doi.org/10.36721/PJPS.2026.39.12.357.1

# Effect of Chinese medicine compound on intestinal bifidobacteria recovery and CD4+ T-Cell activation in postoperative chemotherapy patients with breast cancer

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**Abstract**: **Background**: Postoperative chemotherapy for breast cancer often leads to intestinal dysbiosis and immune suppression. Traditional Chinese medicine shows potential in regulating gut microbiota and enhancing immune function, offering a promising adjunctive therapeutic strategy. **Objectives**: To explore the effects of a traditional Chinese medicine compound on the recovery of intestinal bifidobacteria and CD4+ T-cell activation in breast cancer patients undergoing postoperative chemotherapy. **Methods**: Female patients with newly diagnosed breast cancer were randomly assigned to a control group (triamcinolone tablets) and an observation group (control treatment plus kidney-tonifying and compound traditional Chinese medicine soup). Intestinal flora and immune indices were measured and analyzed using SPSS 22.0. **Results**: After chemotherapy, the observation group showed significant increases in bifidobacteria to 9.01±1.49 lg (CFU/g) and in CD4+ T-cell counts to 969/μL. The expression levels of peripheral blood CD4+ T cells and the differences in S2, nm23 and Her-2 indices were significant between the groups, with the observation group showing greater CD4+ T cell activation. The incidence of adverse reactions was significantly lower in the observation group (*P-values*: 0.002, 0.006 and 0.030). The observation group also demonstrated better control of gastrointestinal conditions and disease progression (P<0.001). **Conclusion**: The traditional Chinese medicine compound effectively maintained intestinal flora balance, promoted beneficial bacterial growth, reduced adverse reactions and offered better therapeutic prospects for breast cancer patients.

**Keywords**: Breast cancer patients; Triamcinolone acetonide tablets; Kidney tonic compound Chinese medicine; Immune index; CD4 cell expression level

*Submitted on 22-04-2025 – Revised on 30-09-2025 – Accepted on 07-11-2025 – Available online on 05-09-2026 – Published on 01-12-2026*

## INTRODUCTION

In recent years, the prevalence and death rate of breast cancer have been high among females and its incidence has been on a continuous upward trend, which seriously threatens women's life, health and quality of life (Guo *et al*., 2023). Surgical resection of the tumor is the key means of breast cancer treatment, but postoperative adjuvant chemotherapy is often required to reduce the risk of recurrence and prolong patient survival. Although chemotherapy plays an important role in inhibiting the growth and spread of tumor cells, it also causes a range of side effects that cannot be ignored (Al-Hilli and Wilkerson, 2021; Baranova *et al*., 2022). Surgical resection of the tumor occupies a fundamental and crucial position in the treatment system of breast cancer; however, in order to effectively curb tumor recurrence and metastasis and prolong the survival time of patients after surgery, adjuvant chemotherapy has become an indispensable therapeutic means (Yang *et al*., 2021). Through the use of cytotoxic drugs, chemotherapy, to a certain extent, accurately combats tumor cells and inhibits their uncontrolled growth and spread. This directly leads to the impairment of the intestinal barrier function and harmful bacteria take the opportunity to multiply and transpose, which in turn causes a series of intestinal dysfunctions such as diarrhea, constipation, abdominal pain, etc., which seriously affecting the quality of daily life of the patients, leading to malabsorption of nutrients, decreased physical strength and may also force the chemotherapy process to be interrupted or delayed. This greatly affects the therapeutic outcomes and prognosis of patients (Akamandisa *et al*., 2023; Dzugan *et al*., 2024).

Traditional Chinese Medicine (TCM), as a unique health resource of China, has advantages such as regulating the overall function of the body, enhancing immunity and alleviating the side effects of chemotherapy. It has potential application value in the postoperative treatment of breast cancer. At present, surgery combined with chemotherapy is the main treatment for breast cancer, but Western medicine has not recognized a safe and effective solution for the symptoms produced after surgery and the series of side effects caused by chemotherapy. Traditional Chinese Medicine (TCM) can effectively alleviate patients' postoperative symptoms and reduce side effects by combining identification of evidence and disease, applying both attack and tonic and supporting the positive and eliminating the evil. This paper fully leverages the advantages of traditional Chinese medicine (TCM) in cancer treatment, comprehensively analyzes existing

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research and reviews a large number of documents. Through a controlled study of 105 women who were first diagnosed with breast cancer and admitted to the hospital, the study systematically explores the role of kidney-qi-tonifying Chinese herbal decoctions in the adjuvant treatment of breast cancer. The study selected a kidney-qi-tonifying Chinese herbal compound combined with chemotherapy and triamcinolone tablets to scientifically evaluate the effects of TCM on the recovery of intestinal bifidobacteria and the activation of CD4-positive T cells in breast cancer patients after surgery. These research findings are of great significance for guiding clinical practice, optimizing treatment plans and improving the quality of life of patients.

