis. Inclusion criteria: age 60–74 years old, completed surgical treatment in our hospital, and diagnosed as glioma by pathological examination. Depression was clinically diagnosed before postoperative rehabilitation by trained clinicians according to the International Classification of Diseases, Tenth Revision (ICD-10) criteria, rather than by SAS or SDS scores alone; the Self-rating Anxiety Scale (SAS) and Self-rating Depression Scale (SDS) were used to assess baseline anxiety and depressive symptom severity. Exclusion criteria were cognitive dysfunction, physical activity disorders, serious psychiatric or psychological diseases unrelated to comorbid depression, known allergy to FAA, and other tumors. Allergy to FAA was screened by reviewing the documented allergy history and asking patients about previous exposure to *Artemisia*-related products, topical herbal preparations, or mugwort-containing products; patients with a known allergy or previous local irritation after such exposure were excluded. This study strictly followed the Helsinki Declaration, and all participants signed informed consent forms. 52 of the patients received conventional rehabilitation therapy after surgery and were considered as the control group; the other 52 patients also used FAA on top of conventional rehabilitation therapy and were considered as the research group.

2.2 Methods

All patients underwent brain tumor surgery in our hospital operated by the

same physician. The control group received acupoint massage on the third day after surgery: Neiguan, Hegu, Ganyu, Baihui, Touwei, and Fengchi acupoints were selected. The patient was instructed to maintain a comfortable position and relax the whole body. The operator used the pulp of the thumb or forefinger to press and knead at the corresponding acupoints. During the massage, the movements were coordinated and coherent, with a sensation of local soreness and numbness of the massaged site as the best. The massage was performed for 15 min per session once daily for 7 days. All acupoint massage procedures were delivered by nurses who had completed unified protocol training, and the selected acupoints, massage sequence, session duration, and pressure standard were kept consistent. On this basis, the research group received FAA hair washing: 100 g of FAA was decocted in 1,000 mL of clear water for 10–15 min, filtered, and kept at 38–40°C in a thermostatic medicinal bath pot. Approximately 500 mL of mugwort water was used per wash, and each session lasted 10–15 min; the intervention was performed every two days for 14 days, resulting in seven sessions in total. Treatment completion and documented adverse reactions, including skin allergy, local irritation, dizziness, nausea, vomiting, and odor intolerance, were reviewed from nursing records.

2.3 Ratings Survey

Baseline assessments and blood sampling were completed before the first postoperative rehabilitation intervention, and post-treatment assessments and blood sampling were performed within 24 h after completion of the 14-day intervention. Patients' psychological status was investigated at these two time points using the Self-rating Anxiety Scale (SAS) and Self-rating Depression Scale (SDS) [12]. Higher SAS/SDS scores suggest more serious depression and anxiety. Patients were also scored before and after treatment by the Pittsburgh Sleep Quality Index (PSQI) [13] for their sleep states from seven dimensions: sleep quality, sleep duration, sleep efficiency, sleep disturbances, sleep latency, use of sleep medication, and daytime dysfunction. The score is negatively related to the sleep quality. The Nutrition Risk Screening (NRS 2002) [14] was used for the assessment of the malnutrition risk level from the severity of the disease, impaired nutritional status, and age, with a higher score indicating a higher nutritional risk level. (5) Satisfaction: Patients completed a self-administered 10-point satisfaction questionnaire at discharge, with the results divided into very satisfied (10 points), satisfied (7–9 points), reluctantly accepted (4–6 points), and dissatisfied (1–3 points); the total satisfaction rate was calculated as (very satisfied + satisfied)/total number of patients $\times 100\%$. This questionnaire was used for discharge satisfaction assessment in this retrospective study and was not an externally validated instrument. (6) Prognostic follow-up was calculated from the date of surgery, and patients were followed for 1 year through regular review, with the interval between reviews not exceeding 2 months. The prognostic survival of the two groups of patients was recorded, and the survival curve was plotted accordingly.

2.4 Detection of serum nutritional markers

Three mL of blood from the elbow vein of patients before and after treatment were drawn into procoagulant tubes, and the serum was centrifuged (3000 rpm/min for 10 min) after standing for 30 min at room temperature. Albumin (ALB), Transferrin (TRF), Prealbumin (PA) and Total protein (TP) levels were measured using an automatic biochemical analyzer.

