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RESEARCH ARTICLE

Efficacy of Huaier granule in patients with breast cancer

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Abstract

Background Huaier extract has been demonstrated to exhibit potent anti-tumor effects in various types of cancer cells. However, the clinical benefit of Huaier granule in breast cancer has not been reported. In this study, we aimed to evaluate the efficacy of Huaier granule in breast cancer patients.

Methods Our study included 284 breast cancer patients treated with or without Huaier granule between January 2005 and October 2016 at Qilu Hospital, Shandong University, Jinan, China. Retrospective data obtained included demographics, clinicopathological characteristics, disease-free survival (DFS), serum concentrations of tumor markers, the Karnofsky performance scale (KPS), and incidences of emotional symptoms. DFS was the main outcome measure.

Results Of the patients included, 144 were classified into the control group and 140 into the Huaier group. Baseline characteristics were well balanced between the study arms. Median DFS was 91.43 months for control group and 112.61 months for Huaier group (hazard ratio (HR) = 2.97, 95% confidence interval (CI) = 1.57–5.61, $p < 0.01$). After Huaier granule treatment, the serum levels of tumor markers could be reduced to the normal range. In addition, breast cancer patients with Huaier granule treatment had higher KPS scores and less emotional symptoms.

Conclusions Our data demonstrated that patients orally administrated Huaier granule got longer DFS. Furthermore, Huaier granule could reduce serum tumor markers, improve the functional status, and decrease the incidences of emotional symptoms in breast cancer patients. Therefore, Huaier granule was an effective therapy for women with breast cancer.

Keywords Breast cancer · Huaier · Efficacy · Tumor marker · Retrospective

Introduction

Around the world, breast cancer is the most frequently diagnosed malignancy in female, with approximately 1.67 million new cases for 1 year [1]. According to the recent data in the US, breast cancer alone accounts for 30% of all new diagnosed cancers and 14% of cancer-related deaths among women [2, 3]. In 2018, an estimated 268,670 new cases will occur and 41,400 of them will die of breast cancer in both sexes [2]. In the past decades, the incidence and mortality of cancer are increasing in China, with estimated 4.29 million new cases and 2.81 million deaths in 2015 [4]. Breast cancer is also the most common cancer among women in China, and is expected to account for 15.7% of all the cancers [5]. Therefore, it is of great importance to search for novel approaches to improve the survival of breast cancer patients.

In the recent years, the traditional Chinese medicines (TCMs) are attracting great interests all over the world, due to their potential anti-tumor effects [6, 7]. Increasing evidences indicated that TCMs were non-toxic and effective treatments for various tumor types [8, 9]. Milicevic et al. demonstrated that Rhus verniciflua stokes extracts could significantly improve DFS and overall survival (OS) in advanced non-small cell lung cancer patients [10]. Furthermore, no severe adverse effects were observed [11]. In addition, for locally advanced or metastatic pancreatic cancer patients, *Viscum album* [L.] extract was an effective therapy to improve OS without a significant toxicity [12]. Thus, searching for novel drugs from TCMs could be a promising method to improve the prognosis and life quality of cancer patients.

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Huaier belongs to officinal fungi and has attracted increasing attentions because of its anti-tumor effects. The previous studies demonstrated that Huaier extract exerted potent cytotoxic functions in a variety of cancers, such as gastric cancer, hepatocellular carcinoma, and cervical cancer [13–15]. Recently, according to a multicenter, randomized clinical trial including 1044 patients, Chen et al. demonstrated that Huaier granule could significantly prolong the recurrence-free survival and reduce extrahepatic recurrence after curative liver resection [16]. We have focused on exploring the mechanisms underlying the inhibitory effects of Huaier extract for several years. According to our data, Huaier extract suppressed breast cancer proliferation and migration through inhibiting lncRNA-H19/miR-675-5p signaling pathway and activation of autophagic cell death [17, 18]. In addition, Huaier extract could inhibit tumor angiogenesis and cancer stem cells, and induce immunomodulatory effects [19–21]. Nowadays, Huaier granule has been approved for clinical use. However, no evidence was provided for its clinical benefit in breast cancer.

In the present study, we focused on the efficacy of Huaier granule for its clinical treatment, and we tried to find the potential mechanisms. Our data suggested that Huaier granule significantly prolonged DFS. In addition, we assumed that Huaier granule exerted its clinical benefit partly through reducing the serum tumor biomarkers and improving quality of life in breast cancer patients.

