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ORIGINAL ARTICLE

Evaluation of *in vitro* Cytochrome P450 Inhibition and Hepatotoxicity Potential of a Herbal Formula (Xiang Bei Yang Rong Tang) for Treatment of Cancer-Related Fatigue

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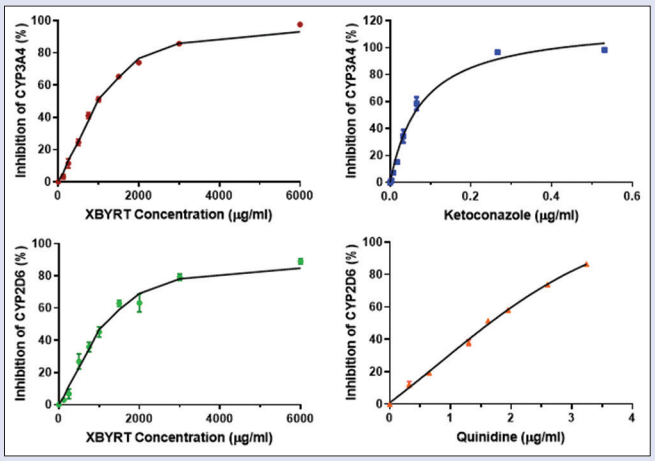
ABSTRACT

Background: The modified Xiang Bei Yang Rong Tang (XBYRT) is a Chinese herbal medicine formulated to mitigate symptoms of cancer-related fatigue. XBYRT comprises of 15 herbal components which are commonly used in traditional Chinese medicine. **Objectives**: In this study, the *in vitro* inhibition of cytochrome P450 3A4 (CYP3A4) and cytochrome P450 2D6 (CYP2D6) activities, along with the *in vitro* liver cell toxicity were evaluated for XBYRT and the individual herbal components. **Materials and Methods:** CYP3A4 and CYP2D6 inhibitions were assayed using the Vivid® CYP450 screening kits and liver cell toxicity in L-02 cells was analyzed using the 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl) -2-(4-sulfophenyl)-2H-tetrazolium cell viability kit. The half maximal inhibitory concentrations (IC_{50}) for CYP450 inhibition and cell viability were determined using the non-linear regression from GraphPad Prism. **Results:** The IC_{50} for CYP3A4 and CYP2D6 activities were 980 (942–1019) μg/ml and 1159 (1066–1261) μg/ml, respectively, for the XBYRT. The herbal components with the lowest IC_{50} values for CYP3A4 activity were *Radix Paeoniae Alba* (144 μg/ml) and Rhizoma Cyperi (278 μg/ml), while herbal components with the lowest IC_{50} values for CYP2D6 activity were *Radix Codonopsis pilosulae* (437 μg/ml) and *Fructus Ligustri Lucidi* (447 μg/ml). At the concentration of 256 μg/ml, XBYRT did not exhibit liver cell toxicity, with a 100% cell viability. **Conclusion:** The herbal components assessed did not demonstrate potent inhibitions of CYP3A4 and CYP2D6; however, precaution is recommended for breast cancer patients taking tamoxifen as the long-term impact of the herb-drug interaction is unclear. Further, *in vivo* and pharmacokinetic studies are required to ascertain the actual clinical significance of potential herb–drug interactions.

Key words: Cancer-related fatigue, Chinese herbal medicine, cytochrome P450 2D6, cytochrome P450 3A4, drug interaction

SUMMARY

- The modified Xiang Bei Yang Rong Tang (XBYRT) and its herbal components did not show potent inhibitions of CYP3A44 and CYP2D6, but further *in vivo* and pharmacokinetic studies are needed to determine the potential herb-drug interactions.



Abbreviations used: CHM: Chinese herbal medicine; CRF: Cancer related fatigue; CYP2D6: Cytochrome P450 2D6; CYP3A4: Cytochrome P450 3A4; DMEM: Dulbecco's Modified Eagle Medium; FBS: Fetal bovine serum; GMP: Good Manufacturing Practice; IC_{50}: Half maximal inhibitory concentration; $NADP^+$: Nicotinamide adenine dinucleotide phosphate; NADPH: Dihydronicotinamide adenine dinucleotide phosphate; QOL: Quality of life; RT: Room temperature; TCM: Traditional Chinese medicine; XBYRT: Xiang Bei Yang Rong Tang.

