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ORIGINAL ARTICLE

Alleviation of Doxorubicin-induced Hepatic Toxicity with Fermented *Cordyceps sinensis* via Regulating Hepatic Energy Metabolism in Rats

Ping-An Yao, Rong Wu, Yao-Li Zhang[1], Xiao-Hua Cui, Ke-Zhao Wei, Xu Xu[1], Jian-Ping Gao

Department of Pharmacology, School of Pharmacy, Shanghai University of Traditional Chinese Medicine, [1]Department of Chemistry, School of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai, China

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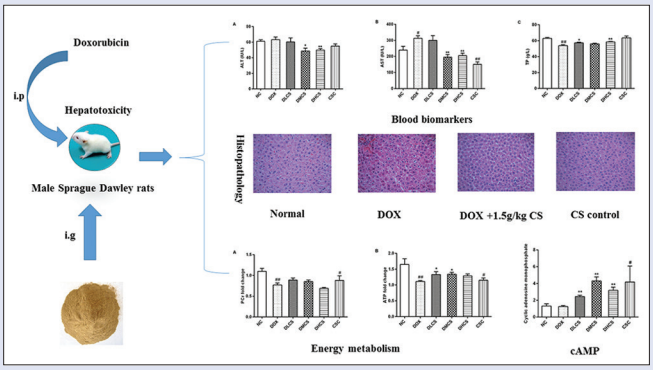
ABSTRACT

Background: *Cordyceps sinensis* (CS), a traditional Chinese herbal medicine, has many pharmacological effects. Doxorubicin (DOX) is a spectrum antitumor drug, but it has toxicity to multiple organs. **Objective:** The objective of this study was to evaluate the effect of fermented CS on regulating hepatic energy metabolism against DOX-treated hepatic toxicity in rats. **Materials and Methods:** Male Sprague-Dawley rats were randomly divided into six groups and administrated orally for 23 days as follows: normal control group, CS (1.50 g/kg/d) control group, DOX control group, and DOX + CS (0.75 g/kg/d, 1.5 g/kg/d, 3.00 g/kg/d) groups. Rats in DOX-treated groups were intraperitoneally injected with DOX at a dose of 2.5 mg/kg at every 48 h, and repeated for six times. At the end of the experiment, the mortality and liver index, blood biomarkers about the hepatic injury, hepatic histopathological changes, hepatic energy metabolism, and hepatic cyclic adenosine monophosphate (AMP) were measured. **Results:** DOX-induced a higher mortality, the damage of the liver manifests itself in the increase of liver weight index and the activity of serum aspartate aminotransferase, the decrease of serum contents of total protein and albumin, and histopathological changes, and the disorders of energy metabolism including the decrease of phosphocreatine, adenosine-triphosphate, adenosine diphosphate, AMP, and total adenine nucleotides in hepatic tissues. Fermented CS not only could attenuate those changes but also could increase the content of hepatic cAMP. **Conclusion:** DOX induces hepatic injury accompanying hepatic energy metabolism disorders. Fermented CS regulates the disorders of hepatic energy metabolism, thereby attenuating liver injury caused by DOX.

Key words: Cyclic adenosine monophosphate, *Cordyceps sinensis*, doxorubicin, energy metabolism, hepatic injury

SUMMARY

- The aim of this study was to explore the potential mechanism of fermented *Cordyceps sinensis* ameliorate liver injury. Results: Fermented *Cordyceps sinensis* ameliorates hepatic injury caused by DOX by regulating the hepatic energy metabolism.



Abbreviations used: CS: *Cordyceps sinensis*; DOX: Doxorubicin; cAMP: Cyclic adenosine monophosphate; AST: Aspartate aminotransferase; TP: Total protein; ALB: Albumin; ATP: Adenosine triphosphate; ADP: Adenosine diphosphate; AMP: Adenosine monophosphate; TAN: Total adenine nucleotides.

