

# Reduction of inflammatory response by way of oxidative stress with san huang decoction (SHD) in perioperative care: a randomized clinical trial

## Abstract

The microenvironment has a profound impact on both wound healing and on cancer progression. Optimizing the microenvironment has implications for both cancer and postoperative recovery. Reduction of inflammatory factors by reducing oxidative stress provides a promising avenue for clinical effect. Traditional Chinese medicine treatments have been shown to be beneficial and safe for optimal regulation of oxidative stress during the postoperative period. This clinical trial evaluated the effectiveness of a promising Chinese herbal formula, SHD, on oxidative stress and inflammatory response after mastectomy.

**Methods:** 60 breast cancer patients who met the inclusion/exclusion criteria were randomized to either treatment (n=30) or control group (n=30). Patients in the treatment group were given liquid SHD taken twice a day with/without food. Treatment was given for 1 day prior to surgery and 7 days postop. Participants in the control group received placebo in the same schedule as for the treatment group. Outcomes measured included oxidative stress scores, quantity and duration of drainage fluid and serological levels of TAOC, NO, SOD, CRP IL-6, TNF-alpha, and VEGF on day 1 pre-operatively and days 1, 4 and 7 postop.

**Results:** Oxidative stress scores decreased significantly in the treatment group compared to those of the control group ( $p < 0.05$ ) with the maximal differences of found on day 4 after operation ( $p = 0.028$ ). Drainage fluid was reduced significantly with 572.2ml in the treatment group compared to 700.4ml in the control group. Participants in the treatment group were able to have their drainage tubes removed an average of 2.73 days before the control group. Serum levels of TAOC, NO, SOD, CRP IL-6, TNF-alpha, and VEGF were significantly lower in the treatment group at postop days 4 and 7 compared those in the control group.

**Conclusions:** Perioperative treatment with SHD effectively reduced oxidative stress, the inflammatory response and demonstrated improvement in recovery amongst women having mastectomy for breast cancer.

**Keywords:** shd, breast cancer surgery, oxidative stress, inflammatory response, integrative oncology, complementary medicine, traditional chinese medicine, TCM

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## Introduction

Breast cancer is now the most common cancer in Chinese women; cases in China account for 12.2% of all newly diagnosed breast cancers and 9.6% of all deaths from breast cancer worldwide.<sup>1</sup> Surgery remains the most curative option for breast cancer.<sup>2</sup> Post operative healing from breast surgery is complicated by a variety of factors including psychological pressure, anesthetic drugs and injury.<sup>3</sup> It is known that the body healing process starts from wound and injury with the basic steps from the phase of inflammatory, proliferation to that of plastic.<sup>4</sup> Though inflammation is a necessary first step towards wound reparation, many studies show that a sustained and prolonged inflammatory response hinders recovery.<sup>5</sup> In addition, sustained strong inflammatory cytokines in the microenvironment can increase cancer cell growth and potential for metastasis.<sup>6</sup> In the context of cancer control, it is important to manage the intensity and duration of inflammation without hampering recovery.<sup>7</sup>

Breast cancer surgery has evolved over the last 40 years from profound and aggressive treatment to much more conservative procedures that minimize tissue trauma and physical deformity.<sup>8</sup> These changes came about after Fisher's discovery that breast cancer is a systemic disease 1976.<sup>9</sup> Implications of this are that aggressive

treatments leading to more extensive injuries may intensify worse prognoses and also support the beneficial roles of systemic integrated approaches that may reduce cancer progression. Pro-inflammatory factors can be contributors to the development and progression of breast cancer.<sup>10</sup> Serum pro-inflammatory cytokines including IL-6 and IL-8 are higher in breast cancer patients and may serve as an indicator for prognoses in patients with breast cancer.<sup>11</sup> Anti-inflammatory therapies may also yield a viable approach to supporting an internal environment less conducive to cancer progression. It was reported that anti-drug of aspirin can prevent breast cancer occur and prolong breast cancer survival.<sup>12,13</sup>

Surgery induces an acute inflammatory response which could impact negative clinical outcomes in breast cancer. It was found that elevated inflammatory mediators including vascular endothelial growth factor-C (VEGF-C), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-1 $\beta$  (IL-1 $\beta$ ) are associated with increased rates of breast cancer recurrence and metastasis of following surgery.<sup>14-16</sup> Inflammatory biomarkers and scores are potentially important prognostic factors in breast cancer during the perioperative period.