***Relevant works***

*Relationship between Bifidobacterium intestinalis and breast cancer*

It has been found that chronic psychological stress can lead to alterations in the gut microbial community and promote cancer metastasis. The promotion of metastasis by chronic stress was confirmed by breast cancer (BC) spontaneous metastasis model and CRC tail vein injection metastasis model and the involvement of microorganisms was also demonstrated by sterile animals and fecal mass transplantation (FMT) experiments. 16SrRNA gene sequencing was used to select key bacteria and auxin and mass spectrometry analyses were used to elucidate hormone-epithelial interactions and identify key metabolites that drive microbial alterations (He *et al*., 2024). A study investigated the antitumor effects of the culture supernatants of Mimospora fragilis and Bifidobacterium bifidum on mouse mammary carcinoma. The in vivo study showed a 98-99% reduction in tumor volume in mice receiving B.F.S (Karami *et al*., 2023). An oral protein vaccine platform has been developed using genetically engineered modified Bifidobacterium longum to anchor Wilms' tumor 1 (WT1) protein for intestinal immunotherapy of acute myeloid leukemia. The ability of the oral vaccine to induce intestinal immunity suggests a potential application of Bifidobacterium bifidum in cancer therapy, with broader implications for restoring intestinal bifidobacteria in patients undergoing postoperative chemotherapy for breast cancer. It suggests that cancer therapy can be assisted by regulating intestinal bifidobacteria, providing a new strategy for cancer immunotherapy (Nakagawa *et al*., 2023).

***Role of CD4-positive T cells in breast cancer***

A new mouse mammary tumor model has been used for immune cell population depletion experiments in which tumor cells maintain an epithelial phenotype in adaptive immune response-deficient mice, while EMT occurs in the presence of T cells. Among them, CD4T cells played a major promotional role, rather than CD8T cells or B cells. Intratumoral immune infiltration and transcriptome analysis of mammary tumors in mice with different EMT phenotypes showed a negative correlation between the proportion of mesenchymal tumor cells and intratumoral neutrophils, revealing a role for CD4T cells in adaptive immunity in promoting EMT (Chen *et al*., 2024). The Tumor-Infiltrating Lymphocyte (TIL) counts have been compared in primary and corresponding recurrent breast cancers and the relationship between tumor TIL composition and recurrence-free survival time and tumor clinicopathology has been analyzed. It was found that interstitial CD3, CD8 and CD56 lymphocytes decreased in primary breast cancer recurrence, while CD4 remained similar. Given the unique role of CD4-positive T cells in breast cancer recurrence, monitoring and regulating their levels may be important for preventing recurrence and optimizing therapeutic efficacy in patients undergoing postoperative chemotherapy (Mutka *et al*., 2023). Lu, C. *et al*. reviewed the mechanisms of radiotherapy resistance in breast cancer, Chinese herbal medicines and their active ingredients have a variety of pharmacological effects including anti-tumor and their active ingredients have a variety of pharmacological effects including anti-tumor, providing a wide range of possibilities for identifying effective that can overcome radiotherapy resistance and Chinese herbal medicines can improve the therapeutic effect of radiotherapy resistance in breast cancer (Lu *et al*., 2021).