Correspondence:

Prof. Jian-Ping Gao,
Department of Pharmacology, School of Pharmacy, Shanghai University of Traditional Chinese Medicine, No. 1200 Cailun Road, Shanghai 201203, China.
E-mail: zydgjp@aliyun.com

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INTRODUCTION

Doxorubicin (DOX), the same as the other anticancer drug, has considerable side effects. It has toxicity to multiple organs such as vascular toxicity[1] and serious liver dysfunction.[2] Some studies reveal that the mechanisms of DOX-induced hepatotoxicity mainly includes oxidative stress, apoptotic response and so on.[2-4] The liver is one of the most important metabolic organs in the body and requires a great deal of energy in the process of metabolism. Mitochondria are susceptible to DOX injury,[5] due to the high affinity of DOX to the mitochondrial membrane.[6] Mitochondrion plays an important role in the process of energy supply. It has revealed that mitochondrial damage in the liver can cause the inhibition of oxidative phosphorylation (adenosine-triphosphate [ATP] synthesis).[7] Energy metabolism plays an important role in hepatic injury, and the disorder of hepatic energy metabolism can further aggravate hepatic damage.[8] Hence, hepatic energy metabolism damage may play an important role in DOX-induced hepatic injury. However, no research has been found if there is a relationship between DOX hepatotoxicity and impairment of energy metabolism.

Cordyceps sinensis (CS) is a widely used traditional Chinese medicine. Due to the limited resources of natural CS, fermented CS is often used

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as a substitute for the natural CS. Some studies reveal that CS has certain protective effects on the different hepatic injury of animal models.[9,10] However, there is no any report mentioned if fermented CS can regulate the hepatic energy metabolism against hepatic injury, especially the DOX-induced hepatotoxicity. Based on the mentioned above, in this study, we investigated the effect of CS on DOX-induced hepatic injury in rats and explored whether the effect is related to the regulation of energy metabolism in the liver or not.

MATERIALS AND METHODS

Materials

DOX hydrochloride was purchased from Zhejiang Hisun Pharmaceutical Co., Ltd., (Taizhou, China). Fermented CS is a substitute for natural CS.[11] It is produced by the purification and artificial fermentation of the fungus isolated from fresh Qinghai CS. The products of fermented CS meet the standard of the Pharmacopoeia of the People's Republic of China (2015 Edition).[12] In the previous study, it had reported the comparison of the chemical compositions and bioactive ingredients between natural CS and fermented CS.[13] The natural CS contains 29.1% protein, 8.62% fat, 24.2% carbohydrate, 2.85% ash, 8.93% moisture, 18.1% amino acid, and 5.4% cordycepin. Fermented CS contains 39.4% carbohydrate, 14.8% protein, 9.23% amino acid, 6.63% fat, 6.4% moisture, 2.95% ash, 1.4% cordycepin, and 0.21% adenosine. Fermented CS was supplied by Hangzhou Zhongmei Huadong Pharmaceutical Co., Ltd., (Hangzhou, China).

High-performance liquid chromatography assay for the quantification of fermented *Cordyceps sinensis*

According to the Pharmacopoeia of the People's Republic of China (2015 Edition),[12] adenosine content is required to verify the qualification of fermented CS. The quantification of fermented CS was assayed in accordance with the way reported in the Pharmacopoeia of the People's Republic of China (2015 Edition) by high-performance liquid chromatography (HPLC, Ultimate 3000, thermo fisher scientific, America). The result shows that the adenosine content in 0.5 g fermented CS is 1.07 mg and this meets the requirement of the Pharmacopoeia of the People's Republic of China (2015 Edition).

Animals

Male Sprague-Dawley rats (200 ± 5 g) were used to carry on this study, and all of them were from Shanghai Slac Laboratory Animal Co., Ltd. Rats were kept in the Laboratory Animal Center of Shanghai Traditional Chinese Medicine (temperature 22°C–24°C, humidity 40% ± 5%, and 12 h light/dark cycle) with free access to food and water. The treatment procedures of the rats were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996), and the operation specification released by the Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine.

