RESEARCH ARTICLE

Protective Effect of *Astragalus* polysaccharides on Liver Injury Induced by Several Different Chemotherapeutics in Mice

Wen Liu[1], Fang-Fang Gao[1], Qun Li[3], Jia-Wei Lv[2], Ying Wang[1], Peng-Chao Hu[1], Qing-Ming Xiang[2], Lei Wei[1]*

Abstract

Side effects are an unavoidable consequence of chemotherapy drugs, during which liver injury often takes place. The current study was designed to investigate the protective effect of *Astragalus* polysaccharides (APS) against the hepatotoxicity induced by frequently-used chemical therapy agents, cyclophosphamide (CTX), docetaxel (DTX) and epirubicin (EPI)) in mice. Mice were divided into five groups, controls, low or high dose groups (DTX_L, CTX_L, EPI_L or DTX_H, CTX_H, EPI_H), and low or high dose chemotherapeutics+APS groups (DTX_L+APS, CTX_L+APS, EPI_L+APS or DTX_H+APS, CTX_H+APS, EPI_H+APS). Controls were treated with equivalent normal saline for 28 days every other day; low or high dose group were intraperitoneal (i.*p*) injected with low or high doses of CTX, DTX and EPI for 28 days every other day; low or high dose chemotherapeutics+APS group were separately intraperitoneal (i.*p*) injected with chemotherapeutics for 28 days every other day and i.*p* with APS (100 mg/kg) for 7 days continually from the 22th to the 28th days. The body weight, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), histopathological features, and ultrastructure morphological change of liver tissues, protein expression level of caspase-3 were estimated at different time points. With high dose treatment of DTX, CTX and EPI, weight gain was inhibited and serum levels of ALT and AST were significantly increased. Sections of liver tissue showed massive hepatotoxicity in CTX_H group compared to the control group, including hepatic lobule disorder, granular and vacuolar degeneration and necrosis in hepatic cells. These changes were confirmed at ultrastructural level, including obvious pyknosis, heterochromatin aggregation, nuclear membrane resolution, and chondrosome crystal decrease. Western blotting revealed that the protein levels of caspase-3 increased in CTX_H group. The low dose groups exhibited trivial hepatotoxicity. More interestingly, after 100 mg/kg APS, liver injury was redecued not only regarding serum transaminase activities (low or high dose chemotherapeutics+APS group), but also from pathological and ultrastructural changes and the protein levels of caspase-3 (CTX_H+APS group). In conclusion, DTX, CTX and EPI induce liver damage in a dose dependent manner, whereas APS exerted protective effects.

Keywords: Hepatotoxicity - cyclophosphamide - docetaxel - epirubicin - *Astragalus polysaccharides* - ALT - AST

Asian Pac J Cancer Prev, **15 (23)**, 10413-10420

Introduction

Cancer is among the common diseases which seriously endanger human health and lifespan, with its morbidity and mortality increasing year by year. According to Cancer Report 2014 launched by he International Agency for Research on Cancer (IARC), in 2012, the worldwide new cancer cases a year is about 14 million, a figure expected to climb annually to an estimated 19 million by the year 2025 and 24 million by the year 2035, namely an approximate fifty percent increase within the next two decades. Worldwide cancer deaths are projected to rapidly rise from 8.2 million per year to 13 million.

Chemotherapy is by far one of the primary methods employed in cancer treatment, the efficiency of which is generally elevated by using a combination of different types of antineoplastic drugs (Fang et al., 2014). However, chemotherapy can also produce different degrees of damage to the body's normal tissues because of its low selectivity to normal tissues and cancer tissues. Antineoplastic agents circle the body and destroy cancer cells. Side effects are expected to occur when treated with these agents and many of these side effects associated with antineoplastic agents are generated because chemotherapy treatment destroys not only abnormal cancerous cells but also body's normal cells meanwhile. Numerous studies were reported about the outcomes of all these anticancer drugs (Murialdo et al., 2014). DTX, CTX and EPI have

*[1]Department of Pathology and Pathophysiology, Research Center of Food and Drug Evaluation, School of Medicine, Wuhan University, [2]Zhongnan Hospital of Wuhan University, the Second College of Clinical Medicine of Wuhan University, [3]Renmin Hospital of Wuhan University, the First College of Clinical Medicine of Wuhan University, Wuhan, Hubei, China *For correspondence: leiweifr@hotmail.com*

been widely used for chemotherapy of different cancers, including breast cancer (Chen et al., 2013; Murialdo et al., 2014). However, although they have some acceptable therapeutic effects on various cancers, severe toxicity and undesirable side effects are unavoidable (Field et al., 2008; Shin et al., 2014).