Materials and methods

Patients

Patients with pathologically confirmed breast cancer treated with or without Huaier granule from January 2005 to October 2016 at Qilu Hospital, Shandong University (Jinan, China) were included. Data were retrospectively obtained from the medical records of patients and 10 year follow-up. Patients with insufficient data were excluded from the analysis. The study was approved by the Ethics Committee on Scientific Research of Shandong University, Qilu Hospital. All patients provided written informed consent before study entry.

Eligibility criteria

We determined the eligibility of patients according to the following criteria: (1) eastern cooperative oncology group (ECOG) performance status ≤ 2; (2) adequate function of principal organs (leucocytes $\geq 3000/mm^3$, platelets $\geq 100{,}000/mm^3$, hemoglobin ≥ 10 g/dL, and serum creatinine ≤ 2 mg/dL); (3) absence of active infection; (4) negative pregnancy test; (5) available data on patient outcomes; (6) available medical records containing detailed information; (7) no distant metastasis.

Treatment

Huaier granule applied in this study was an approved drug and had a marketing authorization under the name "JinKe". Patients orally received 20 g Huaier granule three times a day. Treatments were lasted until disease recurrence or unacceptable toxicity, or until the patients determined to terminate treatment. The median treatment time was 6 months. During the course of the study, all patients received the best supportive care.

Assessment of response

Efficacy was assessed by DFS. DFS was defined as the time from date of operation to either the first relapse (local, regional, or distant), contralateral breast cancer, or death of any cause before recurrence. Efficacy was evaluated by ultrasound, CT, MRI, bone scan, and physical examination every 3 months.

Detection of serum tumor markers

Peripheral blood samples were collected from each patient. The serum levels of carcinoembryonic antigen (CEA), cancer antigen 153 (CA153), and cancer antigen (CA125) were measured using a Roche Elecsys Immunoassay analyzer (Roche, Penzberg, Germany) through the electrochemiluminescence assays.

Functional status and emotional symptom assessment

136 patients were included for the measurement of functional status and emotional symptoms. KPS was a standard method for evaluating the ability of cancer patients to perform daily and working activities, self-care, and the need for assistance [22]. KPS was a clinical score ranging from 0 to 100, with a score of ≥ 80 indicating independent living. In addition, we recorded the emotional symptoms in breast cancer patients who refer to the European organization for research and treatment of cancer quality-of-life questionnaire C30 (EORTC QLQ-C30). Each patient was provided with the questionnaire by the experienced interviewers, who were previously trained in reading the questions and response options.

Statistical analysis

The study was designed to evaluate the efficacy of Huaier granule for the treatment of breast cancer patients. To check

the balance of demographic and clinicopathological baseline characteristics, we used the Chi-square test to compare frequency distributions for categorical variables, and a two-tailed *t* test or the Wilcoxon rank sum test to compare continuous variables, where appropriate. Kaplan–Meier method was used to estimate actuarial DFS. The log-rank test was used to compare DFS rates between strata. The COX proportional hazard regression model was applied to estimate the HR and corresponding 95% CI. Differences with p value < 0.05 were considered statistically significant. The software SPSS V18.0 was used for statistical analysis.

Results

Patient characteristics

Between January 2005 and October 2016, a total of 294 breast cancer patients in Qilu hospital were included in this study. Ten patients were lost to follow-up. The remaining 284 patients were suitable for DFS analysis. 144 were included in the control group and 140 in the Huaier group.

Clinicopathological characteristics of evaluated patients are described in Table 1. The median age of the patients was 50.59 years (range 22–80), with no significant differences between two groups ($p = 0.85$). More than half (57.04%) of the patients were in postmenopausal status. There were no significant differences on menopausal status between the two groups ($p = 0.66$). 53.17% of the patients were hormone receptor (HR) negative, and 169 of the patients were HER-2 ∓. Chemotherapy had been initially administered to 113 (78.47%) and 104 (74.29%) patients, respectively, in the control and Huaier groups ($p = 0.41$). The median cycles of chemotherapy were both 6. In addition, 127 (44.72%) patients in total were previously treated with endocrinotherapy, and 115 (40.49%) patients had received radiotherapy ($p = 0.87$).