2.5 Detection of neurotransmitter

1mL of blood was drawn from the elbow vein of the patient before and after

treatment, 50µL of methanol:acetonitrile (1:1) solution was added, and 10µL of internal standard solution isoproterenol epinephrine (10µg/mg) was added after mixing. Subsequently, the supernatant was obtained by centrifugation, and 100 µL was taken for chromatographic analysis to detect the contents of neurotransmitters 5-hydroxytryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA), dopamine (DA), and norepinephrine (NE).

2.6 Endpoints

(1) Psychological status: SAS, SDS. (2) Sleep status: PSQI. (3) Serum neurotransmitter levels: 5-HT, 5-HIAA, DA, and NE. (4) Nutritional status: ALB, TRF, PA, TP levels, and NRS2002. (5) Treatment satisfaction. (6) Prognosis. The flow of this study is shown in **Figure 1**.

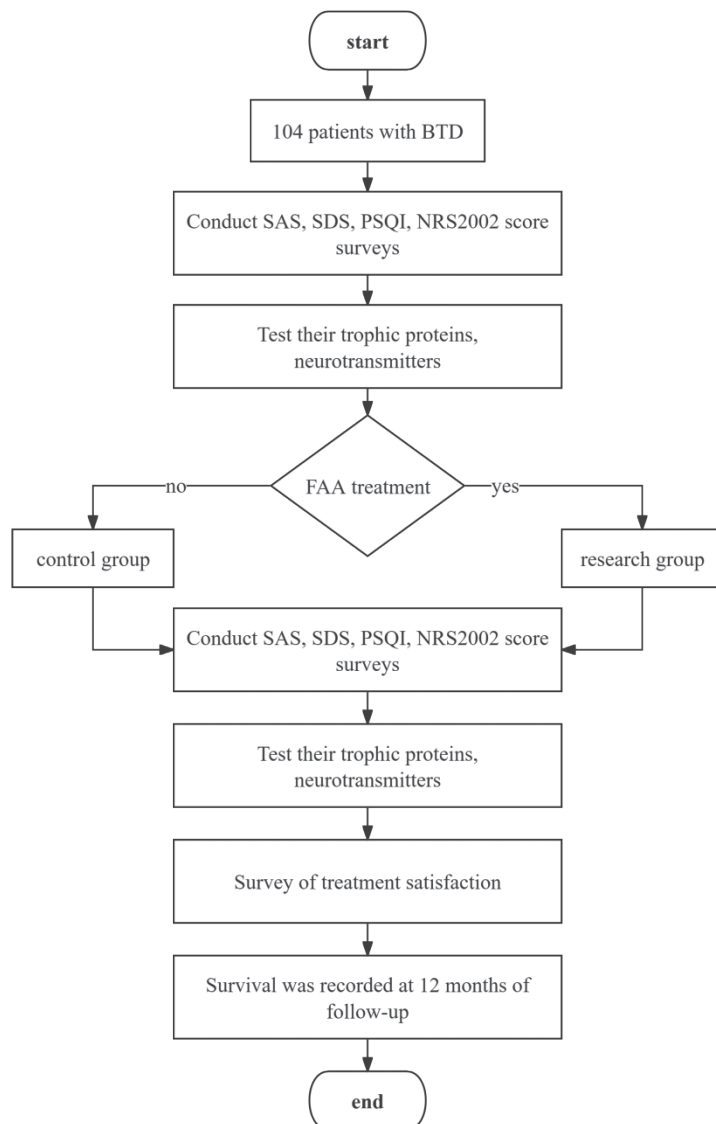


Figure 1. Flow chart of this study.

2.7 Statistical analysis

Statistical analysis was conducted using SPSS 25.0. Continuous variables were expressed as mean ± standard deviation and compared between groups using independent-samples t-tests; within-group pre- and post-treatment comparisons were

performed using paired t-tests. Categorical variables were expressed as n (%) and compared using the chi-square test or Fisher's exact test when appropriate. Survival was estimated using the Kaplan-Meier method, and survival curves were compared using the log-rank test. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1 Comparison of clinical data

Age, disease duration, sex distribution, pathological subtype, tumor location, WHO grade, extent of resection, postoperative adjuvant therapy, and recorded concomitant medications were comparable between the two groups ($P > 0.05$, **Table 1**).