Liver is the center of drug metabolism and bioconversion, rich in a variety of biotransformation enzymes. Drug bioconversion in liver usually generates inactive metabolic end products which are excreted from the body, but can also generate toxic metabolites. Therefore, liver is both drug detoxification and side effect targeting organ. Patients always develop liver injury during the process of chemotherapy, and chemotherapy induced liver injury has attracted much attention in recent years, So how to reduce the liver damage caused by the chemotherapy agents and strengthen the drug efficacy is a clinical problem to be solved.

Herbal Chinese medicine was reported to be able to rescue body immune function, protect the liver, regulate liver function, improve blood circulation in liver, repair hepatocyte damages effectively and relieve symptoms of liver diseases (Lu et al., 2010). The utility of traditional Chinese medicine is related to the concepts that it can alleviate toxic effects associated with chemical treatments and improve quality of life (McQuade et al., 2012; Fan et al., 2014). The astragalus compound, derived from the dried roots of the Astragalus membranaceus, has a more than 2000-year history of medicinal use in traditional Chinese medicine, primarily employed in treatment of the common cold, diarrhea, fatigue, anorexia, and cardiac diseases (Wang et al., 2014). Moreover, a variety of potential therapeutic roles of Astragalus have been demonstrated in the treatment of immunodeficiency syndromes and cancer (Anonymous, 2003). For instance, it has been reported that Astragalus extract inhibits destruction of gastric cancer cells to mesothelial cells through anti-apoptosis (Na et al., 2009). The active pharmacological constituents of Astragalus membranaceus contain various bioactive compounds, such as flavones, polysaccharides and saponins as well as L-arginine or L-canavanine (Ma et al., 2002b). Among these, Astragalus polysaccharides (APS) has been most extensively studied, which is demonstrated to have substantial pharmacological applications, including anti-cancer, anti-inflammatory, immunomodulatory and antioxidant effects. APS plays its affiliated anticancer role by strengthening immune function (Liu et al., 2010; Zhao et al., 2011), counteracting the negative effects of chemotherapeutic drugs and enhancing the sensitivity of chemotherapeutics (Tian et al., 2012).

It was reported that astragalus polysaccharide (APS) displayed hepatoprotective and antioxidant properties acting as a synergistic impact in CCl4 induced liver damage in mice (Jia et al., 2012). However, whether APS can antagonize liver injury induced by different chemotherapeutics is seldom reported. Therefore, the current study was designed to evaluate the hepatotoxic effects of DTX, CTX, and EPI and investigate the protective effect of APS on liver injury induced by several different chemotherapeutics in mice.

Materials and Methods

Animals and chemicals

The experiment was performed as the Regulations of Animal Experimentation of College of Veterinary Medicine, Wuhan University, which is in accordance with the protocols and guidelines of the International Committee on Laboratory Animals. Kunming strain male mice (a closed strain coming from Kunming, Yun nan province, P.R. China) were purchased from the ABSL-III laboratory at Wuhan University, 7-8-week-old. Mice were fed by Laboratory animals-mice formula water and feeds. Cyclophosphamide (CTX)and Docetaxel (DTX) were provided by Hengrui Pharmaceutical Co., Ltd., Jiangsu, China. Epirubicin Hydrochloride (EPI) was provided by Haizheng Pharmaceutical Co., Ltd., Zhejiang, China. Astragalus polysaccharides (APS) were provided by American Generalisation Pharmaceutical Company, Stanford University Science Park, Stanford, CA, USA.

Experimental groups

Seventy eight Kunming strain male mice were divided into five groups, control group (n=6), low dose group (DTX_L, CTX_L, EPI_L group) (n=36), high dose group (DTX_H, CTX_H, EPI_H group) (n=36), low dose chemotherapeutics +APS group (DTX_L+APS, CTX_L+APS, EPI_L+APS group) (n=36) and high dose chemotherapeutics +APS group (DTX_H+APS, CTX_H+APS, EPI_H+APS group) (n=36). Control group were treated with equivalent normal saline for 28 days every other day; low dose group and high dose group were intraperitoneal (i.*p*) injected with low or high dose of CTX (10 mg/kg or 20 mg/kg), DTX (0.5 mg/kg or 2 mg/kg) and EPI (0.8 mg/kg or 3 mg/kg) for 28 days every other day respectively; low dose chemotherapeutics +APS and high dose chemotherapeutics +APS group were separately intraperitoneal (i.*p*) injected with chemotherapeutics for 28 days every other day and intraperitoneal (i.*p*) injected with APS (100 mg/kg) for 7days continually from the 22th day.

Body weights and serum levels of ALT and AST were measured at the indicated time points. On the 29th day, all of the mcie were killed under diethylether anesthesia. Liver samples were quickly harvested, Each liver was divided into two halves: one half was processed for histopathology analysis and transmission electron microscopy (TEM) examination, and the other half was kept at -80°C subsequent analysis.