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INTRODUCTION

Cancer patients often experience a myriad of long-term side effects caused by cancer itself or anticancer therapies, and among the most commonly reported and distressing symptom is cancer-related fatigue (CRF).[1] CRF is a manifestation of persistent physical, emotional, and mental tiredness, which can disrupt the daily functioning and significantly affect the quality of life (QOL) of affected individuals. In addition, CRF is often accompanied by other symptoms such as depression, anxiety, pain, and insomnia.[2] Due to the complex etiology of CRF, there is currently no effective pharmacological intervention for treating CRF.

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The use of Traditional Chinese Medicine (TCM) has been gaining popularity and acceptance among cancer patients, especially in Asia.[3-5] In China and Taiwan, TCM treatment is often integrated with conventional chemotherapy.[6-8] Chinese Herbal Medicine (CHM), being the most common form of TCM used by cancer patients, is usually taken to alleviate cancer or treatment-related symptoms and improve the QOL.[3] From the TCM perspective, CRF is caused by the depletion of Qi or energy and blood deficiency, as a result of chemo- or radiotherapy.[9] The use of herbal medicines for revitalizing Qi and nourishing blood to alleviate fatigue and improve general health is an established concept within the TCM practice.[10] Xiang Bei Yang Rong Tang (XBYRT) is a TCM formulation described in the classical TCM text "Yi Zong Jin Jian," which is designed to nourish patients' Qi and blood.[11] The modified XBYRT (香贝养荣汤加减) comprises of 15 herbal components and these components are selected based on their TCM indications to enhance Qi, nourish blood, improve appetite, and calm the mind. Currently, XBYRT is being investigated for its efficacy and safety for managing CRF in cancer survivors in a double-blind randomized placebo-controlled trial (ClinicalTrials.gov NCT04104113).[12]

With the increased usage of herbal medicines as complementary therapy, along with the number of clinical trials investigating the clinical efficacy of herbal medicines, the potential interactions between herbal and conventional medicines as well as hepatotoxicity risk remain a significant concern. However, due to the complexities of compounds found in CHM which involves the combination of a number of herbal components, the interactions with cytochrome P450 (CYP450) enzyme activities expressed in human hepatocytes are often unknown. Both cytochrome P450 3A4 (CYP3A4) and cytochrome P450 2D6 (CYP2D6) are significant CYP450 enzymes which are involved in the metabolism of many clinical drugs.[13,14] Inhibition of CYP metabolism can potentially enhance the toxicity and/or reduce the efficacy of medications. Therefore, it is pertinent to assess the potential inhibition of XBYRT on CYP3A4 and CYP2D6 metabolism, in order for proper precautions to be taken with the concomitant administration of drugs which are major substrates of the corresponding CYPs. In this study, the *in vitro* inhibition of XBYRT and the individual herbal components on CYP3A4 and CYP2D6 activities were investigated, along with the *in vitro* liver cell toxicity.

MATERIALS AND METHODS

Materials

The herbal granules were purchased from Good Manufacturing Practice certified KinHong Pte Ltd, Singapore and China Resources Sanjiu Medical and Pharmaceutical Co. Ltd. The granules were obtained by boiling the dried raw herbal ingredients in water for 2 h, followed by vacuum concentrating and spray drying the extract concentrate. The amount of raw ingredients and final extracted amount as granules for the daily dosage of XBYRT are shown in Table 1. The daily dosage of the raw ingredients in the formulation was in accordance with the safety and efficacy guidelines from the "Pharmacopoeia of the People's Republic of China" and "General Eleventh Five-Year National Planning Textbook for Higher Education: Chinese Materia Medica."[15,16] The final daily dosage for the XBYRT consists of 23.97 g of extracted dried granules.

Ketoconazole and quinidine (≥98% purity) were purchased from Sigma, USA. Vivid® CYP3A4 Green and CYP2D6 Blue screening kits were purchased from ThermoFisher Scientific, USA. The CellTiter 96® AQueous One Solution Cell Proliferation Assay was obtained from Promega. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Gibco, while the fetal bovine serum (FBS), Penicillin-Streptomycin, and molecular biology grade dimethyl sulfoxide (DMSO) were purchased from Sigma. DMSO was used as a solvent for positive controls and the final concentration of DMSO in assays was 0.5%.