During the perioperative period, many non-physiological factors such as surgical related trauma, psychological stress and so on, can

trigger oxidative stress,<sup>17</sup> followed by peak systemic inflammatory response. Oxidative stress is defined as a disturbance in the balance between oxidant-antioxidant states, favoring the oxidant environment.<sup>18</sup> Generally reactive oxygen species (ROS) expression is increased in cancer cells and plays an important role in the initiation and progression of cancer through rising the expression of the related factors of tumor metastasis and relapse, such as hypoxia-inducible factor (HIF-1 $\alpha$ ), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), chemotactic factor CXCL12 and metalloproteinase-1 (MMP-1), etc.<sup>19,20</sup>

During the perioperative period, states of increased stress and high levels of inflammatory factors have been shown to be related to worse recovery, followed by progression of cancer proliferation and metastasis.<sup>21</sup> The concept of “fast-track surgery” (FTS)<sup>22</sup> was presented over the last two decades and is also known as “enhanced recovery after surgery” or “multimodal rehabilitation after surgery” with evidence-based approaches to optimize patient outcome during perioperative period. Through FTS with minimally invasive surgery of laparoscopic radical gastrectomy, it was found that postoperative VEGF levels were decreased and the 3-year survival rate was increased. FTS may facilitate postoperative recovery by reducing physical and psychological stresses.<sup>23</sup> Now it becomes an integrated protocol covering preoperative appropriate preparations and assessments, sophisticated operative manipulation, and standardized postoperative treatment and nurse care, have been proposed to maintain physiological function and thereby facilitate to enhance recovery. Each of these interventions such as “Gastrectomy Fast Track Surgery Guideline” formulated by 2014 European FTS Association, has been shown to independently be favorable and beneficial to the patient undergoing surgery.<sup>24</sup>

Traditional Chinese Medicine (TCM) is primarily used as a complementary alternative medicine approach built on a foundation of more than 2,500 years of Chinese medical practice.<sup>25</sup> TCM's view of the body mainly addresses functional entities rather than to look for the fixed somatic structures that perform the activities. Because of this, Chinese medicine pays attention to the recovery of the functions balance to maintain the health rather than restore the west concept of biochemical or anatomical properties. While health is perceived as a harmonious interaction of these entities with the outside world, disease is interpreted as a disharmony. Harmonious balances are disrupted by cancer burden, surgical injury, psychological depression and other imbalances in function or structure. There may be benefits achievable using TCM principles to support the creation of harmony inside the body through tools including herbal medicine, acupuncture, massage, qigong, and dietary modifications.<sup>26</sup>

San Huang Decoction (SHD) is a type of herbal medicine composed of astragalus, turmeric and rhubarb. This formula is a traditional one used within TCM that has the effect of reducing oxidative stress and suppressing tumor angiogenesis.<sup>27,28</sup> Our current protocol focuses on clinical observation of enhanced recovery through an attempt to reduce oxidative stress and inflammatory response in the perioperative period using the SHD decoction. Goals of the trial and decoction are to increase breast cancer control and improve the quality of life.

## Materials and methods

### Methods and study protocol

The research protocol was approved by Institutional Review Board of Human Research in Affiliated Hospital of Nanjing University of Traditional Chinese Medicine. Patients from the breast disease department of Jiangsu Provincial Hospital of TCM were identified,

screened and enrolled in the study following informed consent from January 2013 to December 2015. Patients in this study were recruited from a population of patients undergoing routine follow-up at the clinic during the perioperative period. The subjects were invited to participate in the study if they fulfilled all of the following criteria: all patients had to have a diagnosis of breast cancer with planned modified radical surgery between aged from 30 to 80 years old. Informed consent document should be clearly understood and signed by the subject. Patients with other types of surgery instead of modified radical surgery, metastatic breast cancer, serious internal organ diseases and/or accepting oral anticoagulation and suppressive anti-inflammatory medicines like aspirin and steroids were excluded from the study. If the subjects would like to withdrawal of consent or failure to adhere to the research protocol or serious adverse events happened, the study of the participant would be suspended and recorded as withdrawn.