Pathological findings

A portion of the liver were collected and fixed in 4% neutral formalinand embedded in paraffin. Then they were sectioned at 5μm, stained with Hematoxylin and Eosin (H&E) and examined buy a Motic BA200 microscope (Motic Instruments, Inc, Baltimore, MD).

Ultrastructural observation

The livers tissues were diced into 1 mm^3, excised and prefixed in 2.5% glutaraldehyde. Then Post-fixation was in cold 1% aqueous osmium tetroxide for 1h. After rinsing with phosphate buffer again, specimens were dehydrated in graded ethanol, embedded in Epon 812. Images were

taken with a Hitachi HT7700-SS transmission electron microscope.

Measurement of Parameters in Sera

The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured spectrophotometrically by standard methods using commercial Kits with a Semi-automatic Biochemical Analyzer (Vital, Holland) described as Instrument specifications

Western blotting assay

100 mg of liver tissues was lysed in 1ml lysis buffer (RotiLoad (Roth, Germany)) using glass homogenizer. Liver tissue lysates was measured for protein concentration by the BCA protein assay kit (Pierce, USA). Equal amounts of total protein (20 μg) were loaded and separated by SDS-PAGE (12% gels) and transferred onto poly vinylidene fluoride (PVDF) membranes (Millpore, Billerica, MA). The membranes were blocked with for 1h at room temperature with 5% nonfat milk in Tris-buffered saline for 2h, then probed with Caspase-3 (1:1000; SantaCruz Biotechnology, Santa Cruz, CA, USA) andβ-actin (1:3000; Cell signaling, USA) antibody 4°C overnight, washed extensively with Tris Buffer Solution Tween (TBST), followed by incubation with HRP-linked secondary antibodies (1:10, 000 Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1h at room temperature, and detected by ECL reagents (Pierce). The experiments were repeated three times.

Statistical analysis

All results were performed at least in triplicate and expressed as mean±standard deviation (x±S.D). The statistical significance of differences between means was calculated using One-way Analysis of Variance (ANOVA) or Student's t test followed by Prism GraphPad Software for multiple comparisons with the control group (*p* value <0.05).

Results

Effect of APS on body weight with DTX, CTX and EPI

The body weight gain in different groups was shown in Figure 1A to 1D. The body weight of mice in each group indicated a gradually increase from day 4 to day 28, but the percentage of body weight gain differed remarkably among each group. As show in Figure 1A, on day 28, in high dose group, mice in DTX_H, CTX_H and EPI_H group showed a body weight gain respectively by 18.8%, 14.5% and 17.4%, and when compared with control group (28.8%), the differences were statistically significant ($p<0.01$). These results illustrated that these chemotherapeutics had some inhibitory effects on weight gain in mice, among which CTX showed the most obvious inhibitory effect. As show in Figure 1B, on day 28, in low dose group, mice in DTX_L, CTX_L and EPI_L group showed a body weight gain respectively by 23.3%, 22.7%, 24.3%, and when compared with control group (28.8%), the differences were statistically significant ($p<0.01$), which indicated that low dose treatment group showed similar effects as high dose group, despite for a less striking inhibitory degree on weight gain in mice than high dose group.

To investigate whether APS exerts a protective effect on the inhibition of weight gain induced by the chemotherapeutics, we further observed the impact of 100 mg/kg APS on mice weight. As shown in Figure 1C, on day 24, in high dose chemotherapeutics +APS group, mice in DTX_H+APS, CTX_H+APS and EPI_H+APS group showed a body weight gain respectively by 17.9%, 13.5%, 14.8%, and when compared with relevant high dose group (DTX_H:17.5%, CTX_H:12.9%, EPI_H: 14.1%) respectively, there displayed no statistically significant differences ($p>0.05$). On day 28, in high dose chemotherapeutics +APS group, mice in DTX_H+APS and EPI_H+APS group showed a body weight gain respectively by 21.1% and 19.4%, slightly higher than that in the relevant high dose group (DTX_H:18.8%, EPI_H: 17.4%), but the differences were not statistically significant ($p>0.05$). While mice in CTX_H+APS group showed a body weight gain by 18.2%, which was 3.7% higher than that in the CTX_H group (14.5%), and the difference was statistically significant, $p<0.05$. In addition, on day 24 and day 28, when comparing every high dose group and high dose chemotherapeutics +APS group with control group at corresponding time point, the differences were all statistically significant ($p<0.01$). Similarly, when treating mice with low dose chemotherapeutics, the additional application of APS exhibited a similar trend of effect. The results demonstrated that APS displayed a trend of reversing the inhibition of weight gain induced by chemotherapeutics to a certain degree, especially by CTX on day 28 which was most distinct.