Inhibition assay

In vitro CYP3A4 and CYP2D6 inhibitions were analyzed using the Vivid® CYP450 screening kits according to the manufacturer's instructions. Briefly, at least six varying concentrations of the herbal granules or test compounds were serially diluted in the Vivid® CYP450 reaction buffer. Individual herbal components were serially diluted from a maximum concentration of 2000 μg/ml to 125 μg/ml. The XBYRT was assayed at nine varying concentrations serially diluted from a maximum concentration of 6000 μg/ml to 125 μg/ml. Ketoconazole was used as a positive control for CYP3A4 inhibition, and quinidine was the positive control for CYP2D6 inhibition. Ketoconazole was serially diluted from a maximum concentration of 0.531 μg/ml to 0.004 μg/ml, while quinidine was serially diluted from a maximum concentration of 3.244 μg/ml to 0.324 μg/ml. The test compounds and positive controls were added to the master mix containing the CYP450 Baculosomes and Regeneration System (glucose-6-phosphate and glucose-6-phosphate dehydrogenase). The Regeneration System was required to convert nicotinamide adenine dinucleotide phosphate ($NADP^+$) into dihydronicotinamide adenine dinucleotide phosphate in order to start the CYP450 reaction. After preincubation at room temperature (RT) for 10 min, the CYP450 enzymatic reaction was initiated by the addition of NADP + and the Vivid® Substrate. The substrates for CYP3A4 and CYP2D6 were Vivid DBOMF and EOMCC, respectively. The reaction

Table 1: Amount of raw herbal ingredients and extracted granules in a daily dosage of Xiang Bei Yang Rong Tang

Number	Latin name	Chinese pinyin	Part used	Raw herbs (g)	Extracted granule weight (g)
1	Radix *Astragali seu hedysari*	Huang Qi	Root	15	1.50
2	Radix *Codonopsis pilosulae*	Dang Shen	Root	15	3.00
3	Rhizoma *macrocephalae*	Bai zhu	Root	12	2.00
4	Poria	Fu Ling	Sclerotium	15	1.50
5	*Radix Paeoniae Alba*	Bai shao	Root	15	1.50
6	*Fructus Lycii*	Gou Qi zi	Fruit	12	4.80
7	*Fructus Ligustri Lucidi*	Nü Zhen Zi	Fruit	12	1.20
8	*Plantago asiatica*	Che Qian Zi	Seed	12	0.80
9	Endothelium corneum gigeriae galli	Ji Nei Jin	Inner wall of chicken gizzard	10	1.00
10	*Hordeum vulgare* L.	Sheng Mai Ya	Germinated grain	15	1.00
11	Fructus *Alpinia oxyphylla*	Yi Zhi Ren	Fruit	10	1.00
12	Rhizoma Cyperi	Xiang Fu	Root	10	1.00
13	Radix Polygalae	Yuan Zhi	Root	10	1.67
14	Bulbus *Fritillaria thunbergii*	Zhe bei mu	Flower bulb	10	1.00
15	*Smilax glabra* Roxb.	Tu Fu Ling	Root	15	1.00

incubation time for CYP3A4 assay was 10 min at RT and CYP2D6 assay was 35 min at RT. The oxidation of the substrates released highly fluorescent metabolites. The reactions were terminated by the addition of the stop reagent (0.5M Tris), and fluorescence was detected using the Tecan Infinite M200 microplate reader. The fluorescence excitation and emission wavelengths for the CYP3A4 assay were 490 nm and 520 nm, while 415 nm and 460 nm were used for the CYP2D6 assay. Assays were performed in triplicates.

Cell culture

The human fetal hepatocyte cell line, L-02 which was characterized by Hu *et al*. was used for the hepatotoxicity study.[17] The L-02 cell line was chosen as it was shown to express normal hepatocyte markers and any toxicity detected will represent potential toxicity to normal liver cells.[17] The L-02 cell line used in this study was a kind gift from the laboratory of Associate Professor Yu Chun Kong Victor, National University of Singapore. Cells were grown in a humidified incubator at 37°C, with 5% CO_2. The growth medium was DMEM with 10% FBS and 1% Penicillin-Streptomycin.

Cell viability assay

Cell viability after incubation with the test compounds was determined using the colorimetric CellTiter 96® AQueous One Solution Cell Proliferation3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) Assay. Cells were seeded at a cell density of 5000 cells in 100 µL culture medium per well in a 96-well plate. After 24 h, test compounds serially diluted by 2-fold from a maximum concentration of 256 µg/ml to 1 µg/ml in the culture medium, were added to the cells. The positive control used was doxorubicin. Doxorubicin was serially diluted by 2-fold from a maximum concentration of 57 µg/ml to 0.226 µg/ml in the culture medium. Cells were incubated with the test compounds and positive control for 24 h. Subsequently, the CellTiter 96® Assay Reagent was added and incubated for 4 h. In metabolically active or viable cells, the MTS tetrazolium compound is converted to a colored formazan product which is soluble in culture medium. Assays were performed in triplicates. The absorbance was determined at 490 nm using the Tecan Infinite M200 microplate reader.