Treatment activity

During the study, patients in the Huaier group orally received 20 g Huaier granule three times a day. No patients in the control group received Huaier granule. All patients in both groups were provided with the best supportive care. At the time of data cutoff and analysis, 59 patients experienced local recurrence ($n = 5$), distant metastasis ($n = 46$), or contralateral breast cancer ($n = 8$). In the overall population, median DFS was 100.68 months. In the Huaier group, the median DFS was 112.61 months, which was significant longer than that in the control group (91.43 months, $p < 0.01$). Overall survival data were immature at the time of the analysis. The Kaplan–Meier DFS is shown in Fig. 1. A

Table 1 Patient characteristics in the total population and within different groups

	Total	Control group	Huaier group	*p* value
Number of patients (%)	284	144 (50.70%)	140 (49.30%)	
Median age (years, range)	50.59 (22–80)	50.71 (22–77)	50.47 (24–80)	0.85
Menopausal status				0.66
Postmenopausal	162 (57.04%)	84 (58.33%)	78 (55.71%)	
Premenopausal	122 (42.96%)	60 (41.67%)	62 (44.29%)	
HR status				0.893
Positive	133 (46.83%)	68 (47.22%)	65 (46.43%)	
Negative	151 (53.17%)	76 (52.78%)	75 (53.57%)	
HER-2 status				0.78
+++	70 (24.65%)	38 (26.39%)	32 (22.86%)	
++	45 (15.85%)	22 (15.28%)	23 (16.43%)	
±	169 (59.50%)	84 (58.33%)	85 (60.71%)	
Chemotherapy				0.41
Yes	217 (76.41%)	113 (78.47%)	104 (74.29%)	
No	67 (23.59%)	31 (21.53%)	36 (25.71%)	
Endocrinotherapy				0.42
Yes	127 (44.72%)	61 (42.36%)	66 (47.14%)	
No	157 (55.28%)	83 (57.64%)	74 (52.86%)	
Radiotherapy				0.87
Yes	115 (40.49%)	59 (40.97%)	56 (40.00%)	
No	169 (59.51%)	85 (59.03%)	84 (60.00%)	

HR, hormone receptor

*p value < 0.05 were considered significant

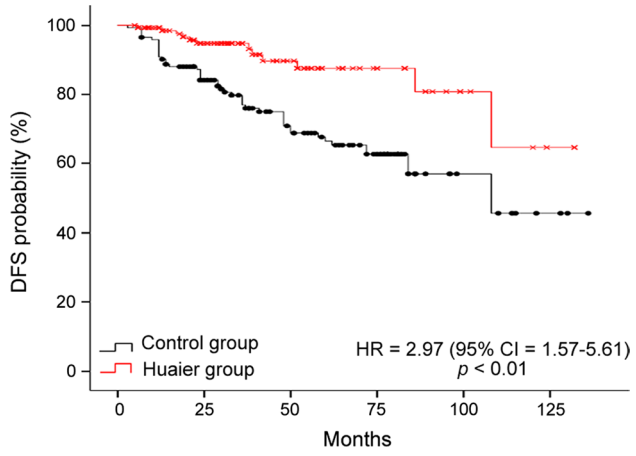

Fig. 1 Kaplan–Meier curves showing disease-free survival of breast cancer patients with or without Huaier granule treatment. CI confidence interval; HR hazard ratio

prognosis-group-adjusted HR of 2.97 (95% CI = 1.57–5.61, $p < 0.01$) was estimated by the group-sequential procedure.

Effect of Huaier granule on serum tumor markers

CEA, CA153, and CA125 were serum tumor markers, which were widely used for the management of the prognosis, metastasis, and recurrence of breast cancer patients [23–25]. To investigate the effects of Huaier granule on tumor markers, we detected the serum levels of CEA, CA153, and CA125 before and after Huaier granule treatment in breast cancer patients. Forty-one cases were found to have abnormal concentrations of tumor markers before Huaier granule treatment (20 cases in the CEA group, 12 cases in the CA153 group, and 9 cases in the CA125 group, Fig. 2).

In the CEA group, the CEA levels were reduced in 18 cases after administration with Huaier granule for 3 months. To be noticed, the concentrations of CEA in 14 patients were decreased to the normal range (0–5 ng/ml). For the 12 patients with elevated CA153, 11 of them had lower CA153 levels after Huaier granule treatment for 3 months. 7 cases had been restored to the normal CA153 levels (0–25 U/ml). In addition, among the 9 cases with elevated CA125, all patients had decreased levels of CA125 after 3 months of Huaier granule administration (6 cases were reduced to the normal range, 0–35 U/ml).