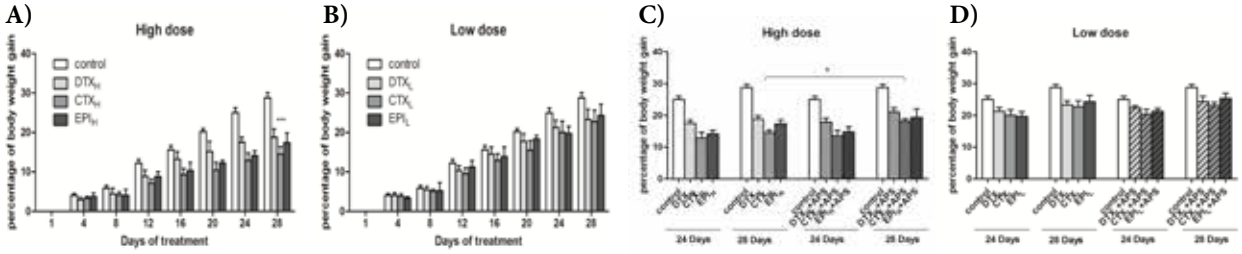

Figure 1. The Effect of APS on Body Weight Restraint Induced by DTX, CTX and EPI. The details of the animal grouping and experiment design were specified in materials and methods. Body weights were measured twice weekly at 1, 4, 8, 12, 16, 20, 24 and 28 days post-treatment. **A and B)** are the percentages of body weight gain with high or low dose of different chemotherapeutics. Compared with control group; ***$p<0.01$. **C and D)** showed, on day 24 and day 28, the percentages of body weight gain with high or low dose chemotherapeutics +APS (*$p<0.05$; **$p<0.01$; ***$p<0.001$). Three independent experiments were performed. Results were presented as means±SD

APS reverse elevation of the serum levels of ALT, AST induced by DTX, CTX and EPI

The influences of DTX, CTX, EPI and APS on serum levels of ALT, AST were represented in Figure 2. As show in Figure 2A to 2D, the serum levels of ALT, AST in both low and high dose of DTX, CTX and EPI were significantly higher than the control group at all of the indicated time points (all $p<0.05$). Notably, on day 28, the serum levels of ALT, AST in CTXH group increased respectively by 366.7% (Figure 2A) and 263.3% (Figure 2B) compared with control group, and the serum levels of ALT, AST in CTX_L group increased respectively by 224.2% (Figure 2C) and 159.5% (Figure 2D) compared with control group, and the differences were all statistically significant ($p<0.01$). This result indicated that these chemotherapeutics could all induce dose dependent liver damage, of which CTX had the most remarkable inductive effect.

Subsequently, we further investigated the protective effect that APS plays on liver injury induced by the chemotherapeutics. As shown in Figure 2E, on day 28, in high dose chemotherapeutics +APS group, the serum levels of ALT in mice treated with high dose of DTX+APS, CTX+APS and EPI+APS were respectively 75, 110 and 71U/L; correspondingly, in high dose group, the serum levels of ALT in mice treated with high dose of DTX, CTX and EPI were respectively 110, 154 and 102U/L, and the differences between these two groups were all statistically significant (all $p<0.05$). As shown in Figure 2F, on day 28, in high dose chemotherapeutics +APS group, the serum levels of AST in mice treated with high dose of DTX+APS, CTX+APS and EPI+APS were respectively 164, 184 and 162U/L; in high dose group, the serum levels of AST in mice treated with high dose of DTX, CTX and EPI were respectively 209, 287 and 200 U/L, and the differences between these two groups were also statistically significant (all $p<0.05$). The results demonstrated that, compared with single injection of high dose chemotherapeutics, mice in groups with simultaneous injection of APS and chemotherapeutics suffered a drop in the serum levels of ALT and AST to various degrees, which was similar with the results of mice in low dose