Table 1. Comparison of clinical data

	Control group (n = 52)	Research group (n = 52)	t/ χ^2 /Fisher	P value
Age, years	65.77±6.97	64.40±5.20	1.133	0.260
Duration of disease, months	8.69±1.67	8.46±1.60	0.718	0.474
Sex, n (%)			0.650	0.420
Male	34 (65.38)	30 (57.69)		
Female	18 (34.62)	22 (42.31)		
Pathological subtype of glioma, n (%)			1.160	0.560
Astrocytoma	37 (71.15)	40 (76.92)		
Glioblastoma	12 (23.08)	11 (21.15)		
Other glioma subtype	3 (5.77)	1 (1.92)		
WHO tumor grade, n (%)			1.636	0.441
Grade II	26 (50.00)	24 (46.15)		
Grade III	20 (38.46)	25 (48.08)		
Grade IV	6 (11.54)	3 (5.77)		
Tumor location, n (%)			0.702	0.873
Frontal lobe	17 (32.69)	15 (28.85)		
Temporal lobe	16 (30.77)	16 (30.77)		
Occipital lobe	17 (32.69)	20 (38.46)		
Other location	2 (3.85)	1 (1.92)		
Extent of surgical resection, n (%)			1.016	0.602
Gross total resection	34 (65.38)	29 (55.77)		
Subtotal resection	12 (23.08)	15 (28.85)		
Partial resection	6 (11.54)	8 (15.38)		
Postoperative radiotherapy, n (%)			0.377	0.539
Yes	7 (13.46)	5 (9.62)		
No	45 (86.54)	47 (90.38)		

Continuation Table:

	Control group (n = 52)	Research group (n = 52)	t/ χ^2 /Fisher	P value
Postoperative chemotherapy, n (%)			0.102	0.75
Yes	6 (11.54)	5 (9.62)		
No	46 (88.46)	47 (90.38)		
Glucocorticoids, n (%)			Fisher	1.000
Yes	2 (3.85)	3 (5.77)		
No	50 (96.15)	49 (94.23)		
Antiepileptic drugs, n (%)			0.414	0.52
Yes	14 (26.92)	17 (32.69)		
No	38 (73.08)	35 (67.31)		
Analgesics, n (%)			Fisher	1.000
Yes	3 (5.77)	4 (7.69)		
No	49 (94.23)	48 (92.31)		
Antidepressants, n (%)			0.377	0.539
Yes	5 (9.62)	7 (13.46)		
No	47 (90.38)	45 (86.54)		

Note: Other glioma subtype refers to histologically confirmed glioma subtypes other than astrocytoma and glioblastoma; all enrolled patients had postoperative pathological confirmation of glioma. WHO tumor grade was extracted from pathological reports and included in the revised baseline comparison.

3.2 Comparison of psychological and sleep states

Patients' psychological and sleep states were assessed using the SAS/SDS and PSQI, respectively. Baseline SDS scores were comparable between the control and research groups (58.92 ± 4.24 vs. 58.48 ± 4.73 , $P = 0.617$), indicating a similar baseline depressive symptom burden. The results showed no statistical inter-group differences before treatment ($P > 0.05$); the above scores of both groups decreased after treatment, with even lower scores in the research group ($P < 0.05$, **Table 2**).

Table 2. Comparison of psychological and sleep states

Groups	n	PSQI		SAS		SDS	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	52	8.62 ± 1.73	$6.67 \pm 1.91^*$	63.25 ± 3.56	$52.37 \pm 5.47^*$	58.92 ± 4.24	$48.21 \pm 5.97^*$
Research group	52	8.79 ± 1.66	$4.23 \pm 1.42^*$	63.94 ± 5.27	$46.60 \pm 4.96^*$	58.48 ± 4.73	$41.73 \pm 6.84^*$
t		0.521	7.403	0.784	5.630	0.502	5.144
P		0.604	< 0.001	0.435	< 0.001	0.617	< 0.001

Note: comparison with before treatment * $P < 0.05$.