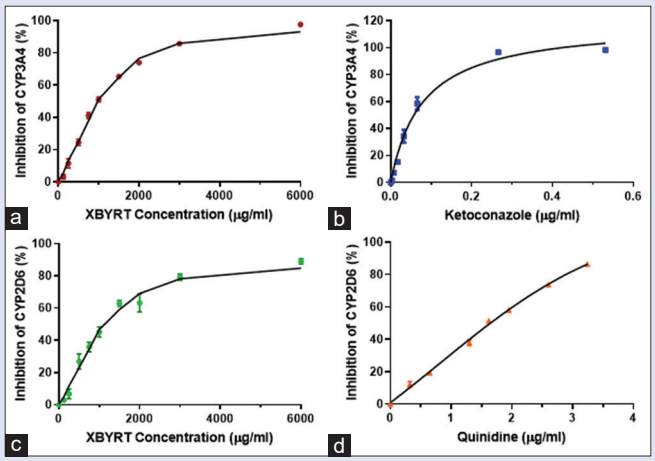

Figure 1: Concentration dependant inhibition of CYP activities. (a) Inhibition of cytochrome P450 3A4 activity by Xiang Bei Yang Rong Tang, (b) Inhibition of cytochrome P450 3A4 activity by ketoconazole, (c) inhibition of cytochrome P450 2D6 activity by Xiang Bei Yang Rong Tang, (d) inhibition of cytochrome P450 2D6 activity by quinidine

Statistical analysis

The half maximal inhibitory concentrations (IC_{50}) for CYP450 inhibition and cell viability were calculated using the non-linear regression from GraphPad Prism V8 (USA).

RESULTS

Inhibition of herbal ingredients on cytochrome P450 3A4 and cytochrome P450 2D6 activities

The XBYRT formulation [Figure 1] and the individual herbal components inhibited CYP3A4 and CYP2D6 in a dose-dependent manner. The IC_{50} values for CYP3A4 and CYP2D6 inhibitions of XBYRT and the individual herbal components are shown in Table 2. Among the herbal components, *Radix Paeoniae Alba* and Rhizoma Cyperi had the lowest IC_{50} for CYP3A4 activity at 144 (134–154) µg/ml and 278 (259–299) µg/ml, respectively. The herbal medicines with the lowest IC_{50} values for CYP2D6 activity were Radix *Codonopsis pilosulae* at 437 (403–472) µg/ml and *Fructus Ligustri Lucidi* at 447 (401–498) µg/ml. The XBYRT exhibited IC_{50} of 980 (942–1019) µg/ml for CYP3A4 activity and 1159 (1066–1261) µg/ml for CYP2D6 activity.

L-02 cell viability test for herbal medicines

The IC_{50} of doxorubicin was 0.633 ± 0.092 µg/ml. The L-02 cell viabilities after incubation with *Hordeum vulgare* L. and *Smilax glabra* Roxb. at 256 µg/ml were 52.1% ± 1.2% and 50.0% ± 0.7%, respectively. However, the viability of cells incubated with both of these herbal components at 128 µg/ml was >90%. The other herbal medicines including XBYRT had IC_{50} of >256 µg/ml [Table 3].

Table 2: Half maximal inhibitory concentration (IC50) of herbal medicines on cytochrome P450 3A4 and cytochrome P450 2D6 activities

TCM	IC_{50} (µg/ml) mean±95% CI	
	CYP3A4	CYP2D6
Radix *Astragali seu hedysari*	1786 (1651-1894)	1066 (992-1144)
Radix *Codonopsis pilosulae*	>2000	437 (403-472)
Rhizoma atractylodis macrocephalae	>2000	>2000
Poria	>2000	572 (523-624)
Radix Paeoniae Alba	144 (134-154)	475 (454-496)
Fructus Lycii	>2000	1923 (1712-2160)
Fructus Ligustri Lucidi	425 (392-460)	447 (401-498)
Plantago asiatica	>2000	>2000
Endothelium corneum gigeriae galli	1264 (1101-1449)	493 (468-520)
Hordeum vulgare L.	766 (720-816)	933 (885-984)
Fructus *Alpinia oxyphylla*	449 (427-473)	672 (643-703)
Rhizoma Cyperi	278 (259-299)	517 (429-623)
Radix Polygalae	953 (873-1042)	>2000
Bulbus *Fritillariae thunbergii*	>2000	578 (502-665)
Smilax glabra Roxb.	468 (435-504)	796 (636-996)
XBYRT	980 (942-1019)	1159 (1066-1261)
Positive controls	**IC_{50} (µg/ml), mean±95% CI**	
Ketoconazole	0.051 (0.046-0.056)	
Quinidine		1.566 (1.499-1.635)