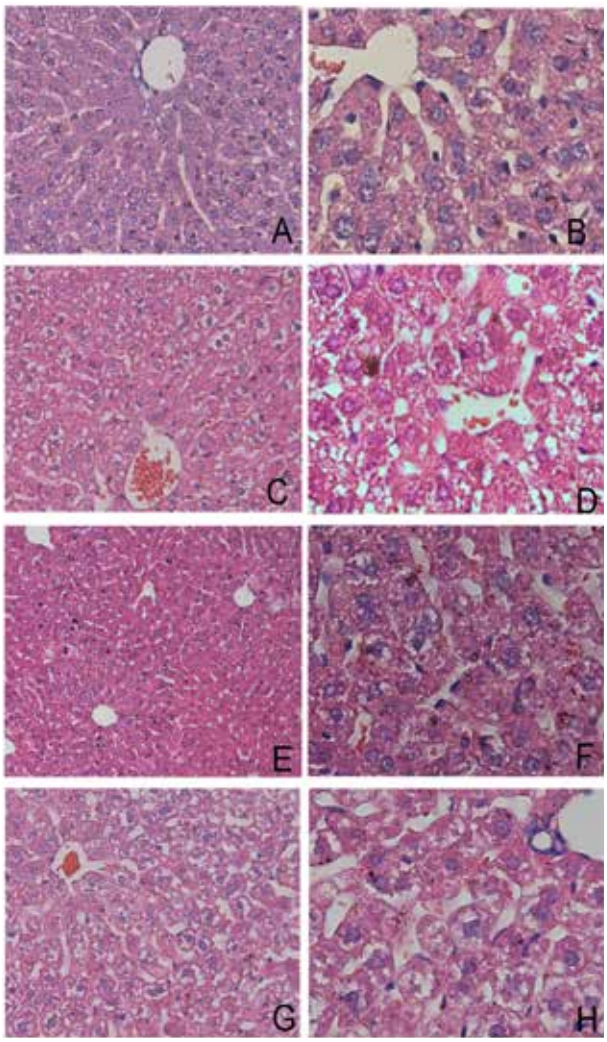

Figure 3. APS Reverse Liver Damage Induced by DTX, CTX and EPI from the Ultrastructural Feature. On the 28th day, all of the mice were killed, and liver samples were quickly harvested for histopathology analysis. **A-B)** Histology of normal control mice liver; **C-F)** The liver histology of CTX_H groups; **G-J)** The liver histology of CTX_L groups; **K-L)** The liver histology of CTX_H+APS group (original magnification: E×1000; A, C, G×2000; B, D, F, H×4000.) Three independent experiments were performed

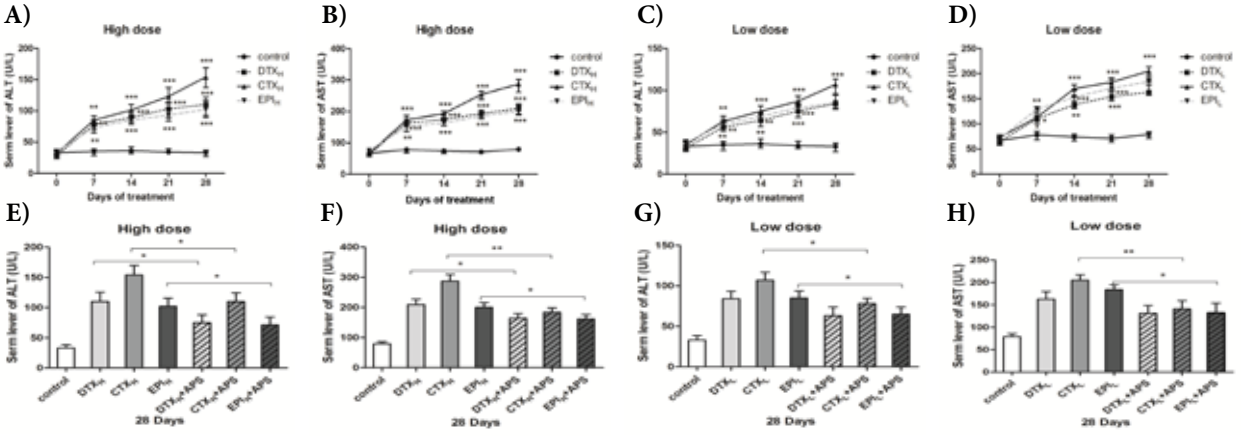

Figure 2. APS Reverse Elevation of the Serum Levels of ALT, AST Induced by DTX, CTX and EPI. Blood samples from the orbit were taken at 1, 7, 14, 21 and 28 days post-treatment. **A and B)** respectively indicated the serum levels of ALT and AST treated with high dose of DTX, CTX and EPI; **C and D)** are the serum levels of ALT and AST with low dose chemotherapeutics; **E-F)** showed changes of serum ALT and AST levels in mice in high or low dose chemotherapeutics +APS group on day 28 (*$p<0.05$; **$p<0.01$; ***$p<0.001$). Three independent experiments were performed. Results were presented as means±SD

groups (Figure 2G and 2H). This illustrates that APS can reverse elevation of the serum levels of ALT, AST induced by DTX, CTX and EPI

APS reverse liver damage induced by DTX, CTX and EPI from the pathological changes in H&E slides