***Application of traditional Chinese medicine in the treatment of breast cancer***

Zhang, Y. *et al*. pointed out that TCM plays an important role in the whole process of cancer prevention and treatment, especially in the treatment of breast cancer and the adverse effects of radiotherapy and chemotherapy showed positive results. TCM can treat a variety of diseases, acting on multiple sites and targets (Zhang and Shi, 2024). In seven related studies, covering the years 2014-2023, four examined breast cancer risk in women with menopausal symptoms and other co-morbidities. The role of traditional Chinese medicinal herbs in preventing breast cancer and improving survival in breast cancer patients was explored. The studies found that the use of traditional Chinese herbal medicines was associated with lower breast cancer incidence and improved survival. All studies reported that the rate of patients receiving adjuvant TCM was lower than that of patients receiving only CM (Hou *et al*., 2024). Chinese medicines and their active ingredients have a variety of pharmacological effects, including anti-tumor, providing a wide range of possibilities for identifying effective treatments that can overcome radiotherapy resistance and Chinese medicines can improve the therapeutic effects of radiotherapy resistance in breast cancer (Zhao *et al*., 2024).

***Research methodology***

*Clinical information*

One hundred and five female patients admitted to a hospital with a first diagnosis of breast cancer from May 2024 to January 2025 were selected for the study.

*Inclusion criteria*:
The inclusion criteria were that the patients needed to be clearly diagnosed with breast cancer by pathologic examination and had not used antimicrobials and probiotics within 2 weeks before enrollment. The physical status score was in the range of 0 to 2 and the results of blood, liver and kidney function tests performed before chemotherapy were normal. Postoperative wound healing, Karnofsky Functional Status Score ≥60 and expected survival of more than 6 months (Barbieri *et al*., 2024).

*Exclusion criteria*:
(1) Those who had used antimicrobial drugs or had unexplained diarrhea within 2 weeks before enrollment.
(2) Patients with abnormal results of blood routine, liver and kidney function tests before chemotherapy.
(3) Those with physical status score of 3-5.
(4) Those who were not expected to complete 4 cycles of chemotherapy after evaluation (Wei *et al*., 2024).
(5) Those with significant concomitant evidence, combined cardiac, hepatic, renal and other multi-system primary conditions.
(6) Those with gastrointestinal disorders such as ulcerative colitis, symptomatic inflammatory bowel disease, chronic diarrhea and intestinal obstruction after gastrointestinal resection.
(7) Those who had poor medication compliance and failed to take medications as prescribed. The patients were divided into 52 cases in the traditional Chinese medicine combination group and 53 cases in the control group using the random number table method and the two groups were compared in terms of general information such as age, primary tumor-regional lymph node-distant metastasis (TNM) stage and lymph node metastasis (Alsakkaf *et al*., 2024).

Table 1 shows a comparison of general information for the two groups of patients. All patients involved in this study were informed of the study content and signed informed consent forms. The study has been approved by the hospital's ethics committee to ensure compliance with ethical standards.

***Treatment***
The control group was treated with tamoxifen citrate tablets (triamcinolone acetonide) produced by Shanghai Fudan Fuhua Pharmaceuticals, a commonly used drug for breast cancer treatment, with each tablet containing 10mg of tamoxifen citrate. The dose was 2 tablets each time, 20mg each time, twice a day. In the Chinese medicine combination group, based on the control group's treatment regimen, an additional kidney-tonifying compound Chinese medicine soup was added to support the treatment. The prescription of this herbal soup contains 14g of chasteberry, 10g of cornelian cherry, 10g of ripe earth, 10g of angelica, 10g of eucommia, 10g of eucommia, 5g of Sichuan hyssop, 12g of white peony, 18g of raw astragalus, 12g of chai hu, 10g of scutellaria baicalensis, 10g of chickweed, 10g of scented herbs, 30g of xiaku cao, 30g of fumitory wheat and 5g of roasted licorice. Each dose of Chinese medicine should be decocted twice and a total of 200 ml of juice should be extracted after combining the two decoctions and the patient should take it orally twice a day. For patients who have difficulty in taking the medicine, the same dose of granules can be used for dosage and the whole treatment takes 3 months as a complete course of treatment.

In the postoperative recovery period of breast cancer, adjuvant traditional Chinese medicine should focus on tonifying deficiency, with Yangrong decoction as the basic formula and combined with herbs for soothing the liver and resolving depression, regulating the Chong and Ren meridians and tonifying qi and blood according to syndrome differentiation. The kidney-tonifying compound traditional Chinese medicine decoction used in this study integrates the effects of kidney-tonifying, qi and blood-tonifying and liver-soothing and depression-resolving, which is in line with the principles of traditional Chinese medicine in the postoperative recovery period of breast cancer.