L-02 cell viability test for herbal medicines. XBYRT: Xiang Bei Yang Rong Tang; TCM: Traditional Chinese medicine; IC: Inhibitory concentrations; CYP3A4: Cytochrome P450 3A4; CYP2D6: Cytochrome P450 2D6; CI: Confidence interval

Table 3: L-02 cell viability after treatment with Xiang Bei Yang Rong Tang and its herbal components

TCM	IC_{50} (μg/ml)	Viability at 256 μg/ml (%)
Radix Astragali seu hedysari	>256	100
Radix Codonopsis pilosulae	>256	100
Rhizoma atractylodis macrocephalae	>256	100
Poria	>256	100
Radix Paeoniae Alba	>256	83.5±2.3
Fructus Lycii	>256	100
Fructus Ligustri Lucidi	>256	100
Plantago asiatica	>256	100
Endothelium corneum gigeriae galli	>256	100
Hordeum vulgare L.	>256	52.1±1.2
Fructus *Alpinia oxyphylla*	>256	100
Rhizoma Cyperi	>256	66.8±1.8
Radix Polygalae	>256	100
Bulbus *Fritillariae thunbergii*	>256	100
Smilax glabra Roxb.	256	50.0±0.7
XBYRT	>256	100
Doxorubicin (positive control)	0.633±0.092	-

XBYRT: Xiang Bei Yang Rong Tang; TCM: Traditional Chinese medicine; IC: Inhibitory concentrations

DISCUSSION

Patients often take CHM during and after chemotherapy treatment. However, there is a paucity of preclinical or clinical studies assessing clinically important herb–drug interactions. In this study, we assessed the *in vitro* inhibition of the XBYRT formulation and its individual herbal components on CYP3A4 and CYP2D6 activities, along with the liver cell toxicity potential. XBYRT contains 15 herbal components and many are often included as ingredients in other TCM formulations for treating a variety of conditions including fatigue.[6,7,18-20] The results of this study are novel and worthy to report as the potential of CYP3A4 and CYP2D6 inhibition for a number of the tested components has not been evaluated elsewhere.

Among the herbal components found in XBYRT, *Radix Astragali seu hedysari* and *Radix C. pilosulae* are commonly prescribed for treating fatigue and improving the QOL in cancer patients.[6,7,18,20] In XBYRT, *Radix Astragali seu hedysari* is one of the main ingredients for enhancing Qi and energy levels. Other studies have evaluated the inhibition of *Radix* Astralagus extract on CYP activities due to its common usage in TCM. Lau *et al*. reported inhibition of *Radix Astralagus* on CYP3A4 activity *in vitro* with an IC_{50} of 61 (36–103) μg and Pao *et al*. showed CYP3A4 inhibition in rats given 15 mg/kg of *Radix Astralagus* extract.[21,22] On the contrary, Or *et al*. have reported only weak inhibition of *Radix Astralagus* on CYP3A4 and CYP2D6 (IC_{50} >1 mg/ml) activities *in vitro*.[23] We observed similar findings with our results. In this study, *Radix Astralagus* did not significantly inhibit CYP3A4 or CYP2D6 activities (IC_{50} >1 mg/ml). *Radix C. pilosulae* is also used in TCM for replenishing Qi. In cancer patients, the Shenqi Fuzheng injection, comprising *Radix Astralagus* and *Radix Codonopsis* is given as an adjuvant for enhancing the efficacy and reducing toxicity of chemotherapy.[6,7] Lau *et al*. demonstrated a CYP3A4 IC_{50} of 83 (26–269) μg for *Radix Codonopsis*, but Pao *et al*. showed no inhibition at 2 mg/ml.[21,22] *Radix Codonopsis* assessed here did not exhibit CYP3A4 inhibition but a possible weak CYP2D6 inhibition (IC_{50} >400 μg/ml). Crude herbal components often contain mixtures of unknown compounds with varying concentrations which may differ between samples from different plants or sources and this may account for the variability in IC_{50} observed.