It was important to note that the inhibitory effects of Huaier granule on tumor markers were seemed to be revertible. In one case, the initial concentration of CA153 was 25.22 U/ml (Fig. 3a). After 3 month administration of Huaier granule, the level of CA153 was reduced to 24.37 U/ml. Another 4 month treatment reduced the CA153 concentration to 21.10 U/ml. However, the patient determined to

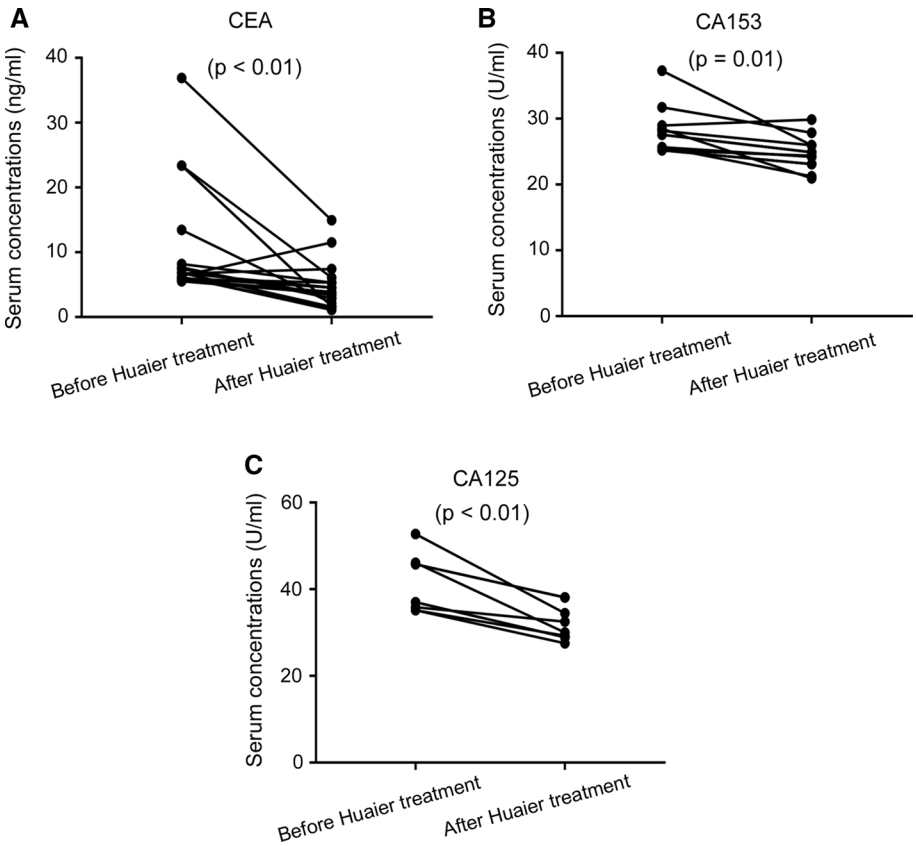

Fig. 2 Serum concentrations of several tumor biomarkers before and after Huaier granule treatment: **a** CEA, **b** CA153, and **c** CA125

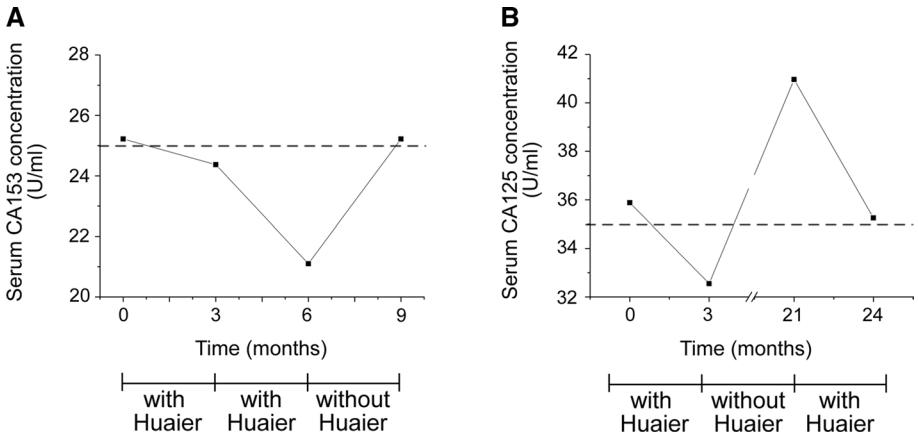

Fig. 3 Serum concentrations of tumor biomarkers influenced by Huaier granule treatment