### Study design

Assuming  $\alpha=0.05$ , 80% power and an expected effect size of 20% improvement approximately 60 subjects were needed. The 60 patients who met the inclusion/exclusion criteria were allocated the digital numbers of A to the experimental and those of B to the control group equally with computer random central allocation and the delegates of A and B were blind to both researchers and subjects.

### Treatment technique

**Treatment group:** 200ml liquid medicine of SHD given orally twice a day in the morning and afternoon plus the same basic treatments as those of control group. Treatment was applied for 1 day pre-operatively and 7 days post-operatively. The SHD decoction consisted of Chinese herbs of 30g astragalus, 10g rhubarb and 10g turmeric blended in 500ml water and heated to 100°C for one hour until 200ml of liquid medicine left. A total of 400ml liquid medicine was obtained by the above step repeated twice and mixed together.

**Control group:** The patients received intravenous drip of cefazolin sodium (2.0g) for half-hour before surgery, continued intravenous drip of cefazolin sodium (2.0g) for one-hour during surgery, intravenous drip of Omeprazole 42.6mg for suppressing acid. The total of fluid infusion was 1500ml which included sugar 750ml and salt 750ml, in addition add Vitamin C 2.0, Vitamin B 0.2 and potassium chloride 1.0g. Liquid diet was required for 6 hours after surgery and normal eating was permitted for 12 hours after surgery.

To partly prevent the bias of the subjects, the volume of 200ml liquids of sodium chloride were applied with the same package of non-transparent plastic bag twice per day. Because all the patients drank the liquid with the same appearances of plastic bags and different tastes, they were partly blind to know which group had been allocated.

### Outcome measures

#### Main index

The scores of clinical symptoms focused on oxidative stress state was referenced to Stanford acute stress reaction questionnaire (SASRQ).<sup>29</sup> The questionnaire here mainly concentrated on the acute stress symptoms during the perioperative period, including the degree of incision pain, the influences on diet and sleep, skin and joint and attentiveness. The scores were evaluated as the sum of each clinical symptom in the column of Table 1.

The amounts of drainage fluid and the duration the drainage tubes remained after operation were recorded at the day 1, day 4 and day 7 postoperatively.

**Serological test indexes:** Serum nitric oxide (NO) and superoxide dismutase (SOD) were measured using the Shimadzu spectrophotometer (Shimadzu Company, Japan). According to operational instructions, the absorbance value (OD) was measured at 550nm and based on a comparison with the standard curve, the content of NO and SOD was calculated. Serum levels of tumor necrosis factor (TNF- $\alpha$ ), interleukin-6 (IL-6) and human c-reactive protein (CRP) were evaluated by using enzyme linked immunosorbent assay (ELISA) kits. Total antioxidant capacity (TAOC) was evaluated using the Ferric reducing antioxidant potential assay (FRAP) measured at 570nm. ELISA kits of NO and SOD were purchased from Nanjing Jiancheng Bioengineering Institute of cata No 20150914 and 20150923 respectively. ELISA kits of TNF- $\alpha$  and IL-6 were purchased from Beijing 4A Biotech Co., Ltd. of cata No. 20150109 and 20150113 respectively. The ELISA kit of CRP was purchased from Shanghai Mibio Bio-tech Co., Ltd. of cata No. ml027874. The TAOC kit was purchased from Shanghai Yubo Bio-tech Co., Ltd. of cata No. YB-E13560.

**Dates for outcome measures:** All of the outcomes were measured at 1 day before operation, and on day1, day4 and day7 postoperatively for every subject.

### Statistical analysis

The SPSS17.0 statistical software was applied for data analysis. Continuous data was evaluated with a T-test, grade data with Redit analysis and categorical data with X2 test.

## Results

### Participant recruitment and characteristics

A total of 74 patients were assessed for eligibility and 60 were enrolled as the subjects in the study as from Jan. 2014 to Dec. 2015. Participants flown through the trial are depicted as per Figure 1. All subjects received modified radical mastectomy and 73.3 percent of the participants in each group were found to have positive lymph nodes. 86.3 percent of all subjects were diagnosed with invasive ductal or lobular invasive carcinoma with 67.7 percent of sum with TNM stages of IIa to IIc. At baseline there were no significant differences between treatment groups for age, the scores of stress, the serological levels of TAOC, NO, SOD, CRP, IL-6, TNF- $\alpha$ , and VEGF before operation (Table 2).