After 28 days of treatment with different dose of several different chemotherapeutics, there were obvious pathological changes appeared in the liver of mice. The control hepatic tissue showed normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in-between the hepatic cords with fine arrangement of Kupffer cells in Light microscopic (Figure 3A-B). After 28 days of high dose treatment of different chemotherapeutics, dramatic hepatotoxicity appeared, especially in CTX_H group. There were obvious pathological changes in which lobules of liver became invisible; central vein became congested; hepatic cords were indiscriminate; sinus hepaticus was compressed and became narrow; degenerations were expanded; hepatocytes appeared hydropsia and ballooning degeneration, their volume was increased; cytoplasm becoming hypochromatic; nucleus became swollen with punctiform and piecemeal necrosis where the volume of hepatic cells was decreased and there were karyopycnosis and anachromasis with chromatin distributed unevenly (Figure 3C-D). CTX_L group showed trivial hepatotoxicity which displayed that the lobules of liver was still visible, however, the volume of some hepatocytes increased with cytoplasm becoming pultaceous, hypochromatic and transparent. Some of the nucleus became swollen while still maintained its structure. Little degeneration was observed (Figure 3E-F).

While after subjected to 100 mg/kg APS for 1 week, hepatotoxicity was relieved, especially in CTXH +APS group. We found that hepatic cells hydropsia, hepatic cords disorder and sinus hepaticus compression was ameliorated; cells volume was decreased; cytoplasm became cancellated which acted as low dose group. However, cells necrosis didn't retrieve (Figure 3G-H).

APS reverse liver damage induced by DTX, CTX and EPI from the ultrastructural feature

Electron microscope revealed specific ultrastructural changes in hepatic cells and renal tubular epithelial cells under experimental conditions. Details of a normal hepatic cell and its organelles were illustrated in Figure 4. Control hepatocytes were normally polygonal, with oval-shaped nuclei in the center accompanied by more euchromatin and less heterochromatin, cytoplasm were crowded with mitochondria, rough and smooth endoplasmic reticulum, golgi apparatus, ribosomes and glycogen particles (Figure 4A). The hepatocytes of liver from group treated with high dose of CTX displayed obvious pyknosis, heterochromatin aggregation, nuclear membrane resolution, chondrosome increase, chondrosome cristal disorder with cytoplasma filled with ribsome and amylon (Figure 4B). Low dose group under the electron microscope showed similar morphological change. The hepatocytes of liver from group treated with low dose of CTX showed shrunken nuclei with irregular nuclear membrane, the cytoplasm included vesiculated rough endoplasmic reticulum and atrophied mitochondria with non-differentiated cisternae (Figure 4C). However, after subjected to 100 mg/kg APS liver injury was relieved (Figure 4D).

APS reverse the increase of Caspase-3 expression level in liver tissues induced by CTX

To explore mechanisms of the protective effect that APS plays on liver injury induced by the chemotherapeutics, we detected the protein expression levels of caspase-3 in liver tissues of mice. As shown in Figure 5, on day 28, the protein level of caspase-3 in liver tissues of mice in CTX_H group increased by 140.5% compared with control group ($p<0.01$), however, after additional injection of APS at 100 mg/kg, the protein level of caspase-3 in liver tissues of mice decreased by 28.3% ($p<0.05$) (Figure 5A). This result

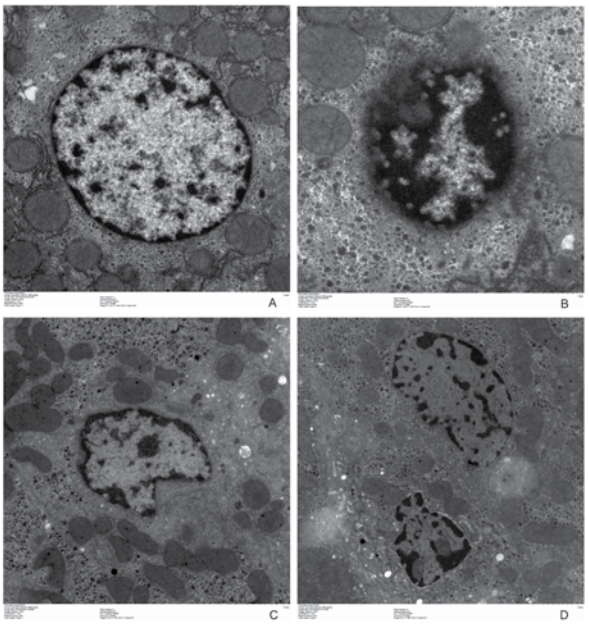

Figure 4. APS Reverse Liver Damage Induced by DTX, CTX and EPI from the Ultrastructural Feature. Liver tissues derived from the mice on the 28th day. **A)** Mice liver tissues in control group. **B)** mice liver treated with high dose of CTX; **C)** Mice liver tissues treated with low dose of chemotherapy; **D)** mice liver treated with high dose of CTX (20 mg/kg) every other day for 28days and APS 100mg for the last 7days (Lead citrate and uranyl acetate: A&C×3000, D×2500, B×5000). Three independent experiments were performed