3.3 Comparison of serum neurotransmitter levels

Compared with pre-treatment, serum 5-HT, 5-HIAA, DA, and NE levels did not change significantly in the control group ($P > 0.05$). In the research group, serum 5-HT, DA, and NE levels increased after treatment and were higher than those in the control group, whereas serum 5-HIAA decreased and was lower than that in the control group ($P < 0.05$, **Table 3**). These changes suggested a more favorable serum monoamine profile, because 5-HT, DA, and NE are involved in mood regulation, arousal, motivation, and sleep-wake regulation, whereas 5-HIAA reflects serotonin metabolism rather than direct neurological recovery.

Table 3. Comparison of neurological function

Groups	n	5-HT (ng/mL)		(ng/mL)		(ng/mL)		(ng/mL)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	52	4.38±1.01	4.69±1.20	19.68±3.23	19.07±2.46	10.62±1.73	10.48±2.25	152.06±9.11	155.06±22.24
Research group	52	4.61±1.11	5.25±1.31	19.46±2.57	16.07±3.05	10.80±1.97	15.31±1.88	150.39±14.43	169.47±19.43
t		1.069	2.276	0.385	5.513	0.477	11.880	0.502	3.517
P		0.288	0.025	0.701	< 0.001	0.635	< 0.001	0.616	< 0.001

Note: comparison with before treatment * $P < 0.05$.

3.4 Comparison of nutritional status

Serum nutritional markers, including ALB, TRF, PA, and TP, did not differ significantly between groups before treatment ($P > 0.05$); these markers remained unchanged in the control group after treatment ($P > 0.05$), whereas they increased in the research group and were higher than those in the control group after treatment ($P < 0.05$). The NRS2002 scale was used for malnutrition risk assessment. The distribution of NRS2002 categories differed between groups ($\chi^2 = 8.142$, $P = 0.043$), with a higher proportion of low-risk patients and a lower proportion of high-risk patients in the research group than in the control group (**Table 4**).

Table 4. Comparison of nutritional status

Groups	n	ALB (g/L)		TRF (g/L)		PA (mg/L)		TP (g/L)		NRS2002			
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	None	Low	Medium	High
Control group	52	39.20±4.79	40.83±4.72	2.88±0.62	2.92±0.64	325.30±57.66	326.61±60.64	72.49±6.93	71.81±6.61	12 (23.08)	22 (42.31)	11 (21.15)	7 (13.46)
Research group	52	39.78±4.72	45.72±4.10*	2.91±0.89	3.28±0.89*	323.70±55.57	353.38±42.33*	71.72±8.26	79.94±8.15*	10 (19.23)	34 (65.38)	7 (13.46)	1 (1.92)
t/ χ^2		0.623	5.633	0.230	2.363	0.144	2.610	0.517	5.583	0.231	5.571	1.075	4.875
P		0.535	< 0.001	0.819	0.020	0.886	0.010	0.606	< 0.001	0.631	0.018	0.300	0.027

Note: comparison with before treatment * $P < 0.05$. For NRS2002 classification, χ^2 and P values were calculated using the chi-square test rather than t-tests.

3.5 Satisfaction comparison

Finally, the satisfaction survey conducted upon discharge showed that the total satisfaction rate of the research group was 88.46%, much higher than that of the control group ($P < 0.05$, **Table 5**).

Table 5. Satisfaction comparison

Groups	n	Very satisfied	Satisfied	Reluctantly accepted	Dissatisfied	Total satisfaction rate
Control group	52	17 (32.69)	18 (34.62)	13 (25.00)	4 (7.69)	35 (67.31)
Research group	52	26 (50.00)	20 (38.46)	5 (9.62)	1 (1.92)	46 (88.46)
χ^2						6.755
P						0.009

3.6 Prognostic survival

All 104 patients completed the 1-year follow-up from the date of surgery, with no attrition. At 1 year, 48 of 52 patients in the research group and 40 of 52 patients in the control group were alive, corresponding to unadjusted overall survival rates of 92.31% and 76.92%, respectively; Kaplan-Meier analysis showed a difference between survival curves (log-rank = 4.438, $P = 0.035$; **Figure 2**). Because this retrospective study could not fully adjust for tumor grade, extent of resection, adjuvant therapy, baseline physical condition, and nutritional status, this survival result was interpreted as exploratory.

