Study Protocol

Cardioprotective effect of saffron total glycoside tablets in patients with breast cancer receiving anthracycline-based chemotherapy: study protocol for a multicentre, randomised, parallel, double-blind, placebo-controlled clinical trial

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Background: Breast cancer is the most common cancer worldwide. Anthracyclines, alone or in combination with other chemotherapeutic agents, are the most effective chemotherapy agents against breast cancer. However, the dose-dependent cardiotoxicity of anthracyclines is a serious drawback in clinical treatment. Considerable efforts have been made to establish suggestions to avoid anthracycline-induced cardiotoxicity. Crocin extracted from saffron has potential cardioprotective effects against anthracycline-induced cardiotoxicity. The aim of this study was to estimate the cardioprotective effects and safety of saffron total glycoside tablets relative to placebo in patients with breast cancer undergoing anthracycline-based chemotherapy.

Methods: This is a multicentre, randomised, double-blind, placebo-controlled clinical trial. A sample of 200 participants (100 per group) with breast cancer will be randomly assigned to receive either saffron total glycoside tablet or placebo (four tablets each time, three times each day) for 6 months. Each participant will be interviewed three times: baseline (visit 1), after 3 months (visit 2), and after 6 months (visit 3). The primary outcome is to confirm if administration of saffron total glycoside tablets reduces the rate of cardiotoxicity relative to that with placebo. Secondary outcomes include new arrhythmic events, and cardiac troponin I and N-terminal pro-B-type natriuretic peptide levels. The quantity, quality, and severity of the adverse events will be carefully documented.

Discussion: We look forward to obtaining high-quality evidence that can be used to formulate clinical practice guidelines. Thus, the findings of this study are expected to help fill the current gap in cardiotoxicity prevention drugs.

Trial registration: This trial was published in the Chinese Clinical Trial Registry (No. ChiCTR2000041134, registered on 19th December 2020).

Keywords: Anthracycline-induced cardiotoxicity; traditional Chinese medicine; saffron total glycoside tablet; randomised controlled trial

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Introduction

According to the latest global cancer data, the breast cancer burden will increase to 2.3 million new cases in 2020. Breast cancer has now overtaken lung cancer as the most common cancer and is the fifth most common cause of cancer death worldwide (1). Despite improvements in the long-term survival rate of patients with cancer, outcomes are still affected by untoward side effects associated with treatment (2). Chemotherapy-related cardiac dysfunction accounts for a notable proportion of severe syndromes, especially in female patients with breast cancer (3). Anthracyclines, one of the most widely used chemotherapeutic agents (4), are associated with dose-related cardiotoxicity, which frequently leads to heart failure (5,6). While improving the survival rate of cancer patients, the clinical adverse effects of anthracycline-induced cardiotoxicity (AIC) are also on the rise (7). It is important for patients to receive timely prevention and treatment. Several prevention strategies have been proposed to reduce AIC (8). Dexrazoxane is the only specific agent for AIC to obtain FDA approval and has been widely used to prevent cardiotoxicity after anthracycline administration, including in paediatric cancer patients (9,10). As an iron-chelating agent, dexrazoxane can rapidly penetrate through the cell membrane, reduce the trivalent iron ion and anthracycline complexes, and alleviate the generation of oxygen free radicals (11-14). Nevertheless, dexrazoxane has failed to meet the expectations raised based on preclinical studies, and there are concerns about its side effects, such as leukocytopenia (15-17).

Saffron is a traditional medicine obtained from the stigma of the flower of *Crocus sativus L.*, whose medical history traces back nearly 3,000 years based on current evidence (18,19). The main active compound of saffron is crocin (20,21). Studies have shown that crocin has therapeutic value with multiple pharmacological effects, including antioxidant, anti-inflammatory, and cardioprotective effects (22-25). Razmaraii (26) conducted an animal experiment to evaluate the cardioprotective effect of crocin against AIC and found that crocin reduced heart damage, ventricular dysfunction, and structural changes in the myocardium. Elsherbiny (27) and Chu (28) found that crocin has a positive effect on the production of anthracycline-induced reactive oxygen species. The essential component of saffron total glycoside tablets (STTs) is crocin (*Table 1*). Therefore, STTs may have cardioprotective effects preventing AIC. The STT is still more economical and practical than dexrazoxane. However, there are currently no high-quality trials evaluating the safety and effectiveness of STT. The purpose of this study is to estimate the cardioprotective effect and safety of STT relative to those of placebo in patients with breast cancer undergoing anthracycline-based chemotherapy. Therefore, this study will be applied to evaluate the viability of a large-scale clinical trial that might provide an alternative, evidence-based prevention of AIC. We present the protocol in accordance with the SPIRIT reporting checklist (available at https://dx.doi.org/10.21037/apm-21-444).