Radix Paeoniae Alba and Rhizoma Cyperi demonstrated the lowest IC_{50} for CYP3A4 inhibition among the herbal components assessed. *Radix Paeoniae Alba* displayed an IC_{50} of 144 (134–154) μg/ml for CYP3A4 activity. The most commonly studied bioactive compounds from *Radix Paeoniae Alba* are albiflorin and paeoniflorin and both compounds demonstrated IC_{50} of >1000 μM for CYP3A4 and CYP2D6 activities.[24] Although it is unclear which compound exerted the inhibition of CYP3A4 activities in this study, it is likely other compounds might be responsible for the CYP3A4 inhibition of *Radix Paeoniae Alba* extract. Other bioactive compounds from *Radix Paeoniae Alba* include paeonilactone-B, paeonilactone-C, paeoniflorigenone, and benzoylpaeoniflorin.[25] Rhizoma Cyperi had an IC_{50} for CYP3A4 of 278 (259–299) μg/ml. A study conducted by Essaidi *et al*. showed stronger CYP3A4 inhibition of the methanolic extract from Rhizoma Cyperi (IC_{50} 13.4 μg/ml).[26] Herbal methanolic extracts contain higher concentrations of phenols and flavonoids such as quercetin and kaempferol which could inhibit CYP3A4 activity, compared to aqueous extracts.[26] Nonetheless, CHM is often boiled or extracted with water and aqueous extracts represent the typical form taken by patients. The herbal components with the highest CYP2D6 inhibitions were *Radix C. pilosulae* and *Fructus Ligustri Lucidi*, displaying possible weak inhibitions with IC_{50} of 437 (403–472) μg/ml and 447 (401–498) μg/ml, respectively. Unlike single compound drugs, the interpretation of *in vitro* inhibition values for herbal medicines is challenging, owing to the presence of complex mixtures in crude extracts. However, studies on herbal crude extracts have classified potentially significant inhibitors as having an *in vitro* IC_{50} value of <100 μg/ml and based on this reference value, the herbal components in XBYRT did not show strong CYP3A4 or CYP2D6 inhibition.[27-29] The herbal formula XBYRT as a whole, had relatively high IC_{50} values for both CYP3A4 and CYP2D6 activities.

Compared to CYP3A4, there are limited studies on the CYP2D6 inhibition for the herbal components included in this study and this presents the first report of *in vitro* CYP2D6 inhibition for many of these herbal medicines.[21,22] The potential interaction with CYP2D6 is of particular concern for CHM which are used for breast cancer patients. CYP2D6 inhibition could affect the metabolism of tamoxifen, a selective estrogen receptor modulator that is commonly prescribed for the treatment or prevention of breast cancer. Tamoxifen is metabolized by CYP3A4 and CYP2D6, but CYP2D6 is the rate-limiting enzyme which converts tamoxifen to its primary active metabolite, endoxifen.[30] Moreover, the abundance of CYP2D6 is relatively lower than CYP3A4 and potential inhibition will likely reduce the efficacy of tamoxifen. Although XBYRT and the herbal components did not demonstrate potent *in vitro* CYP2D6 inhibition, *Radix C. pilosulae* and *Fructus Ligustri Lucidi* showed possible weak inhibition. At the moment, the clinical consequences of herb–drug interaction as a result of long-term concomitant usage of tamoxifen with XBYRT and its herbal components are still unclear. For this reason, it is recommended that precautions should be taken for patients who require long-term tamoxifen.

In this study, the hepatotoxicity potential of XBYRT and its individual herbal components was also evaluated using the MTS cell viability assay. The herbal components with the lowest IC_{50} were *S. glabra* Roxb and *H. vulgare* L with an approximate IC_{50} of 256 μg/ml. At a concentration of 128 μg/ml, both herbal components had an inhibition of <10% on cell viability. The MTS assay used in this study detects cells which are metabolically active and hence, it is not possible to distinguish whether these compounds are exerting a cytotoxic or cytostatic effect, based on the high IC_{50} values. *H. vulgare* L. is barley, a common cultivated grain which is a popular health food and the barley sprout is used in TCM to

promote digestion and improve appetite. The consumption of *H. vulgare* L or *S. glabra* Roxb has not been associated with reports of liver injury and *in vivo* studies have shown hepatoprotective properties of both herbal extracts.[31,32] Therefore, XBYRT and the herbal components tested are unlikely to exhibit direct toxicity to liver cells considering the high IC_{50} values. In clinical trials, potential CHM-induced liver injury can be closely monitored for clinical symptoms and biochemical indicators that reflect liver injury. In addition, cancer patients who are taking CHM should be encouraged to inform their attending physicians or oncologists regarding their usage of herbal medicines, to enable the early detection and proper surveillance of possible CHM-related adverse effects, such as hepatotoxicity.