***Observation indicators***
*The observation indexes contain the following aspects*:
(1) Take 3- 5 g of fresh feces from patients before and after chemotherapy, respectively, in a sterile bottle and send it for examination within 0.5 h. Take 0.5g of feces and dilute it by adding sterile diluent, dilute it to 10 by 10-fold serial dilution method and inoculate it into the medium of intestinal flora, in which Lactobacillus and Bifidobacterium were inoculated into Lactobacillus medium and Bifidobacterium medium and Enterobacter and Enterococcus were inoculated into Erythromycin agar medium and Enterococcus selective medium. Enterococci and Enterobacteriaceae among aerobic bacteria were placed in a 37°C incubator for 24 h for colony counting and Lactobacillus and Bifidobacterium among anaerobic bacteria were anaerobically incubated with Gas-Pak for 48-72 h for colony counting (Zhang *et al*., 2024).

(2) 1 day before treatment, 3ml of peripheral venous blood was drawn from 2 groups and processed by centrifugation at 3000r/min for 10min with a radius of 8cm, leaving the serum behind. CA125 level was detected using fully automatic electrochemiluminescence analyzer and collected whole blood samples were mixed with fluorescently labeled monoclonal antibodies against CD4, CD25 and CD69 proportionally. Usually, the volume of the antibody was 5 microliters and the volume of the whole blood was 50 microliters and the FAR level was detected using a fully automatic biochemical coagulation analyzer (Huang *et al*., 2024). The normal reference ranges for serum indices were used to distinguish CD4-positive T cells and to calculate their number based on the intensity and distribution of fluorescent signals.
(3) Adjust the voltage and compensation of the instrument's

forward angle (FSC), lateral angle (SSC), fluorescence 1 (FL1), fluorescence 2 (FL2) and fluorescence 3 (FL3) with FACSComp and adjust the position of the marker with the corresponding isotype control, respectively. Treg cells were isolated using flow cytometry and tissue sections were immunohistochemically stained using specific antibodies to detect the expression of proteins such as S2, nm23, Her-2, ER, PR and p53. Based on the staining results, the samples were classified as negative or positive, or as having low or high expression, based on staining intensity. Cellquest software was used to analyze the data and the results were expressed as a percentage by subtracting the non-specific control values.

(4) Observation indicators and efficacy determination criteria: Observe and compare the incidence of clinical manifestations such as diarrhea, nausea, vomiting and fever, the incidence of intestinal dysbiosis and the incidence of adverse drug reactions between the two groups during the period of chemotherapy and draw blood to check the blood and urine routine and liver and kidney functions of the two groups before and after treatment. Diarrhea assessment criteria: total amount of defecation >200g/d or the number of defecations>3 times/d, in which fecal water is more than 85% of the total amount (Liu *et al*., 2024; Asano *et al*., 2023). The diagnostic criteria of intestinal flora dysbiosis is to dilute 0.5g of fresh stool to 10 times with sterile saline, inoculate it on two kinds of media, bacillus and coccus and then calculate the number of colonies after placing in culture by applying plate viable bacteria counting method, of which the number of coccus bacteria is greater than or equal to 40% can be diagnosed as intestinal flora dysbiosis.

***Statistical methods***

Data were processed using SPSS 22.0. Measurement data are presented as "mean: standard deviation." For comparisons between groups, independent samples tests were used, while paired-samples tests were used for comparisons within groups. Count data were analyzed using chi-square tests and ordinal data were analyzed using rank-sum tests. The significance level was set at $\alpha=0.05$. For the final analysis, only patients with complete records for the reported variables were included. No sensitivity analyses were performed.

***Findings***

The flow of study participants from recruitment to final analysis is shown in Fig. S1.