Methods

Study design and setting

This trial is designed as a multicentre, double-blind, placebo-controlled trial with two parallel groups to assess the efficacy of the STT. The protocol was formulated in accordance with the SPIRIT 2013 statement and the Declaration of Helsinki (as revised in 2013). The start and end of recruitment had been planned to be May 1, 2021, and June 30, 2024, respectively.

Before trial commencement, the researchers will conduct a training program to ensure that medical staff participating in the trial are adequately aware of each part of the trial. The trial will be performed across four clinical centres in China, namely Guang'anmen Hospital, the Second People's Hospital of Dezhou, the First Affiliated Hospital of Hebei North University, and Xingtai People's Hospital. After acquiring informed consent and confirming eligibility, a total of 200 patients with breast cancer will be randomised into either the STT or control group at a 1:1 ratio. The study consists of four stages: enrollment, allocation, intervention, and close-out. Each participant will be registered only once. On a background of anthracycline-based chemotherapy, participants will receive either STT or placebo treatment. Each participant will be interviewed three times: baseline (visit 1), after 3 months (visit 2), and after 6 months (visit 3). At visit 1, participants are recruited from the mammary surgery department, where they will undergo physical examination and qualification assessment and receive three months of medication. Participants will be asked to return for visit 2 to undergo metaphase clinical assessment and obtain the next three months of medication. Visit 3 will arranged to conduct clinical evaluations at the end of treatment. The participant flowchart is shown in *Figure 1*, and the study schedule is presented in *Table 2*.

Table 1 Ingredients of saffron total-glycoside tablet (intervention drug) and assay

Main composition	Chemical formula	Ultraviolet spectrum (nm)	Amount (mg/tablet)
Crocin-1	$C_{44}H_{64}O_{24}$	458±2	4.1
Crocin-2	$C_{35}H_{52}O_{19}$	432±2	1.6