This analysis serves to evaluate the enzymatic inhibitory activities and hepatotoxicity of the XBYRT formulation and the herbal components, which may guide the prescription of CHM decoctions containing similar components in patients taking concomitant medications. However, the enzymatic inhibitory activities or hepatotoxicity of individual specific compounds found in the herbal components were not determined and results presented here were derived from *in vitro* analyses. Further *in vivo* and pharmacokinetic studies are required to ascertain the actual clinical significance of these herbal components on drug metabolism.

CONCLUSION

None of the herbal components showed strong *in vitro* CYP3A4 or CYP2D6 inhibition, and the liver cell viability results indicate that XBYRT is unlikely to cause hepatotoxicity. However, as the clinical effects of herb–drug interaction with tamoxifen, a major CYP2D6 substrate is still unclear for XBYRT and its herbal components, precautions should be exercised for patients who require long-term tamoxifen. These results can inform patients and medical professionals on the precautions to be taken for the concomitant administration of the CHM with conventional medications.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Wang XS, Zhao F, Fisch MJ, O'Mara AM, Cella D, Mendoza TR, *et al.* Prevalence and characteristics of moderate to severe fatigue: A multicenter study in cancer patients and survivors. Cancer 2014;120:425-32.
2. Fiorentino L, Rissling M, Liu L. Ancoli-Israel S. The symptom cluster of sleep, fatigue and depressive symptoms in breast cancer patients: Severity of the problem and treatment options. Drug Discov Today 2011;8:167-73.
3. Carmady B, Smith CA. Use of Chinese medicine by cancer patients: A review of surveys. Chin Med 2011;6:22.
4. Chan A, Tan HL, Ching TH, Tan HC. Clinical outcomes for cancer patients using complementary and alternative medicine. Altern Ther Health Med 2012;18:12-7.
5. Kuo YT, Chang TT, Muo CH, Wu MY, Sun MF, Yeh CC, *et al.* Use of complementary traditional Chinese medicines by adult cancer patients in Taiwan: A nationwide population-based study. Integr Cancer Ther 2018;17:531-41.
6. Liu S, Zhang D, Wu J, Wang K, Zhao Y, Ni M, *et al.* Shenqi Fuzheng injection in the treatment of breast cancer: A meta-analysis of randomized controlled trials. Integr Cancer Ther 2019;18:1534735418816824.
7. Yang Y, Ting W, Xiao L, Shufei F, Wangxiao T, Xiaoying W, *et al.* Immunoregulation of Shenqi Fuzheng injection combined with chemotherapy in cancer patients: A systematic review and meta-analysis. Evid Based Complement Alternat Med 2017;2017:5121538.
8. Cheng YY, Hsieh CH, Tsai TH. Concurrent administration of anticancer chemotherapy drug and herbal medicine on the perspective of pharmacokinetics. J Food Drug Anal 2018;26:S88-95.
9. Hsu CH, Lee CJ, Chien TJ, Lin CP, Chen CH, Yuen MJ, *et al.* The relationship between qi deficiency, cancer-related fatigue and quality of life in cancer patients. J Tradit Complement Med 2012;2:129-35.
10. Chen R, Moriya J, Yamakawa JI , Takahashi T, and Kanda T. Traditional Chinese medicine for chronic fatigue syndrome. Evidence-Based Complementary and Alternative Medicine. 2010;7:3-10.
11. Wu Q. Yi Zong Jin Jian. Beijing: People's Medical Publishing House; 2006.
12. Yap NY, Loo WS, Zheng HF, Tan QM, Tan TK, Quek LY, *et al.* A study protocol for HEalth-Related quality of life-intervention in survivors of Breast and other cancers experiencing cancer-related fatigue using TraditionAL Chinese Medicine: The HERBAL trial. Trials 2020;21:909.
13. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Cytochrome P450 3A: Ontogeny and drug disposition. Clin Pharmacokinet 1999;37:485-505.
14. Tirona RG, Kim RB. Introduction to clinical pharmacology. In: Robertson D, Williams GH, editors. Clinical and Translational Science. 2nd ed., Ch. 20. United Kingdom: Academic Press; 2017. p. 365-88.