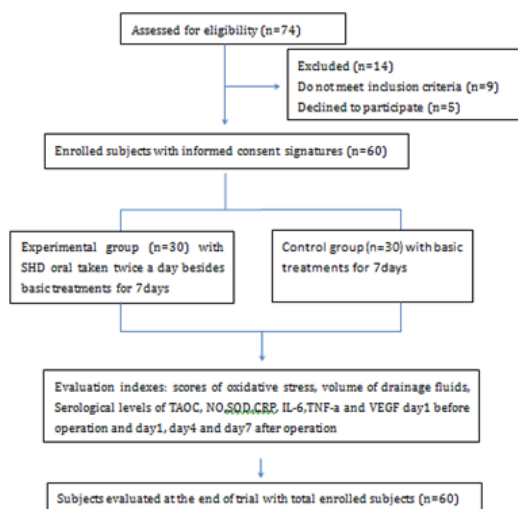


Figure 1 Participant flow through the trial.

### The scores of oxidative stress evaluated by clinical symptoms

On postoperative day1, more severe pain, loss of appetite, fatigue and weakness accompanied with reduced ability for independent daily activity were found in the subjects in the control group compared to treatment. Average score of 6.44 was found in control group compared to those with less pain and fatigue in those in the treatment group (average scores of 5.02). On day4, the subjects in the control group only felt slight relief of pain at incision and some recovery of function postoperatively with scores of 4.31 compared to 2.89 among subjects in the treatment group. Because the stress scores before operation in two group were from 3.43 to 3.45 and the scores on postoperative day4 were 2.89, which is less than the scores before operation, therefore, the patients in the treatment group has recovered to the stress state before operation on postoperative day4. Till 1 week after operation, the stress state of the subjects in the control reduced to 3.48, which was similar to the scores before operation which is 3.43, while the state of subjects in the treatment group continued relief to the scores of 2.35. Significant differences were found between two groups at each point date with all the P values less than 0.05 (Table 3).

### Volume of drainage fluid and duration of drainage placement

On postoperative day1, the volume of drainage fluid per 24 hours in treatment group was 166.87ml, while the volume in control group was 149.80ml. On day4, the volume reduced to 114.67ml of treatment group and 117.33ml of control group respectively. Both did not have significant differences in two groups. On day7, a significant reduction of drainage fluid volume was found in that of treatment group (55.40ml compared to 73.27 ml in that of control group). The total amounts of drainage fluid were also significantly less with 572.20 ml in the treatment group compared to 700.40ml in the control group. The treatment group had 2.73 days less of time with drainage tubes placed postoperatively compared to control group (Table 4).

### The serological test indexes related to stress

The level of TAOC in the control group was decrease, whereas participants in the treatment group had stable and slightly improved TAOC. Serum levels of NO and SOD were stable in the control group, whereas NO decreased continually and level of SOD remarkable increased in the treatment group. The significant differences were found on postoperative day4 and day7 between two group on the above three indices (Table 5).

### Inflammatory markers

The serological indices of all the inflammatory factors increased significantly 1 day after operation with four times increased CRP and two times enhanced IL-6 and TNF- $\alpha$  and gradually decreased along with dates going on. The levels of inflammatory markers in the treatment groups decreased much faster than those in the control group with statistical differences on postoperative day4 and day7. IL-6 and TNF- $\alpha$  on postoperative day4 in the treatment group decreased to the levels found on the day before operation with CRP levels decreased to such level on day7. In the control group values for each marker was still higher than the levels before operation right until day7. VEGF levels were increased significantly on postoperative day1 and enhanced to peak on day4 in the control group, while those in the treatment group decreased gradually postoperatively without being increased. Statistical significant differences were found on day4 and day7 postoperatively between both groups with the levels in the treatment group significantly lowered than those before operation showing somehow inhibiting the potential effects on stimulating vascular growth (Table 6).