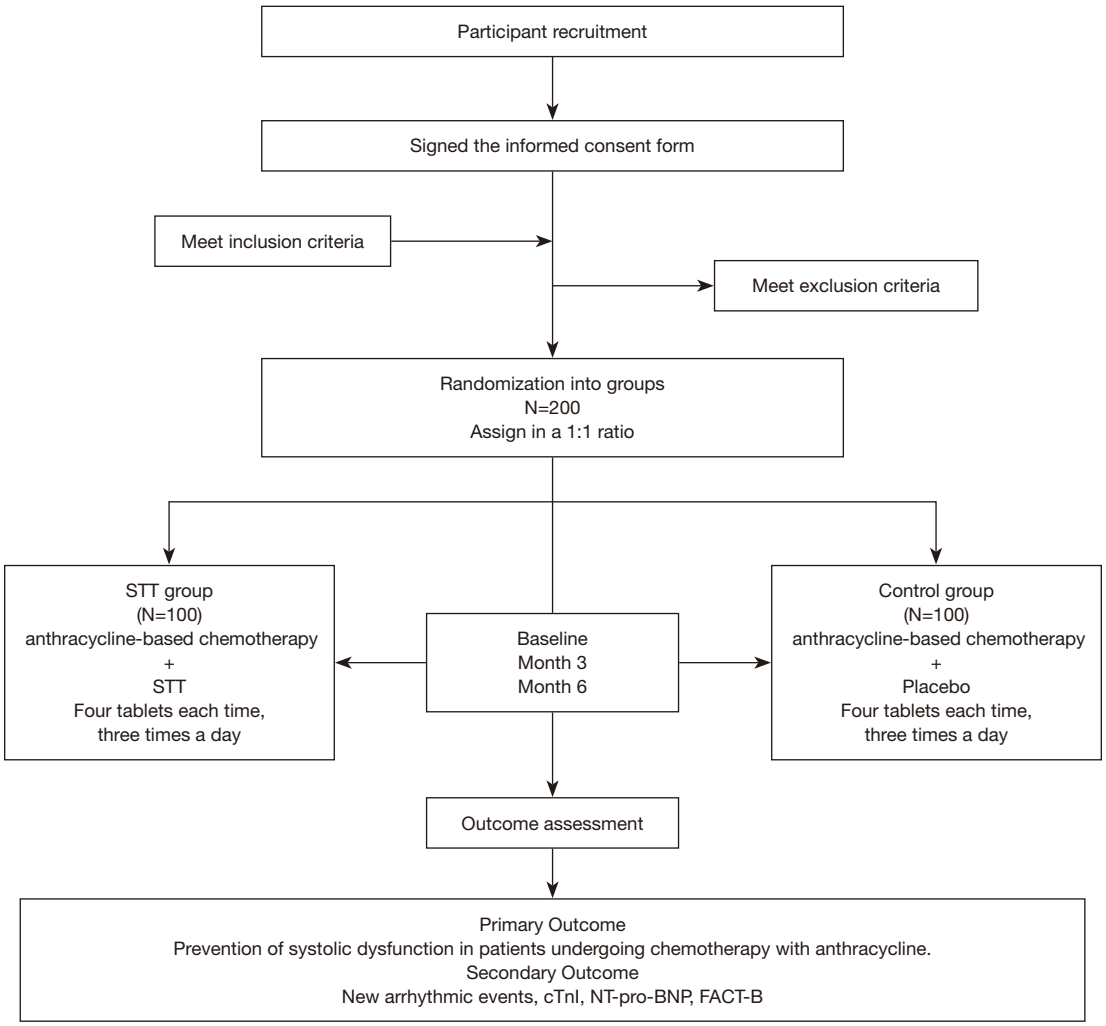

Figure 1 The participant flowchart. STT, saffron total-glycoside tablet; cTnI, cardiac troponin I; NT-pro-BNP; N-terminal pro-B-type natriuretic peptide; FACT-B, functional assessment of cancer therapy for breast cancer.

The study protocol was approved by the Medical Ethics Committee of Guang'anmen Hospital, China Academy of Chinese Medical Sciences (NO. 2020-065-KY) and was registered in the Chinese Clinical Trial Registry (ChiCTR2000041134). If there are any modifications to the study design, the Ethics Committee will be notified promptly. Each participant will be requested to provide informed consent.

Participation and recruitment

Inclusion criteria

The inclusion criteria for the study include the following patient conditions: (I) female sex; (II) aged 18–70 years;

Table 2 Schedule of enrollment, interventions and assessment

Study procedure	Visit 1 (Baseline)		Visit 2	Visit 3
	Enrolment	Allocation	Post-allocation	Close-out
Time point		0	3-month	6-month
Enrolment				
Eligibility screen	•			
Medical history	•			
Clinicopathological evaluation	•			
Informed consent	•			
Allocation		•		
Intervention				
STT				
placebo				
Assessment				
Echocardiography		•	•	•
New arrhythmic events		•	•	•
cTnI		•	•	•
NT-pro-BNP		•	•	•
ECG		•	•	•
KPS		•	•	•
TCM clinical symptom score		•	•	•
NYHA functional classification		•	•	•
FACT-B		•		•
Complete blood count	•			•
Electrolyte examination	•			•
Liver function tests	•			•
Renal function tests	•			•
Other medication	•		•	•
Adverse events		•	•	•

According to SPIRIT 2013 Statement: Defining Standard Protocol Items for Clinical Trials. cTnI, cardiac troponin I; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; ECG, electrocardiogram; KPS, Karnofsky Performance Status; TCM, traditional Chinese medicine; NYHA, New York Heart Association; FACT-B, functional assessment of cancer therapy for breast cancer; STT, saffron total-glycoside tablet; cTnI, cardiac troponin I; NT-pro-BNP; N-terminal pro-B-type natriuretic peptide; FACT-B, functional assessment of cancer therapy for breast cancer.

(III) histologically or cytologically proven primary breast cancer; (IV) receiving anthracycline-based chemotherapy, and accumulated dose ≥240 mg/m^2; (V) no prior bilateral/unilateral radiotherapy to chest; (VI) Karnofsky Performance Status ≥60; (VII) life expectancy ≥6 months; (VIII) willing and able to accept random allocation; and (IX) willing and able to provide informed consent. Eligible participants will meet all inclusion criteria.