Table 2 KPS in different groups

Score	Control group	Huaier group	*p* value
			0.02*
100	27 (39.71%)	51 (75.00%)	
90	15 (22.06%)	9 (13.24%)	
80	7 (10.29%)	2 (2.94%)	
70	5 (7.35%)	2 (2.94%)	
60	4 (5.88%)	1 (1.47%)	
50	2 (2.94%)	1 (1.47%)	
40	3 (4.41%)	1 (1.47%)	
30	1 (1.47%)	0	
20	0	0	
10	1 (1.47%)	0	
0	3 (4.41%)	1 (1.47%)	

*p value < 0.05 were considered significant

terminate Huaier granule treatment for 3 months, and the CA153 levels increased to 26.37 U/ml. In another case, the initial concentration of CA125 was 35.88 U/ml (Fig. 3b). After 3 month administration of Huaier granule, the level of CA125 was reduced to 32.55 U/ml. In the next 18 months, the patient decided to terminate Huaier granule treatment. However, the CA125 levels increased to 40.97 U/ml. Then, 20 g Huaier granule three times a day was regularly administrated for 3 months. In addition, the concentration of CA125 was detected to be 35.25 U/ml.

Effect of Huaier granule on functional status and emotional symptoms

As shown in Table 2, the average KPS score in overall group was 86.91. In the control group, the average KPS score was 80.29, which was significantly lower than that in the Huaier group (KPS score = 93.53, $p = 0.02$). The number of patients with high scores (KPS ≥ 80) were 49 and 62, respectively, in the control group and Huaier group. Thus, more patients in the Huaier group had independent livings. In addition, we calculated the incidences of several emotional symptoms (Table 3). According to our data, more patients in the control group felt tense ($p = 0.04$), irritable ($p = 0.02$), and difficult to remember things ($p < 0.01$). In addition, more patients in the control group felt weak ($p = 0.03$) and had trouble sleeping ($p = 0.04$). However, there was no significant difference on the incidence of appetite loss between the two groups ($p = 0.21$). Therefore, oral administration of Huaier granule could improve the quality of life in breast cancer patients.

Discussion

Medicinal herbs played a critical role in the exploration of new drugs for clinical application [26, 27]. Recently, Huaier extract has been demonstrated to exert potent tumor-inhibitory effects in a variety of cancer cells [28–30]. In the diagnosis and treatment guidelines for hepatocellular carcinoma (2017 edition), Huaier granule has been recommended for the treatment of hepatocellular carcinoma [31]. However, little evidence existed regarding the effectiveness and safety of Huaier extract in breast cancer patients. In this study, we retrospectively evaluated the efficacy of Huaier granule in Chinese breast cancer population for the first time. According to our data, breast cancer patients in the Huaier group had a significantly longer DFS.

The previous in vitro and in vivo studies demonstrated that Huaier extract could suppress the progression of breast cancer through multiple pathways, such as cell cycle arrest [32], inhibition of proliferation [33], and metastasis [34]. In addition, combination therapy of Huaier extract with the traditional chemotherapies could obtain better outcomes [35, 36]. These molecular events might contribute to the clinical benefits induced by Huaier granule in this study.

Due to the simple collection and less invasiveness, serum tumor markers were widely used for diagnosis and monitoring prognosis in breast cancer patients. CEA, CA153, and