**Table 1** Scores of oxidative stress evaluated by clinical symptoms

Incision pain	Diet and sleep	Quality of life	Skin and joint	Attentiveness	Score
None	Normal	Satisfied	Normal	Normal	0
Sometime	Good appetite, mild sleep disorders	Trouble for sometime	Mild pruritus and/or joint fatigued	Mild disturbance without daily life influence	1
Slight pain	Loss of appetite, sleep time less than 6 hours	Too much trouble	Pruritus with formication and joint weaken	Anxiety without continued focus	2
Continued pain	Vomit after eat, sleep time less than 4 hours	Terrible	Obvious formication and/or feeble joint with dysfunction independent	Anxiety without ability of life-independent	3

**Table 2** Baseline Participant Characteristics.

Index	Experiment group	Control group	P value
Age (year)	52.8±6.7	53.9±7.7	0.68a
Lymph node metastasis			
None	8	8	
I-3	12	14	
>3	10	8	
Histological type (n):			1.0b
invasive ductal carcinoma	20	20	
invasive lobular carcinoma	6	4	
adenocarcinoma	2	4	
medullary carcinoma	2	2	
TNM stages (n)			1.00a
I	6	6	
Ila	10	10	
Ilb	10	11	
Illa	4	3	
Stress Score	3.43±1.87	3.45±1.93	0.65a
TAOC (mmol/L)	11.65±2.86	12.31±3.12	0.555 a
NO (U/ml)	85.5±13.9	79.5±15.2	0.25 a
SOD (U/ml)	65.2±2.3	66.9±4.8	0.18 a
CRP (mg/L)	3.62±1.43	3.66±1.34	0.929 a
IL-6 (pg/L)	7.91±3.78	7.45±4.12	0.749 a
TNF-a (pg/L)	10.09±6.60	10.45±7.90	0.895 a
VEGF (pg/L)	104.1±8.5	102.3±8.6	0.79 a

a Student t test for paired data. b Fisher's exact test for categorical data

**Table 3** Postoperative Modified Stress Scores per group

Group	n	1 Day	4 Day	7 Day
Control	30	6.44±1.37	4.31±1.58	3.48±1.42
Experiment	30	5.02±1.45	2.89±1.15	2.35±1.45
P value		0.034	0.028	0.026

**Table 4** Postoperative drainage fluid volume and duration of application per group

Group	Day1	Day4	Day7	Amount Sum(ml)	During Period at Chest (Day)	During Period at Axillary Cavity(Day)
Control	149.80±62.15	117.33±39.09	73.27±31.56	700.40±107.38	6.47±1.06	16.80±1.86
Experiment	166.87±52.73	114.67±48.27	55.40±36.52	572.20±93.95	4.53±0.99	14.07±1.62
p	0.424	0.289	0.012	0.001	0.0189	0.000193

**Table 5** Serological indexes of oxidative stress after operation (each group n=30)

Group	Oxidative stress Indexes	Day1	Day4	Day7
Control	TAOC(mmol/L)	9.87±2.72	7.91±2.25	6.84±1.98
Experiment		9.48±2.74	9.85±2.79	11.01±3.13
P value		0.697	0.046	0.01
Control	NO(U/ml)	77.8±14.7	75.4±11.3	74.5±12.3
Experiment		73.3±15.3	65.1±14.6	60.4±10.3
P value		0.39	0.038	0.024
Control	SOD(U/ml)	64.7±3.2	65.2±3.3	66.0±4.6
Experiment		66.5±4.9	72.1±3.9	75.3±5.1
P value		0.22	0.00018	0.00012

**Table 6** Postoperative serological markers of inflammatory response (each group n=30)

Groups	Inflammatory Marker	Day1	Day4	Day7
Control		12.83±2.98	10.35±2.48	8.20±2.32
Experiment	CRP(mg/L)	13.68±2.74	8.26±1.67	3.09±1.00
P value		0.426	0.012	0.001
Control		15.31±8.12	10.91±5.71	8.05±3.98
Experiment	IL-6(pg/L)	12.79±4.12	7.45±2.63	4.26±1.62
P value		0.293	0.042	0.002
Control		23.66±9.33	19.43±7.84	15.32±7.25
Experiment	TNF-a(pg/L)	22.07±6.68	12.97±5.12	7.27±3.88
P value		0.597	0.013	0.001
Control		113.0±13.9	128.9±9.2	96.8±4.5
Experiment	VEGF(pg/L)	100.3±6.1	88.3±6.1	73.7±4.8
P value		0.29	0.002	0.0006