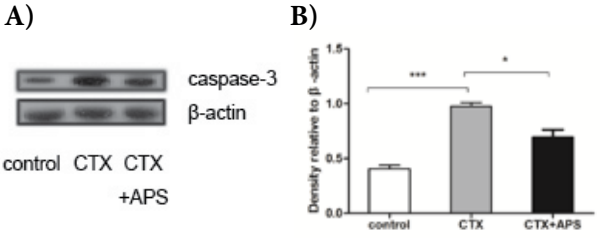

Figure 5. APS Reverse the Increase of Caspase-3 Expression Level in Liver Tissues Induced by CTX. A) On the 28th day, liver tissues were collected. Western blotting analyses of caspase-3 were performed.β-actin was taken as internal control; **B)** The relative density of caspase-3 andβ-actin were densitometrically measured and plotted as a bar graph. Three independent experiments were performed. Results were presented as means±SD. (*$p<0.05$; ***$p<0.001$)

demonstrated that APS is likely to alleviate liver injury induced by the chemotherapeutics through anti apoptosis.

Discussion

Chemotherapy constitutes an important component in the therapy of different tumors. Docetaxel (DTX), Cyclophosphamide (CTX) and epirubicin (EPI) are three kinds of chemotherapeutics that are most commonly used, which can be used alone or in combination with other chemotherapeutics. Although these chemotherapeutics have showed commendable clinical therapeutic effects, their hepatic and renal toxicity can not be ignored.

Chemotherapeutics can be classified into various categories, the anti-tumor mechanism of which varies from each other. Docetaxel is a hemisynthetic compound derived from 10-deacetylbaccatin III (a non-cytotoxic precursor extracted from the needles of the European yew tree, Taxus baccata L (Naume et al., 2014), one of the members of the taxoid family similar to paclitaxel, and can promote microtubule assembly and inhibit depolymerization to free tubulin, which results in the blockage of the cells in the M phase of the cell cycle (Bissery et al., 1995). Cyclophosphamide (CTX) is a nitrogen mustard alkylating agent synthesized in the 1940s, which is broad-spectrum and cell cycle-nonspecific. The anti-tumor mechanism of CTX is primarily cross-linking guanine bases in DNA double-helix strands which results in the disruption of DNA function and cell death (Peng et al., 2005; Shokrzadeh et al., 2014). Epirubicin (EPI) is an anti-tumour derivative of doxorubicin belongs to the anthracycline antibiotics, widely used in the treatment of various types of malignancies due to its good therapeutic efficacy. The precise mechanism of antitumor action of EPI is not well established but is thought to be related with the interference with the synthesis of macromolecules, covalent DNA binding and DNA cross-linking, inhibition of topoisomerase II, arrest of tumor cell cycle progression in G2 phase, induction of apoptosis and generation of reactive oxygen radicals (Lubgan et al., 2006). These drugs cause some side effects while killing tumors, such as bone marrow suppression, alopecia, nausea and vomiting, diarrhea, dizziness, hemorrhagic cystitis, anaphylaxis, fluid retention, cardiotoxicity and elevated transaminase and bilirubin levels, among which liver damage is a rather important toxicity. Clinical evidence of liver injury induced by a Docetaxel has been demonstrated by increased activities of serum enzymes and bilirubin levels, and the development of jaundice (Yeo et al., 2002; Matovina-Brko et al., 2014). In J. Alexandre et al.'s study on a total of 825 heavily pretreated advanced breast cancer patients treated with docetaxel (100 mg/m^2 every 3 weeks), 28% (217 patients) were recorded with minor abnormalities of transaminase and/or alkaline phosphatase [AP] levels (≥1.5 times the upper limit of normal [ULN]), whereas 9.6% (74 patients) displayed evidence of liver dysfunction (transaminase levels >1.5 times ULN and AP level>three times ULN) and 3.7% (28 patients) had hyperbilirubinemia with increased bilirubin levels (≥1.5 times ULN) (Alexandre et al., 2000). George B et al. reported 147 patients with hematologic malignancy who received allogeneic transplantation after pre-treated with CTX (60 mg/kg body weight) and total body irradiation, the result showed that 23 (16%) patients developed moderate or severe sinusoidal obstruction syndrome. This study concluded that exposure to the metabolism of CTX was statistically significantly involved in sinusoidal obstruction syndrome and bilirubin elevation (McDonald et al., 2003). Brigette Ma et al. (2002) reported A cohort of 85 Chinese breast cancer patients who received adjuvant chemotherapy with doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2)every 3 weeks, and these patients were found to have a higher incidence of hepatotoxicity as indicated by raised ALT activity (≥1.5 times the upper limit of normal [ULN]) (n=89.4%) compared to caucasian patients (Acute hepatotoxicity was not reported in the NSABP series (Fisher et al., 1990). Besides, it was thought that endemic chronic hepatitis B infection in the geographical area possibly contributed to the higher incidence of hepatoxicity (Ma et al., 2002a). Indeed, a correlation between EPI dosage and serum ALT levels has been reported in breast cancer patien ts with hepatic dysfunction (Dobbs et al., 2003). This study provides us a clear understanding of modulating the dosage of epirubicin according to patients' physical condition, but hepatotoxicity induced by epirubicin is rare (Thatishetty et al., 2013).