Table 3 Emotional symptoms in different groups

Grade	Control group	Huaier group	*p* value
Feel tense			0.04*
Very much	0	0	
Quite a bit	3 (4.41%)	0	
A little	7 (10.29%)	2 (2.94%)	
Not at all	58 (85.29%)	66 (97.06%)	
Feel irritable			0.02*
Very much	0	0	
Quite a bit	2 (2.94%)	0	
A little	11 (16.18%)	3 (4.41%)	
Not at all	55 (80.88%)	65 (95.59%)	
Difficult to remember			< 0.01**
Very much	4 (5.88%)	1 (1.47%)	
Quite a bit	7 (10.29%)	3 (4.41%)	
A little	16 (23.53%)	4 (5.88%)	
Not at all	41 (60.29%)	60 (88.24)	
Feel weak			0.03*
Very much	3 (4.41%)	1 (1.47%)	
Quite a bit	7 (10.29%)	1 (1.47%)	
A little	8 (11.76%)	3 (4.41%)	
Not at all	50 (73.53%)	63 (92.65%)	
Trouble sleeping			0.04*
Very much	1 (1.47%)	0	
Quite a bit	7 (10.29%)	2 (2.94%)	
A little	12 (17.65%)	5 (7.35%)	
Not at all	48 (70.59%)	61 (89.71%)	
Lack appetite			0.21
Very much	2 (2.94%)	1 (1.47%)	
Quite a bit	6 (8.82%)	3 (4.41%)	
A little	11 (16.18%)	5 (7.35%)	
Not at all	49 (72.06%)	59 (86.76%)	

*p value < 0.05 and **p value < 0.01 were considered significant

CA125 were the most common markers. The previous studies showed that elevated serum levels of CEA, CA153, and CA125 were associated with tumor size, lymph-node status, and breast cancer relapse [37–39]. To identify the molecular mechanisms underlying the Huaier granule effects, we detected the levels of CEA, CA153 and CA125 before and after Huaier granule treatment.

In our study, the serum concentrations of CEA, CA153, and CA125 were obviously decreased after Huaier granule treatment. Twenty-seven cases with elevated levels of tumor markers were reduced to the normal range. Interestingly, the therapeutic effects of Huaier granule on serum tumor markers were seemed to be reversible. The levels of tumor markers increased again after the termination of Huaier granule treatment. These data indicated that Huaier granule could inhibit the secretions of tumor markers and sustain their concentrations at the normal level. Several studies demonstrated that CEA could inhibit apoptosis and promote metastasis in cancer cells [40]. Overexpression of CEA protected cancer cell from inducing anoikis, which was of critical importance for distant metastasis [41]. In addition, CA153 and CA125 promoted the proliferation and metastasis of cancer cells [42, 43]. We hypothesized that Huaier granule could improve the prognosis of breast cancer patients through regulating serum tumor marker levels.

According to our previous study, Huaier extract modulated immune response through suppressing NLRP3 inflammasome activation and impeded the secretion of IL-1β [44]. Various inflammatory agents have been reported to regulate the release of CA125 and CA153, such as interferons, interleukin-1β, and TNF-α [45, 46]. Therefore, we supposed that Huaier could decrease the levels of tumor markers through inhibiting IL-1β. In addition, a great number of miRNAs were predicted to regulate the translations of CEA [47, 48]. Therefore, miRNAs were also putative targets of Huaier granule to reduce the levels of tumor markers. However, detail mechanisms should be explored in the further studies.

KPS was a simple method to evaluate the functional status of cancer patients [49]. The previous studies demonstrated that KPS scores were greatly associated with overall quality of life in cancer patients [50, 51]. According to our data, the Huaier group had a higher KPS than the control group. 62 out of 68 patients in the Huaier group had scores more than 80. However, only 49 out of 68 patients in the control group were functional independent. For the emotional symptoms, there were significant differences between the two groups, apart from appetite loss. In the overall population, hypomnesia was the most common symptom, existing in 33.09% of the breast cancer patients. In addition, 8.82% of patients had ever felt tense, 11.76% felt irritable, 16.91% felt weak, and 19.85% had trouble sleeping. Huaier granule treatment significantly decreased the incidences of emotional symptoms. Therefore, Huaier granule could improve the quality of life in breast cancer patients. Notably, there was no significant difference on the incidence of appetite loss between the two groups ($p = 0.21$). The result was possibly due to the poor palate of Huaier granule.

Possible limitations of our study included the relatively small number of patients and the retrospective nature, such as the potential missing data, the group, and follow-up bias. Thus, only the hypothesis-generating conclusions could be drawn. Besides, because of the complexity of treatment pattern, it was difficult to perform dose-dependent analysis. Other limitation included the single-center design. Large multicenter randomized-controlled trials should be performed to confirm the safety and effectiveness of Huaier granule treatment.

In conclusion, our results suggested that Huaier granule could prolong the DFS and reduce the serum tumor markers

to the normal levels. In addition, Huaier granule improved the quality of life in breast cancer patients. Therefore, Huaier granule could be a promising second-line therapy for breast cancer patients. Further researches were still needed.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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