*Changes in intestinal flora*

Table 2 shows the changes in intestinal flora of breast cancer patients. G is the group, before chemotherapy, CN and CR are the combination of the traditional Chinese medicine group and the control group, respectively, B1 is before chemotherapy and A1 is after chemotherapy. The bifidobacterial count in the control group was 6.98±1.34 lg (CFU/g) and in the Chinese medicine combination group, 7.58±1.85 lg (CFU/g), with no significant difference between the two groups. However, a significant difference was observed after chemotherapy. The number of Bifidobacterium bifidum in the control group decreased to 6.14±1.15 lg (CFU/g) after chemotherapy and the number of Bifidobacterium bifidum had a certain effect, but the decrease was relatively small, reflecting the general inhibitory effect of chemotherapy on the intestinal flora. Meanwhile, the number of Lactobacillus in this group also decreased, from 7.38±1.44 lg (CFU/g) to 6.91±1.14 lg (CFU/g), while the number of Enterobacteriaceae and Enterococci changed relatively little. The changes in bifidobacteria, Lactobacillus, Enterobacteriaceae and Enterococcus in the control group were not significant; in contrast, the number of bifidobacteria in the herbal combination group increased significantly after chemotherapy, reaching 9.01 ± 1.49 log (CFU/g). As with bifidobacteria, chemotherapy may induce changes in the intestinal environment that allow Lactobacillus to survive and multiply more effectively. An increased number of Lactobacillus may help improve intestinal barrier function and enhance intestinal defense against harmful substances. Meanwhile, Enterobacteriaceae and Enterococci were significantly reduced and the competition for nutrients among the dominant flora in the intestinal tract after treatment inhibited the growth of Enterobacteriaceae and Enterococci. This suggests that the compound herbal soup may have a protective effect on the intestinal flora during chemotherapy, helping maintain its balance and health.

***Changes in the number and activation status of CD4-positive T cells***

The CD4-positive T-cell activation of patients in the two groups is shown in table 3. After chemotherapy, the number of CD4-positive T-cells in the traditional Chinese medicine combination group was significantly increased to 969 cells/μL, whereas in the control group, although also increased, the increase was smaller, at 856 cells/μL. The increase was significantly smaller than that in the traditional Chinese medicine combination group. The expression rates of CD25, a CD4-positive T cell activation marker and CD69, a CD4-positive T cell activation marker, were similar in the traditional Chinese medicine combination group and the control group before chemotherapy, with 14.23%, 14.17% and 8.31% and 8.36%, respectively. After chemotherapy, the expression rates of both groups increased, but the increase was greater in the traditional Chinese medicine combination group, reaching 24.98% and the expression rate of CD69 in the traditional Chinese medicine combination group increased to 15.36%, which indicated that chemotherapy had a more significant promotion effect on the expression of CD25, a CD4-positive T cell activation marker and the expression rate of CD69 in patients in the traditional Chinese medicine combination group.

***Peripheral blood CD4 cell expression levels and immunohistochemical indicators***

Table 4 shows Treg cell expression levels and pathological

immunohistochemical indices. In terms of the S2 indicator, the expression level of CD4+ cells in patients in the traditional Chinese medicine (TCM) combined group was slightly lower than that in the control group and in the positive group, the expression level of the TCM combined group was also lower than that of the control group, with statistically significant differences (P=0.015 and P=0.019). Correlation analysis showed that the correlation coefficient of the TCM combined group (r=-0.250) was slightly lower than that of the control group (r=-0.229), but the *P-values* of both groups were less than 0.05, indicating a significant negative correlation between Treg cell expression levels and the S2 indicator in both groups. This suggests that the TCM-combined group can more effectively regulate CD4+ cell levels and avoid excessive reactions.

In terms of the nm23 indicator, Treg cell levels in patients in the TCM combined group were higher than in the control group in the low-expression group, but lower in the high-expression group. Correlation analysis showed that the correlation coefficient of the TCM combined group (r=-0.420) was lower than that of the control group (r=-0.401), indicating an inverse relationship between nm23 expression and Treg cell levels and this relationship was more pronounced in the TCM combined group. In terms of the Her-2 indicator, the level of Treg cells in patients in the TCM combined group was significantly lower than in the control group in the low-expression group, but slightly higher in the high-expression group. Correlation analysis showed that the correlation coefficient of the TCM combined group (r=0.630) was higher than that of the control group (r=0.604) and the *P-values* of both groups were less than 0.001, indicating that the immune system of patients in the TCM combined group can more effectively activate CD4+ cells and enhance immune responses when Her-2 is highly expressed, which is helpful in combating tumors. In terms of the ER, PR and p537 indicators, there was no statistically significant difference in the expression levels of Treg cells between the TCM combined group and the control group, with *P-values* all greater than 0.05.