Exclusion criteria

The exclusion criteria for this study include the following: (I) patients who have received treatment with other cardiac

medications such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, beta-blockers or aldosterone receptor antagonists within one month; (II) patients with a prior diagnosis of cardiomyopathy (ischemic, dilated, restrictive or hypertrophic), moderate or severe aortic and/or mitral valve disease; (III) patients with systolic blood pressure <90 mmHg, or >180 mmHg; (IV) patients with chronic kidney disease with eGFR <30 mL/min/1.73 m^2; (V) patients with serum potassium >5.5 mmol/L; (VI) patients with severe liver failure; (VII) patients with previous use of anthracycline and no exact record of drug usage; (VIII) patients who are currently pregnant or lactating; (IX) patients who are allergic to drugs or have contraindications; and (X) patients participating in other drugs or external therapy clinical trial(s). Candidates that satisfied at least one of the above criteria were excluded.

Randomisation and blinding

The protocol uses a centre-based block randomisation method. The randomisation sequence will be produced by statisticians from a third-party company. Participants will be distributed at a 1:1 ratio to provide uniform baseline characteristics and be assigned a trial code to identify subjects throughout the study. The details of the randomisation sequence have been uploaded to the central random system, CTCRS-IWR. Double blinding of physicians and study participants will be performed in this study. All experimental agents (STT in the experimental group and placebo tablets in the control group) are packaged and labelled uniformly by Reyoung Pharmaceutical Co., Ltd. (Shanghai, China). The placebo tablets mimic the appearance and taste of STT. If severe adverse events are observed in participants during the study, physicians will be expected to execute an emergency break and perform relevant treatment based on the situation. After the study concludes, statisticians will enforce the first unblinding and carry out a statistical analysis based on data from the two groups. After data analysis, the second unblinding will be performed to confirm which group is STT and which is placebo. The personnel responsible for collecting data and evaluating outcomes will all be third-party staff who have not participated in the design or recruitment.

Interventions

All participants will have received anthracycline-based chemotherapy for 6 months according to the 2020 Diagnosis and Treatment Guidelines for Breast Cancer, which was released by the Chinese Society of Clinical Oncology. The specific treatment plan will be formulated by clinicians at each centre based on the participant's condition. In addition, the cumulative dose of anthracycline will be expected to be more than 240 mg/m^2 in all participants. STT (National Medicine Approval No. Z20163079) and placebo will be administered as four tablets each time, three times a day during the 6-month intervention period. Patients will also be asked to return any unused drugs. All drugs used during the study will be deemed to be combined drugs, and their usage will need to be recorded in the case report form (CRF). The trade name, dosage, symptoms, and duration of the medication will be tracked. The researchers will judge if the participants should withdraw from the study in light of specific conditions. Furthermore, the following drugs will not be allowed during the study period: angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, adrenergic beta receptor blockers, and aldosterone receptor antagonists.

Sample size calculation

The primary outcome is the basis of the sample capacity valuation. To the best of our knowledge, this study is the first clinical trial to evaluate the effect of natural bioactive compounds on AIC in a specific mode of traditional Chinese medicine, and no preceding relevant data can be discovered. Hence, after deliberations with clinical specialists, the sample size was calculated with an anticipated incidence of cardiotoxicity of 23% with the use of anthracycline (29,30) and an expected reduction to 7% with the addition of STT. The protocol assumes a statistical significance level of 95% and 80% power. Using PASS 15 statistics software, 75 participants per group (150 participants in total) were calculated to provide sufficient statistical power. Accounting for a 20% drop-out rate, 100 participants in each arm will be recruited for a total sample of 200 participants.

Statistical analysis

The statistical analysis device was formulated by statistical personnel after discussion with the key researchers. The data will be saved in the Data Management Centre of INTELLIGENCE FUTURE SCIENCE& TECHNOLOGY Co., Ltd., and processed by their interior statisticians who are blinded to group allocation. All results will be analysed on the strength of the intention-to-

treat principle. The missing values will be carried forward based on the last observation. SPSS 20.0 for Windows will be employed, and statistical significance is defined as $P<0.05$. Regarding quantitative data, the mean and standard deviation (SD) will be reported. Paired quantitative data will also be reported as differences in the mean and SD. For enumerative data, the frequency and corresponding percentage will be provided. For intra-group comparisons, the paired *t*-test or Wilcoxon signed-rank test will be applied to quantitative indexes, and the chi-squared test will be utilised for qualitative indexes. For comparisons between groups, a two-sample *t*-test or Wilcoxon signed-rank test will be applied to quantitative indicators, and the chi-squared test (or Fisher's exact test when the expected frequency is <5) will be utilised for qualitative indicators. Clinical efficacy should be determined in the evaluation of clinical significance.