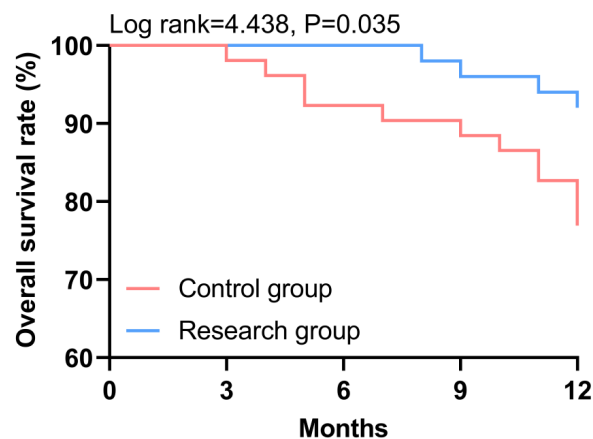


Figure 2. Kaplan-Meier curves for 1-year overall survival in the control and research groups.

4. Discussion

In this study, we found that FAA can effectively alleviate the negative mood of patients with BTD, improve their sleep status, and enhance their nutritional status, which is of great help to improve the prognosis quality of BTD patients and improve the quality of clinical medical services in the future.

First of all, in the investigation of psychological and sleep status, we found more significant reductions in the SAS, SDS, and PSQI scores in the research group after treatment, which shows that FAA has an excellent effect on relieving negative

emotions in patients with BTB. Clinically, craniotomy is considered to be the main treatment for BTB, and its mechanical invasive operation as a stressor will cause strong physical and mental stimulation to patients, inducing anxiety, depression, and huge psychological fluctuations [15, 16]. Cao B et al. reported that the nano-components of FAA carbonisatum can play an anti-anxiety role, and FAA has the effects of warming meridians, dredging collaterals, resisting cold, and relieving pain [17]. We believe that FAA can effectively improve patient comfort, relax patients physically and mentally, and reduce negative emotions. While relieving patients' anxiety and depression, FAA breaks the vicious cycle between high anxiety and depression and poor sleep quality, playing a positive role in improving patients' sleep quality. Furthermore, from a physiological point of view, the relief of anxiety and depression reduces the adverse effects of monoamine neurotransmitter changes during the transition from non rapid eye movement (REM) sleep to REM sleep, thus improving the sleep quality of patients [18]. Similarly, in a previous study, Yang J et al. proposed that FAA can improve sleep efficiency in mice [19], consistent with our results. And in the comparison of nerve function, we found that 5-HT, DA, NE were elevated and more than the control group after the treatment of the research group, while 5-HIAA was reduced and more than the control group, which shows that FAA also has an excellent effect on the improvement of the patients' nerve function. Studies have shown that FAA has a certain inhibitory effect on the nerves, so it helps to alleviate the agitated mood of patients with depression [20]. Meanwhile, FAA has been proved to have the effects of dredging the meridians and channels, activating blood circulation and removing blood stasis in traditional Chinese medicine, which can help accelerate the local blood circulation and improve the function of damaged nerves, helping to alleviate the numbness of the limbs and abnormal sensation of the limbs caused by nerve damage, and is conducive to the repair of the damaged nerves [21].

However, there are also studies that FAA's composition is complex, and overuse may instead have a significant inhibitory effect on the nerves, leading to tinnitus, panic and other symptoms and aggravating sleep disorders [22]. In this study, the patients' neurological function, sleep quality, and psychological condition were all positively improved, and the reason was that FAA was not used orally in this study, which greatly reduced the adverse reactions caused by the excessive dose of FAA. FAA contains natural volatile oils with a recognizable odor, and odor intolerance, nausea, or vomiting may occur in susceptible patients [23]. In the present study, no treatment-limiting odor intolerance or vomiting was documented in the available nursing records, which may be related to topical administration, controlled water temperature, limited exposure time, and the relatively low frequency of use; nevertheless, mild transient discomfort may have been underrecorded because of the retrospective design, and future prospective studies should monitor these reactions systematically.