## Discussion

Modified Radical Mastectomy (MRM) is a commonly used surgical procedure for operable breast cancer that involves extensive tissue dissection,<sup>30</sup> and leads to important release of oxidant and inflammatory factors that are related to debilitating pain and physical impairments after surgery.<sup>31</sup> Furthermore, inflammatory and oxidant microenvironment may have a detrimental impact on prognosis including higher rates of cancer recurrence.<sup>32</sup> The inflammatory environment can contribute to tumor initiation, promotion, angiogenesis, and metastasis. There were studies showed that elevated levels of IL-6 and CRP in peripheral blood were correlated with worse OS.<sup>33</sup> It was reported that a high level of the inflammatory cytokine IL-6 has been associated with increased tumor stage, lymph node infiltration, recurrence, and treatment resistance.<sup>34</sup> Several cytokines regulate the inflammatory tumor microenvironment, of which CRP, IL-6, and TNF- $\alpha$  stimulate cancer cell proliferation and invasion.<sup>35</sup> Our results indicate that patients following breast cancer surgery in the 7days postoperatively experienced elevated serological levels of CRP, IL-6, and TNF- $\alpha$  than before operation. This situation implies that the microenvironment of such patients may be subject to influences supporting proliferation and invasion of any remaining breast cancer cells that reside in the body postoperatively. In addition, the serological level of VEGF which plays a key role in the process of angiogenesis and tumor progression<sup>36</sup> was also found to be transiently increased after surgery. It has been reported that surgical procedure might influence the process of angiogenesis with prognostic significance in breast cancer and potential implications in decisions regarding postsurgical adjuvant therapy.<sup>37</sup>

Surgery for breast cancer causes additional oxidative stress to patients as they suffer both the physical trauma of surgery as well as psychological disturbances. This situation can hamper patient rehabilitation and also produce a microenvironment with higher levels of inflammatory and angiogenic factors that favor tumor cell growth and invasion. Breast cancer may thrive more in a prooxidant environment with enhanced TNF- $\alpha$  and nitric oxide; and this positive correlation was found between the oxidative stress parameters and inflammatory markers in breast cancer patients.<sup>38</sup> Therefore, perioperative antioxidant supplementation as well as anti-inflammatory effects could be beneficial for breast cancer control and prevention. Our results found that patients were under stress with feeling of severe painful, fatigue and anxiety on day1 after operation and recovered slowly with time going on if not intervened optimally. Along with stress symptom changes, the serological microenvironment of oxidative stress increased and antioxidant decreased accordingly. The results of our trial revealed that patients with breast cancer during perioperative period suffered from disorders of stress as well as the

serological microenvironment of substantial high levels oxidative and inflammatory factors.

Most of breast cancer patients during the perioperative period in the research had symptoms of fatigue, anxiety, fixed incision pain, loss of appetite with lusterless complexion, pale tongue and weak pulse, which belongs to syndrome of blood stasis due to qi deficiency according to traditional Chinese medical theory.<sup>39</sup> All such symptoms are in accordance with the appearance of oxidative stress. The Chinese medical decoction of SHD applied have functions of benefiting qi for activating blood circulation and improving symptoms of blood stasis due to qi deficiency as well as oxidative stress simultaneously. Our results demonstrate that oxidative stress can be reduced under the guidance of TCM based formula. Several clinical trials revealed that TCM can provide substantial relief from oxidative stress and inflammatory factors related to diseases like rheumatoid arthritis, senile dementia and for surgery of intestinal cancer.<sup>40,41</sup> Our results demonstrated that the Chinese medical decoction of SHD significantly relieved symptoms of oxidative stress and completed recovery on 1day and on 4day postoperatively respectively. Furthermore, the effects were supported by reductions of pro-oxidative serological level of NO and increases in antioxidant levels of TAOC and SOD accordingly. Therefore, our results showed Chinese medicine SHD could not only ameliorate the symptoms but also the serological microenvironments of oxidative stress. Because enhanced oxidative stress would induce inflammatory reaction in body, the inflammatory factors were shown improved and decreased correlation with changes of extents of oxidative stress in the research.