***Adverse reactions***

Table 5 shows the adverse reactions of the two groups of patients. The number of diarrhea cases in the combination of traditional Chinese medicine group was 10, the number of nausea cases was 16, the number of vomiting cases was 10 and the number of fever cases was 6. The number of intestinal flora disorders in the traditional Chinese medicine combination group was 4, accounting for about 7.69%. The five key dimensions of diarrhea, nausea, vomiting, fever and intestinal flora dysbiosis were significantly lower in the patients in the Chinese medicine combination group than in the control group. The Chinese medicine combination group may have greater advantages in maintaining intestinal flora balance and reducing dysbiosis compared with the control group.

A comparison of gastrointestinal conditions between the two patient groups is shown in table 6. In the distribution of degree I cases, the combined group of traditional Chinese medicine accounts for 5.77%, while the control group accounts for 18.87%. In the distribution of cases of degree II, there were 3.85% in the combined group of traditional Chinese medicine and 26.42% in the control group and in degree III, there were 3.85% in the combined group of traditional Chinese medicine and 9.43% in the control group. The Chinese medicine combination group showed better results in controlling the disease progression to degree I and above compared with the control group, probably due to the fact that the treatment modality adopted by the Chinese medicine combination group played an active role in intervening in the process of breast cancer disease development, effectively reducing the proportion of patients with more severe degrees of breast cancer disease.

## DISCUSSION

Breast cancer belongs to the category of “breast rock” in traditional Chinese medicine and its occurrence and development can be attributed to the weakness of positive qi and the internalization of evil toxins, which are related to the imbalance of yin and yang in the disregulation of Chong Ren (Xu *et al*., 2021). Although surgery can remove localized cancer, it will deplete qi and blood. After radiotherapy, it will deplete qi and injure fluid, further aggravating the patient's state of deficiency. The Kidney Replenishing Compound Formula was prescribed to tonify the liver and kidneys using chasteberry, cornelian cherry, Radix Rehmanniae Praeparata, Radix Angelicae Sinensis, Radix Eucommiae, Radix Achyranthis Bidentatae and Radix Paeoniae Alba. Raw astragalus and chasteberry are used together to benefit qi, promote yang, nourish yin and generate body fluids.

Chaihu is used with Scutellaria baicalensis and Xiangshu to dredge the liver, clear heat and toxins. Xiang Fu is a commonly used herb for detoxifying the liver and regulating qi and Chicken Blood Vine can tonify and invigorate blood, enhancing the effect of regulating qi and promoting blood circulation. Xia Ku Cao belongs to the liver and gallbladder meridians and has the effect of clearing the liver and removing fire. Floating wheat is sweet in taste and cool in nature, nourishing the heart and removing vexation, removing heat and stopping sweating and roasted licorice plays a role in harmonizing all medicines, tonifying the spleen and benefiting the qi. Additionally, it nourishes the liver and kidneys, regulates yin and yang, qi and blood, moistens without dryness and tonifies without stagnation, in line with the traditional Chinese medicine principle of seeking the root of the disease.

**Table 1**: Comparison of general information of the two groups of patients.

| Group | n | Age $\bar{x} \pm s$ (years) | Physical fitness score $\bar{x} \pm s$ (points) | TNM stage, n | | | |
|---|---|---|---|---|---|---|---|
| | | | | Phase I | Phase II | Phase III | Phase IV |
| Chinese medicine joint group | 52 | 54.27± 6.99 | 0.81± 0.21 | 5 | 10 | 21 | 9 |
| Control group | 53 | 53.95±7.85 | 0.89± 0.31 | 6 | 9 | 17 | 7 |
| Statistics | | t=0.028 | t=0.304 | Statistics | | | |
| P value | | 0.954 | 0.744 | P value | | | |

**Table 2**: Changes in intestinal flora in breast cancer patients, lg (CFU/g).

| Group | Bifidobacterium, lg (CFU/g) | | Lactobacillus, lg (CFU/g) | | Enterobacteriaceae, lg (CFU/g) | | Enterococci, lg (CFU/g) | |
|---|---|---|---|---|---|---|---|---|
| | B1 | A1 | B1 | A1 | B1 | A1 | B1 | A1 |
| CN | 7.58±1.85 | 9.01±1.49a | 7.28±1.01 | 8.33±1.96a | 7.59±1.02 | 6.31±1.13a | 6.82±1.08 | 5.36±1.88a |
| CR | 6.98±1.34 | 7.22±1.95 | 7.22±1.95 | 7.26±1.02 | 7.74±1.93 | 7.33±1.05 | 6.85±1.19 | 6.7±1.54 |