On the other hand, when we investigated patients' nutritional status, we found that the nutritional protein levels were higher in the research group than in the control group, and the risk level of malnutrition was lower, indicating that FAA also has an important positive effect on the health of patients with BTB. After any surgical procedure, the stress and trauma will lead to a large amount of metabolism and loss of nutritional proteins and trace elements, plus a greater nutritional loss due to short-term normal diet restriction after surgery than the nutritional intake, resulting in a high incidence of various complications and infections during the early

postoperative period [24]. Pharmacological research on FAA has confirmed that FAA is rich in proteins, especially in amino acids, including leucine, lysine, and arginine. The unsaturated fatty acids contained in it (mainly linoleic acid and linolenic acid) help reduce blood viscosity, improve blood circulation, and prevent cardiovascular diseases; its carbohydrate metabolism is mainly cellulose, which is conducive to promoting intestinal peristalsis, increasing satiety, and preventing constipation; moreover, the various vitamins and minerals it contains, such as vitamins A, C, and K, as well as potassium and calcium, are helpful to maintain the normal physiological function of the human body and enhance immunity [20]. Because FAA was administered topically as hair-washing water in this study, the observed improvements in nutritional markers should not be interpreted as direct nutrient supplementation through scalp absorption. A more cautious explanation is that better sleep, reduced distress, improved comfort during postoperative rehabilitation, and enhanced care compliance may have contributed to more favorable nutritional status; this hypothesis requires prospective mechanistic verification.

Finally, in the satisfaction survey, the total satisfaction rate of the research group showed a more significant increase compared to the control group. This is due to the low price and easy availability of FAA, which play the advantages of TCM's simplicity, efficacy, efficiency, and affordability. In addition, according to the characteristic intervention mode of TCM, FAA is attractive to patients, and acupoint massage is simple and easy to learn, which can arouse their interest, contributing to high patient compliance with intervention. During the perioperative period, we should persist in taking patients as the center, relieve patients' anxiety and depression, and improve their sleep quality to allow them to accept and cooperate with preoperative preparation in the best psychological state, so as to improve patient satisfaction. Not only that, through the prognostic follow-up, we also found that the prognostic survival rate of research group was more significantly improved than that of the control group, which suggests that FAA is also helpful in improving the prognosis of BTM patients. Studies have shown that depression is a key factor affecting the quality of recovery in a variety of diseases (e.g., brain tumors, digestive diseases, cardiovascular and cerebrovascular diseases, etc.) [25]. We believe that the positive effect of FAA on BTM patients' sleep quality and nutritional status lays a more reliable foundation for their recovery, thus improving their prognostic survival.

5. Conclusion

FAA adjunctive hair washing was associated with improved mood, sleep quality, serum nutritional markers, and serum neurotransmitter profiles in patients with glioma and comorbid depression. Nevertheless, this retrospective single-center study had a limited sample size, short follow-up, incomplete adjustment for tumor- and treatment-related confounders, no objective neurological function scale, and no externally validated satisfaction instrument. Larger prospective controlled trials with longer follow-up, standardized adverse-reaction monitoring, objective neurological assessments, and comparisons of different FAA administration methods are needed before this intervention can be recommended as routine clinical care.

Supplementary materials: None.

Author contributions: Conceptualization, JF and JW; methodology, JF; software, JW; validation, JF and JW ; formal analysis, JF; investigation, JW; resources, JW and DY; data curation, JW; writing-original draft preparation, JF; writing-review

and editing, JF and JW; visualization, JF and JW; supervision, JW and DY; project administration, JW and DY; funding acquisition, JW and DY. All authors have read and agreed to the published version of the manuscript.

Funding: None.

Ethical approval: This study was performed in line with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Chinese Second Affiliated Hospital of the Air Force Medical University of the People's Liberation Army (approval No. KY20230139).

Informed consent statement: Written informed consent for the use of clinical data and follow-up was obtained from all participants.

Data availability statement: Original data in this study are available from the corresponding author on reasonable requests.

Acknowledgments: None.

Conflict of interest: Authors have no conflict of interest to declare.

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