After operation, the process of wound healing starts immediately with mainly three overlapping phases of inflammation, proliferation and remodeling, of which the inflammation phase essentially leads to the next phase.<sup>42</sup> Any factors interfering with the normal inflammatory phase will influence the transition from the inflammatory to the proliferative phase which represents a key step during wound healing.<sup>43</sup> It was reported<sup>44</sup> the steroid induces delay in wound healing by way of inhibition of inflammatory phase and reactive oxidative species (ROS) production. However, steroids are strongly suppressive for inflammation. We believe that suitable control of extensive inflammation should benefit for wound healing because excessive and prolonged inflammation results in delayed healing and increased scar formation.<sup>45</sup> The volume of drainage fluid from breast operative wound delegate the delay of wound and the transition from inflammation to proliferation in wound tissue.<sup>46</sup> Our results showed that the duration and amounts of postoperative drainage fluid reduced significantly with SHD treatment. The drainage fluid reduction means the healing process transit from inflammation to proliferation and rapid recovery of postoperative wound. Thus, suitable control of

state of oxidative stress as well as inflammatory response with SHD treatment in postoperative period would not delay the healing process, but improve healing somehow.

It was reported that the state of angiogenesis, inflammation, and oxidative stress in patient suffered from breast cancer would pay important roles on the tumor progress and metastasis<sup>47</sup> and also it was found that there were the interplay between inflammatory pathways and oxidative stress in the pathogenesis of breast cancer.<sup>48</sup> While the patients of breast cancer were waiting for surgery treatment, the states of circadian disruption,<sup>49</sup> improved serum levels of oxidative damage,<sup>50</sup> inflammatory biomarkers and angiogenic factors<sup>51</sup> would make the tumor progress. These researches focused either on oxidative stress or on inflammatory states. Guided by the TCM theories of treating the human body as a holistic and keep health with a balance of yin and yang, our research focused on restoring the imbalance body functions through regulation of oxidative stress with SHD intake. In addition, the levels of inflammatory biomarkers and related angiogenic growth factors of VEGF were also down regulated with the reduction of stress by SHD intervention as well. All of these functions showed in our research should be beneficial to the breast cancer prognosis. Furthermore, the SHD intervention with the functions of stress reduction and inflammatory biomarkers inhibition during perioperative period can reduce the volume of fluid drainage as well. It was reported that the post-surgical drainage fluids would stimulate breast cancer cells growth.<sup>52</sup> Therefore, reduced the volume of drainage fluids may not only improve recovery, but also may decrease to risk of cancer recurrence. Thus, the results in our research showed that SHD can have an additional help to improve the effects of breast cancer during operative period besides the formal western medical measures, which may correspond to the era of precise medical treatments for breast cancer with umbrella trials.<sup>53</sup>

However, due to the limitation of the sum of subjects and duration of evaluations in our research, our results only showed the potential role of SHD to positive adjuvant effects on promotion survival of breast cancer sufferings. We tried our best to avoid the bias produced due to the suspect of subjects who drank the sodium chloride in the same appearance of nontransparent drug bags as the SHD. The differences of liquid tastes did not be overcome in the research. Therefore, it may some bias produced due to the subjects who suspected not be treated with SHD. Nevertheless, all the subjects recruited in our clinical trial did not fall off the project. It may because the duration of the research only lasted for 8days and stay in the ward, which makes easy for subject to sustain the compliance. In the future, it should to design larger samples and long-term observation, such as 3years or 5years of progression free survival, which may need the excellent placebo with the similar taste as SHD to avoid possible bias and reduce the occurrence of fall off.

## Summary

Summary, treatment with SHD can reduce the oxidative stress of breast cancer patients during the perioperative period by the way of its anti-oxidative effects and inflammatory response followed, which would have beneficial effects on rapid recovery and produce a kind of microenvironment with inhibiting breast cancer growth and invasion for tumor prognosis improvement as well.

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## Conflicts of interest disclosures

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