Outcome evaluation

Primary outcome

The primary outcome of the study is the prevention of systolic dysfunction in patients undergoing chemotherapy with anthracycline. According to the purpose of this trial, systolic dysfunction is defined as (31,32): (I) a drop in left ventricular ejection fraction (LVEF) of 10% from baseline in patients with LVEF ≥50%; or (II) a drop in LVEF of at least 5% from baseline in patients whose LVEF decreased to <50%. LVEF will be measured at baseline, 3 months, and 6 months during the study.

Secondary outcome

Secondary outcomes include new arrhythmic events, functional assessment of cancer therapy for breast cancer (FACT-B), and levels of cardiac biomarkers, such as cardiac troponin I and N-terminal pro-B-type natriuretic peptide. The survivors' quality of life will be assessed using the FACT-B at baseline and after 6 months. Other secondary outcomes will be assessed at baseline, 3 months, and 6 months during the treatment period.

Safety evaluation

Safety evaluations will include documentation of adverse events, physical examination, vital signs, and laboratory examinations, such as complete blood count, electrolyte examination, liver function tests, and renal function tests. The above laboratory examination and appearance of unfavourable clinical symptoms after agent administration in patients will be applied to evaluate the safety of STT. Nevertheless, if any adverse events appear, investigators should keep a full record of the onset time, related symptoms, abnormal laboratory indexes, duration, intervention, and prognosis in a timely manner. The relationship with STT will be evaluated based on these data. If a patient presents with any item meeting the termination criteria, their treatment will be urgently unblinded, and they will be safely withdrawn from the study.

Data management and monitoring

During the study, participants will be asked to take the designated agent for six consecutive months, undergo regular review at three time points, and offer three-round biomedical samples. The raw data will be kept in the CRFs. To improve data quality and accuracy, a trained investigator at each participating centre will complete the CRFs. After the completion of data collection, the complete record will be input in duplicate into a computer database using IFUTURE-EDC 1.0.33 (Electronic Data Capture System, Tianjin, China), by two individual data administrators independently using separate authorised identification codes and passwords. The original data will be stored for at least five years after the study is completed. The Data and Safety Monitoring Board (DSMB) for this study will supervise the entire process. All members of the DSMB are independent of the study sponsor and have no competing interests.

To improve the quality and stability of this study, the following measures will be taken. First, all investigators will receive several training courses prior to recruitment to ensure comprehensive understanding of the study. Second, it is necessary for researchers to remind participants to carry the used tablets and then check the medication. Finally, investigators may phone participants three days in advance to remind them of their next visit. For participants with poor compliance, investigators will be required to inquire about possible reasons and encourage them to continue with their study participation.

Discussion

To the best of our knowledge, this study is the largest prospective randomised, double-blind, placebo-controlled clinical trial evaluating the use of natural bioactive compounds to prevent AIC. Since the use of STT to prevent cardiotoxicity in patients receiving anthracycline-based chemotherapy has never been reported, this study

will provide high-quality verification of the cardioprotective effects and safety of STT in breast cancer patients receiving anthracycline-based chemotherapy. In addition, since this study is adopting a multicentre design, the results of this study offer more comprehensive clinical evidence. The design reduces the influence of environmental factors and individual characteristics from the data. The limitations of this study are noteworthy. It is worth noting that the gold standard for diagnosing AIC is still endomyocardial biopsy (EMB) (33). However, EMB is associated with complications and is limited in its clinical application (34,35). Echocardiography and cardiac biomarkers are relatively convenient to execute and have less risk, but can also lead to more ambiguous results in groups.

At present, reports on the pharmacological effects of STT remain at the animal and cellular levels and seldom evaluate its clinical applications. Thus, it is meaningful to perform such studies to prove the potential cardioprotective efficacy of patients with AIC. The data obtained from this study will help establish the feasibility of large-scale clinical trials. We hope that this study will provide high-quality evidence that can be used to formulate clinical practice guidelines. Thus, the findings of this study are expected to fill the current gap in cardiotoxicity prevention drugs.

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Footnote

Reporting Checklist: The authors have completed the SPIRIT reporting checklist. Available at https://dx.doi.org/10.21037/apm-21-444

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/apm-21-444). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The trial will be conducted in accordance with the Declaration of Helsinki (as revised in 2013). The trial was approved by Medical Ethics Committee of Guang'anmen Hospital, China Academy of Chinese Medical Sciences (NO. 2020-065-KY) and informed consent will be taken from all individual participants.

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