15. Gao XM. General Eleventh Five-Year National Planning Textbook for Higher Education: Chinese Materia Medica. Beijing: China Press of Traditional Chinese Medicine; 2009.
16. Pharmacopoeia Commission of the Ministry of Health of the People's Republic of China. Pharmacopoeia of the People's Republic of China. China; 2015.
17. Hu X, Yang T, Li C, Zhang L, Li M, Huang W, *et al.* Human fetal hepatocyte line, L-02, exhibits good liver function *in vitro* and in an acute liver failure model. Transplant Proc 2013;45:695-700.
18. Guo L, Bai SP, Zhao L, Wang XH. Astragalus polysaccharide injection integrated with vinorelbine and cisplatin for patients with advanced non-small cell lung cancer: Effects on quality of life and survival. Med Oncol 2012;29:1656-62.
19. Xu Y, Chen Y, Li P, Wang XS. Ren Shen Yangrong Tang for Fatigue in cancer survivors: A Phase I/II open-label study. J Altern Complement Med (New York) 2015;21:281-7.
20. Wang CH, Lin CY, Chen JS, Ho CL, Rau KM, Tsai JT, *et al.* Karnofsky performance status as a predictive factor for cancer-related fatigue treatment with astragalus polysaccharides (PG2) injection-a double blind, multi-center, randomized phase IV study. Cancers (Basel) 2019;11:128.
21. Lau C, Mooiman KD, Maas-Bakker RF, Beijnen JH, Schellens JH, Meijerman I. Effect of Chinese herbs on CYP3A4 activity and expression *in vitro*. J Ethnopharmacol 2013;149:543-9.
22. Pao LH, Hu OY, Fan HY, Lin CC, Liu LC, Huang PW. Herb-drug interaction of 50 Chinese herbal medicines on CYP3A4 activity *in vitro* and *in vivo*. Am J Chin Med 2012;40:57-73.
23. Or PM, Lam FF, Kwan YW, Cho CH, Lau CP, Yu H, *et al.* Effects of *Radix Astragali* and Radix Rehmanniae, the components of an anti-diabetic foot ulcer herbal formula, on metabolism of model CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4 probe substrates in pooled human liver microsomes and specific CYP isoforms. Phytomedicine: Int J Phytother Phytopharmacol 2012;19:535-44.
24. Gao LN, Zhang Y, Cui YL, Akinyi OM. Comparison of paeoniflorin and albiflorin on human CYP3A4 and CYP2D6. Evid Based Complement Alternat Med 2015;2015:470219.
25. Kim SH, Lee MK, Lee KY, Sung SH, Kim J, Kim YC. Chemical constituents isolated from *Paeonia lactiflora* roots and their neuroprotective activity against oxidative stress *in vitro*. J Enzyme Inhib Med Chem 2009;24:1138-40.
26. Essaidi I, Brahmi Z, Koubaier HB, Snoussi A, Casabianca H, Abe N et al. Phenolic Composition and Antioxidant, Antimicrobial and Cytochrome P450 Inhibition Activities of Cyperus rotundus Tubers; Mediterranean Journal of Chemistry, 2015;4:201-8.
27. Showande SJ, Fakeye TO, Kajula M, Hokkanen J, Tolonen A. Potential inhibition of major human cytochrome P450 isoenzymes by selected tropical medicinal herbs-Implication for herb-drug interactions. Food Sci Nutr 2019;7:44-55.
28. Sevior DK, Hokkanen J, Tolonen A, Abass K, Tursas L, Pelkonen O, *et al.* Rapid screening of

commercially available herbal products for the inhibition of major human hepatic cytochrome P450 enzymes using the N-in-one cocktail. Xenobiotica 2010;40:245-54.

29. Kong WM, Chik Z, Ramachandra M, Subramaniam U, Aziddin RE, Mohamed Z. Evaluation of the effects of *Mitragyna speciosa* alkaloid extract on cytochrome P450 enzymes using a high throughput assay. Molecules 2011;16:7344-56.
30. Klein DJ, Thorn CF, Desta Z, Flockhart DA, Altman RB, Klein TE. PharmGKB summary: Tamoxifen pathway, pharmacokinetics. Pharmacogenet Genomics 2013;23:643-7.
31. Shah P, Parmar M, Thakkar V, Gandhi TR. Protective effect of *Hordeum vulgare* Linn. on acetaminophen-induced liver damage. J Young Pharm 2009;1:336.
32. Xia D, Fan Y, Zhang P, Fu Y, Ju M, Zhang X. Protective effects of the flavonoid-rich fraction from rhizomes of *Smilax glabra* Roxb. on carbon tetrachloride-induced hepatotoxicity in rats. J Membr Biol 2013;246:479-85.