**Table 3**: CD4 positive T cell activation in two groups of patients.

| Group | n | Number of CD4 positive T cells (cells/μL) | | CD4 positive T cell activation marker CD25 expression rate (%) | | CD4 positive T cell activation marker CD69 expression rate (%) | |
|---|---|---|---|---|---|---|---|
| | | B1 | A1 | B1 | A1 | B1 | A1 |
| Chinese medicine joint group | 52 | 841 | 969 | 14.23 | 24.98 | 8.31 | 15.36 |
| Control group | 53 | 852 | 856 | 14.17 | 16.33 | 8.36 | 11.29 |

**Table 4**: Treg cell expression levels (%) and pathological immunohistochemical indicators.

| Immunohistochemical indicators | Chinese medicine joint group | Control group |
|---|---|---|
| S2 negative | 9.00±2.10 | 9.53±2.39 |
| S2 positive | 7.80±1.50 | 8.19±1.66 |
| Correlation (r) | -0.250 | -0.229 |
| P value | *0.015 | *0.019 |
| nm23 low expression | 9.50±1.30 | 9.98±1.44 |
| nm23 high expression | 7.85±1.05 | 8.22±1.13 |
| Correlation (r) | -0.420 | -0.401 |
| P value | *0.035 | *0.043 |
| Her-2 low expression | 7.60±2.15 | 8.05±2.23 |
| Her-2 high expression | 10.20±1.15 | 10.58±1.21 |
| Correlation (r) | 0.630 | 0.604 |
| P value | ***0.000 | ***0.000 |
| ER-negative | 9.40±2.00 | 9.47±2.08 |
| ER-positive | 9.10±2.35 | 9.15±2.48 |
| P value | 0.550 | 0.382 |
| PR-negative | 9.55±1.75 | 9.59±1.81 |
| PR-positive | 9.15±2.45 | 9.13±2.53 |
| P value | 0.420 | 0.441 |
| p53-negative | 9.00±1.80 | 8.98±1.85 |
| p53-positive | 9.40±2.50 | 9.43±2.51 |
| P value | 0.500 | 0.800 |

"*"P<0.05, "**"P<0.01, "***"P<0.001.

**Table 5**: Adverse reactions of patients in the two groups.

| Group | Total sample size, n | Diarrhoea, n (yes/no) | Nausea, n (yes/no) | Vomiting, n (yes/no) | Fever, n (yes/no) | Intestinal flora imbalance, n (yes/no) |
|---|---|---|---|---|---|---|
| Chinese medicine joint group | 52 | 10/42 | 16/36 | 10/42 | 6/46 | 4/48 |
| Control group | 53 | 20/33 | 22/31 | 15/38 | 8/45 | 10/43 |
| Statistics | 0.34 | 0.55 | 7.25 | 0.06 | 0.20 | 4.85 |
| P value | 0.002 | 0.006 | 0.555 | 0.795 | 0.680 | 0.030 |

**Table 6**: Comparison of gastrointestinal conditions between the two groups, n (%).

| Group | n | Ⅰ degree, n (%) | Ⅱ degree, n (%) | Ⅲ degree, n (%) | Total, n (%) |
|---|---|---|---|---|---|
| Chinese medicine joint group | 52 | 3(5.77) | 2(3.85) | 2(3.85) | 7(13.46) |
| Control group | 53 | 10(18.87) | 14(26.42) | 5(9.43) | 34(64.15)) |
| $\chi^2$ value | 20.108 | | | | |
| P value | <0.001 | | | | |

