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# *Schisandra chinensis* extract decreases chloroacetaldehyde production in rats and attenuates cyclophosphamide toxicity in liver, kidney and brain☆

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## ARTICLE INFO

*Chemical compounds studied in this article:*
Cyclophosphamide (Pubmed CID: 2907)
4-ketocyclophosphamide (Pubmed CID: 33676)
2-dechloroethylcyclophosphamide (Pubmed CID: 114861)
carboxyphosphamide (Pubmed CID: 31515)
schizandrol A (Pubmed CID: 23915)
schizandrol B (Pubmed CID: 68781)
schisantherin A (Pubmed CID: 151529)
schisantherin B (Pubmed CID: 6438572)
deoxyschisandrin (Pubmed CID: 155256)
γ-schisandrin (Pubmed CID: 108130)

*Keywords:*
Drug-drug interactions
*Schisandra chinensis* extract
Cyclophosphamide
Pharmacokinetics
Toxicity

## ABSTRACT

*Ethnopharmacological relevance:* *Schisandra chinensis* (Turcz.) Baill (*S. chinensis*) has been used for thousands years in China, and is usually applied in treatment of urinary tract disorders and liver injury. *S. chinensis* extract (SCE) has board protective effects on liver, kidney and nervous system. Schisandra lignans are generally considered as the bioactive components of SCE.

*Aim of the study:* To investigate the pharmacokinetic herb-drug interactions (HDIs) between SCE and cyclophosphamide (CTX). To evaluate the protective effects of SCE against CTX induced damage in rat liver, kidney and brain.

*Materials and methods:* The pharmacokinetic HDIs between SCE and CTX were investigated by determining plasma concentrations of CTX and three metabolites, namely 4-ketocyclophosphamide (4-Keto), 2-dechloroethylcyclophosphamide (DCCTX) and carboxyphosphamide (CPM) using a previously developed UPLC-MS/MS method. To evaluate the protective effects of SCE pretreatment, toxicity and oxidation stress assessments along with histology investigations were carried out in rat liver, kidney and brain.

*Results:* The equimolar produced metabolite DCCTX was chosen to reflect chloroacetaldehyde (CAA, a toxic metabolite of CTX) production in rats. Single-dose pretreatment of SCE significantly reduced CAA production and decreased the $C_{max}$ and $AUC_{0-24\ h}$ of DCCTX by 69% and 49% respectively ($P < 0.05$). After pretreated with SCE for 7 consecutive days, the $C_{max}$ and $AUC_{0-24\ h}$ of DCCTX were still decreased (−25% and −37%, $P < 0.05$) when compared with CTX alone group. Parallel toxicity and oxidation stress investigations showed that single-dose SCE pretreatment significantly decreased plasma BUN and Cr levels (−12% and −46%, respectively) and reduced liver AST activity (−32%). Moreover, SCE pretreatment potently increased the brain GSH content by 7.8-fold, and reduced MDA levels in rat liver, kidney and brain by 39%, 28% and 31%, respectively (compared with CTX alone group). The protective effects of SCE were also supported by histological observations.

*Conclusion:* Our experiment results suggest that *S. chinensis* may find use as a complementary medicine in CTX treatment.

*Abbreviations:* ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate transaminase; AUC, area under concentration-time curve; BMT, blood and marrow transplantation; BUN, blood urea nitrogen; $C_{max}$, peak plasma concentration; CAA, chloroacetaldehyde; CL, clearance; CPM, carboxyphosphamide; Cr, creatinine; CTX, cyclophosphamide; DCCTX, 2-dechloroethylcyclophosphamide; DDI, drug-drug interaction; GSSG, oxidized glutathione; GSH, glutathione; GSH-px, glutathione peroxidase; HDIs, herb-drug interactions; HE, hematoxylin and eosin; IS, internal standard; LSD, least significant difference; MDA, malondialdehyde; MRT, mean residence time; SCE, *Schisandra chinensis* extract; SD, standard deviation; SOD, superoxide dismutase; $t_{1/2}$, terminal half-life; $T_{max}$, time of plasma concentration reach a maximum; TNZ, tinidazole; Vd, apparent volume of distribution; 4-KetoCTX, 4-ketocyclophosphamide

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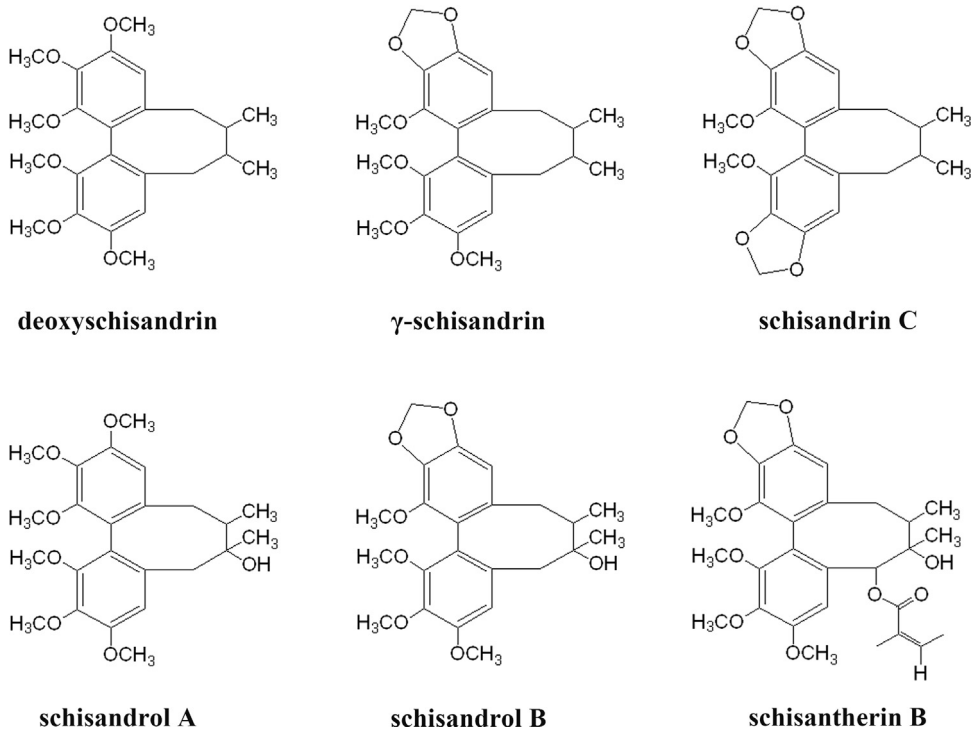