The mechanism of liver injury induced by chemotherapeutics is indicated to probably involve various aspects , not only including the chemotherapeutics itself and its metabolites, but also including variations in host metabolic response and immunologic status, preexisting medical problems, tumor, hepatitis viruses or other infections, and total parenteral nutrition or nutritional deficiencies which may influence a host's sensitivity to liver injury. Therefore, ascribing liver injury to a toxic reaction is incomplete (Thatishetty et al., 2013). Varbiro et al. (2001) and Joerger et al. (2014) showed that PTX induced liver injury might be related with mitochondria damage, which induced mitochondrial permeability transition and ROS formation, and Ozben et al. (2007) considered that probably docetaxel has a similar action mechanism. In fact, research has shown that liver toxicity induced by CTX might be associated with the following mechanisms. CTX is extensively metabolized by the liver cytochrome P450 system, which probably causes sinusoidal obstruction syndrome, resulting in a direct toxic effect on hepatic sinusoidal cells, thus inducing necrosis, obstruction, and obliteration of hepatic veins (de Jonge et al., 2006; Basu et al., 2014). Kebieche et al. (2009) investigated for the first time the implications of oxidative stress in liver toxicity induced by EPI via evaluating the redox status in hepatic cells in EPI-treated rats. Hepatotoxic effects of these chemotherapeutics are not clearly elucidated, thus, we further observed that these chemotherapeutics can induce liver injury to different degrees in animal models, which presents as both dose dependent and time dependent. In our experimental models, DTX, CTX and EPI all caused liver injury, and high dose CTX (20 mg/kg) exhibited to have the most obvious toxicity, compared with other groups. The hepatotoxicity mainly manifested as serum enzyme levels

including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increasing in the biochemical aspect, likewise methane dicarboxylic aldehyde (MDA) and superoxide dismutase (SOD) levels (Horton and Fairhurst, 1987; Al-Sayed et al., 2014). Histological changes including liver congestion, disorganization of hepatic cords and ballooning degeneration could be observed in H&E slides. At the same time we have found a remarkable decrease in body weight gain after mice being treated with CTX, notably in high dose group, implying that hepatotoxicity might have contributed to this weight loss as previously reported (King and Perry, 2001; Basu et al., 2014). In addition, we also discovered that the expression level of apoptosis-related protein caspase-3 increased in the liver of mice after received CTX.

Next, we investigated the protective effect of Astragalus polysaccharides (APS) on Liver Injury Induced by several different chemotherapeutics in mice. Astragalus Polysaccharide (APS) is a sort of water soluble sugar extracted from astragalus root or leguminous plants. It is supposed to be one of the main components that significantly inhibit liver fibrosis induced by hepatitis B and suppress hepatic inflammation (Ding et al., 2008). Moreover, APS has been utilized for chronic liver disease in China for thousands of years and is also known as a kind of agent which could not only restrain tumor progression *in vivo*, but also increase the body weight, spleen and thymus indexes and the phagocytotic function of macrophages in tumor-bearing mice, and further stimulate the secretion of serum TNF-α, IL-2, IL-12, decrease IL-10 level in serum (Yang et al., 2013). It provides us with clues that whether APS could suppress hepatotoxicity induced by multiple kinds of anticancer agents. In our study, after adding APS on the basis of the administration of different chemotherapy drugs, we observed that liver injury was relieved not only from serum transaminase activities (low or high dose chemotherapeutics+APS group), but also from pathological and ultrastructural changes and the protein levels of caspase-3 (CTX_H+APS group). This phenomenon may be interpreted as APS alleviating chemotherapeutics induced liver injury through anti-apoptosis pathway.

In conclusion, our results indicate the hepatotoxic effects of CTX, DTX and EPI and the protective effects of APS against CTX induced hepatotoxicity in experimental mice model *in vivo*. DTX, CTX and EPI can all induce liver injury to different degrees which presents as dose dependent. However, 100 mg/kg APS plays a protective effect against chemotherapeutics induced liver injury, the mechanism of which is anti-apoptosis pathway. In conclusion, chemotherapeutics all have toxicities that aggravate with dose increasing, and our study evaluated hepatotoxicity of these chemotherapeutics and its dose-dependence, as well as further investigation of the protective effect of traditional Chinese medicine against it.

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