Phenoxylamine tablets are non-steroidal anti-estrogenic drugs, long-term use of which causes a decrease in estrogen and prolactin levels in the body, while progesterone levels are 88% high, a trend that becomes more pronounced with the increase in the course of treatment and causes adverse reactions such as menstrual regulation, amenorrhea, vaginal bleeding, hot flashes and flushing of the face (Wang *et al*., 2025). Intestinal flora plays an important role in the metabolism of drugs, which can enhance the efficacy of drugs, promote anti-tumor immunity, reduce chemotherapy-related toxicities and side effects, improve the quality of life of patients and play a role in prolonging the overall survival rate and at the same time, the intestinal flora can mediate the anti-tumor effects of tumors by regulating the function of myeloid cells in the tumor microenvironment. At the beginning of the design of the three present studies, it was considered that the above adverse reactions caused by triamcinolone tablets might be caused by hormone level disorders and attempts were made to change the endocrine environment of the body through traditional Chinese medicines with estrogenic effects, so as to improve these symptoms (Shen *et al*., 2021). Modern pharmacological studies have shown that astragalus polysaccharides, astragalosides and astragalus flavonoids, which are active components in astragalus, can stimulate the immune system and promote the immune proliferation of lymphocytes to achieve the role of enhancing immune function (Du *et al*., 2022). In research on CD4+ T cells and Bifidobacterium, this study further explored, compared with prior studies, the potential mechanisms by which traditional Chinese medicine formulas activate CD4+ T cells. The former study mainly focused on the potential of Bifidobacterium to regulate dendritic cell function and promote the accumulation of CD8+ T cells in the tumor microenvironment, thereby enhancing NK cell secretion of IFNγ and perforin and demonstrating significant anti-cancer effects (Mutka *et al*., 2023). The latter study investigated how the gut microbiota can modulate the cGAS-STING-IFN-I signaling pathway in dendritic cells, thereby regulating the adaptive immune response of CD8+ T cells (Nakagawa *et al*., 2023). Building on these findings, this study further explored how traditional Chinese medicine formulas can modulate CD4+ T cell activation by regulating the gut microbiota, particularly Bifidobacterium. The study found that kidney-tonifying formulas may indirectly influence CD4+ T cell activation by modulating the gut microbiota composition and increasing the abundance of beneficial bacteria, such as Bifidobacterium.

The results of this study show that there was no significant difference in the number of intestinal flora between the traditional Chinese medicine (TCM) combination group and the control group before chemotherapy. However, after chemotherapy, the number of Bifidobacterium in the TCM combination group increased significantly to 9.01±1.49 lg (CFU/g), indicating that the TCM combination group has a positive protective and regulatory effect on the intestinal microbiota. In addition, the expression levels of the activation markers CD25 and CD69 on CD4-positive T cells increased from 14.23% and 8.31% to 24.98%, thereby promoting CD4-positive T cell proliferation and activation and enhancing the immune response. The TCM combination group demonstrated a stronger ability to regulate CD4+ cell levels, effectively activating CD4+ cells and enhancing the immune response. Moreover, the incidence of adverse reactions in the TCM combination group, such as diarrhea, nausea, vomiting, fever and intestinal flora disorder, was lower than that in the control group. The results indicate that a kidney-tonifying compound composed of traditional Chinese medicines, such as Cnidium monnieri, can improve immune function in breast cancer patients after surgery. Although it has no significant effect on the patients' sex hormone levels, it can improve symptoms such as hot flashes, menstrual irregularities and facial flushing caused by endocrine therapy, providing a direction for the traditional Chinese

medicine syndrome differentiation and treatment of such patients. The TCM combination group can enhance the gastrointestinal function of breast cancer patients after surgery and undergoing chemotherapy, regulate intestinal flora, enhance the body's immune function and has a low incidence of gastrointestinal reactions, making it safe and reliable.

This study has several limitations. It was a single-centre study with a relatively small sample size and the study size was determined by the number of eligible patients during the recruitment period rather than by a formal sample-size calculation. The follow-up period was limited to one treatment course and no sensitivity analysis was performed. Therefore, the findings should be interpreted with caution and validated in larger multicentre studies.

***Acknowledgements***
None.

***Authors' Contribution***
Tong Wei: Responsible for research design, experimental operation, data collection, data analysis and manuscript writing; Xuezhen Li: Supervision, project administration, data curation writing–review and editing; Zhitao Guo: Conceptualization, project administration, writing–review and editing.

***Data availability statement***
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

***Funding***
There was no funding.

***Ethical approval***
The study was approved by the Ethics Committee of Guangdong Provincial Second Hospital of Traditional Chinese Medicine (Approval No.: GDET-2024-0317). This study was performed in adherence with the STROBE guidelines. See supplementary file for the STROBE checklist.

***Conflict of interest***
The authors declare no conflict of interest.

***Supplementary data***
https://pjps.pk/uploads/2026/09/SUP1788608972.pdf

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