**Fig. 1.** Chemical structures of deoxyschisandrin, γ-schisandrin, schisandrin C, schisandrol A, schisandrol B and schisantherin B.

## 1. Introduction

Nowadays, herbal medicines are increasingly used in cancer treatments against side-effects of anticancer drugs (Engdal et al., 2009). *Schisandra chinensis* (Turcz.) Baill (*S. chinensis*), also known as "Wu Wei Zi", has been used for thousands years in China for its effects of astringent, tonifying qi, promoting the production of body fluid, nourishing kidney and calming (Lu and Chen, 2009). It is usually applied in treatment of urinary tract disorders and liver injury (But et al., 1997; Fil'kin, 1952; Panossian and Wikman, 2008; Zhu et al., 1999). The extract of *S. chinensis* (SCE) is rich in schisandrae lignans, which are generally considered as the main bioactive components of *S. chinensis*. Among all schisandrae lignans, *S. chinensis* is mainly enriched in schisandrol A, schisandrol B and γ-schisandrin (Lu and Chen, 2009). Recent studies have revealed their board protective effects on the liver, kidney and nervous system (Chiu et al., 2008; Hwang et al., 2013; Kim et al., 2006; Li et al., 2014; Lu et al., 2014) (Fig. 1). The previous findings suggest that *S. chinensis* may relieve unnecessary hepatotoxicity, nephrotoxicity and neurotoxicity in patients during cancer chemotherapy. However, the combination uses of *S. chinensis* with anticancer chemicals have been seldom investigated, and more information is needed to develop the clinical application of *S. chinensis* and to avoid unwanted herb-drug interactions (HDIs).

Cyclophosphamide (CTX, cytophosphane, Fig. 2) is an alkylating anticancer drug and a potent immunosuppressant (Binotto et al., 2003; Emadi et al., 2009). As one of the standard treatment approaches to multiple myeloma (MM) patients, high-dose CTX (HD-CTX, 2–3 g/m$^2$) has been frequently used in autologous peripheral blood hematopoietic stem cell transplantation (ASCT) (Attal et al., 1996). Unfortunately, the application of CTX often causes serious side-effects. As a prodrug, CTX is extensively metabolized into both active and inactive metabolites (Fig. 2). The majority of CTX is transformed into the effective component phosphoramide mustard (main-chain metabolism). However, 5–10% of CTX is transformed into an inactive metabolite 2-dechloroethylcyclophosphamide (DCCTX) and a by-product chloroacetaldehyde (CAA) by CYP3A (side-chain metabolism) (Huang et al., 2000). CAA has been found to produce hepatotoxicity, neurotoxicity and nephrotoxicity (McDonald et al., 2003; Rzeski et al., 2004).

In this study, we have investigated the potential of SCE as a CTX detoxifier for patients in HD-CTX treatment against MM. On one hand, we speculated that SCE may reduce CAA toxicity by its curative effects on liver, kidney and nervous system. On the other hand, we hypothesized that SCE pretreatment may decrease CAA production, considering that *S. chinensis* has been reported to inhibit CYP3A activity *in vivo* (Lai et al., 2009; Wang et al., 2014).

Hence, it was our aim to investigate the pharmacokinetic HDIs between SCE and CTX, and to evaluate the protective effects of SCE against CTX induced damage in rat liver, kidney and brain. Up to now, there is no published data on the predictive value of CTX and SCE interaction.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Plant Material, *S. chinensis* was collected in Liaoning province (batch no. 2010HA) in China and identified by Professor Jun Yin (Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang, China). CTX and tinidazole (TNZ, internal standard) were obtained from Meilun Biotechnology Co., Ltd. (Dalian, China). DCCTX, 4-KetoCTX and CPM standards (purity > 98%) were obtained from Toronto Research Chemicals Inc. (Toronto, Canada). CTX (batch no. 5H071A) and Mesna injections (Endoxan, batch no. 4A177A) were obtained from Baxter International Inc. (Deerfield, America). The standards (purity > 98%) containing schisandrol A, schizandrol B, schisantherin B, deoxyschisandrin, γ-schisandrin and schisandrin C were isolated and purified from SCE, the purities were determined by HPLC with UV detection (Supplement Fig. 1). Acetonitrile and methanol of HPLC grade were obtained from Merck Company (Darmstadt, Germany). HPLC grade of formic acid was obtained from Tedia (Fairfield, OH). All other reagents were of analytical grade.

Superoxide dismutase (SOD), alanine aminotransferase (ALT), aspartate transaminase (AST), malondialdehyde (MDA), creatinine (Cr), blood urea nitrogen (BUN), glutathione peroxidase (GSH-px),