Methods

Experimental design

Based on the consideration of animal protection and the lethality of DOX with high dose, rats were grouped using the following method. All rats were randomly divided into six groups as follows: normal control group (NC), ten rats; CS (1.50 g/kg/d) control (cardiac stromal cell [CSC]) group, five rats; DOX control group, fifteen rats; DOX + higher dose (3.00 g/kg/d) of CS (DHCS) group, fourteen rats; DOX + middle dose (1.50 g/kg/d) of CS (DMCS) group, fourteen rats; and DOX + lower dose (0.75 g/kg/d) of CS (DLCS) group, fourteen rats. All rats were injected with DOX at the dose of 2.5 mg/kg at every 48 h, for six equal intraperitoneal (IP) injections except the rats in NC and CSC groups. The rats in NC and CSC groups were injected with normal saline (the solvent for DOX) in the same way. Two days before DOX IP injected, rats in CS-treated groups were administrated orally with fermented CS at the corresponding dosages daily for successive 23 days, and the other rats were administrated with water.

Sample collection

At the end of the experiment, all rats were anesthetized with IP injection of urethane (1.0 g/kg) to collecting blood. Moreover, the serum was acquired by centrifuging the blood at the speed of 3500 rpm for 10 min. Then, the liver was isolated, and its weight was recorded. Part of the liver (the right part of the liver lobe) was saved in 4% paraformaldehyde for histological analysis, and the remaining liver tissue was stored at −80°C.

Mortality and liver index

During the experiment, the death rate of rats was observed. At the last, the body weight (BW) and liver weight (LW) were recorded, and then, the liver index was evaluated by calculating LW/BW ratio.

Measurement of the blood biomarkers about the hepatic injury

The blood biomarkers about the hepatic injury were examined by Hitachi 7080 biochemical analyzer (Tokyo, Japan). Albumin (ALB), total protein (TP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were included in this study. Moreover, the kits were purchased from Shino-Test Co., Ltd. (Tokyo, Japan).

Histological analysis

Ethanol (70%–100%) and xylene were used to dehydrate and clear the fixed livers. Moreover, then the fixed livers were embedded in paraffin. A volume of 5 μm thick slices were cut and stained with hematoxylin and eosin. Light microscope (Olympus BX51, Japan) was applied to analyze the morphology of liver.

Hepatic energy metabolite and cyclic adenosine monophosphate measurement

Liver tissues were handled with perchloric acid to acquire the extracts. The method is consistent with the methods reported in the reference.[14] Energy levels in extracts were assayed by HPLC (Ultimate 3000, thermo fisher scientific, America), including phosphocreatine (PCr), ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP). Moreover, then ATP/ADP ratio and total adenine nucleotides (TAN) were calculated. Meanwhile, the cyclic AMP (cAMP) contents in hepatic tissues were also measured. The chromatographic column is Venasil MP-C18 (4.6 × 250 mm, 5 μm), and the mobile phase is a mixture of phosphate buffers (40 mmol/L, pH = 6.2) and water/methanol/acetonitrile (2:1:1, v/v/v) mixed in proportion. UV detection wavelength: 210 nm, 254 nm.

Data analysis

All results were expressed as the mean ± standard error of the mean. One-way analysis of variance (ANOVA) was used to analyze the variance. An alternate test, rank-sum test was used to analyze variance heterogeneity. Values of $P < 0.05$ were considered statistically significant. All the analysis procedures were performed by the software of SPSS (Statistical Product and Service Solutions, IBM Co., Ltd., New York, America) version 21.0.

RESULTS

Mortality and liver index

The mortality (40%) was obviously higher in DOX group than that in NC group (0%) ($P < 0.05$). Moreover, in DMCS and DHCS groups, the mortalities were significantly decreased to 0% and 14% ($P < 0.01$, $P < 0.05$), respectively. BW, LW, and liver index are shown in Figure 1. In DOX group, BW was significantly decreased when compared to that in NC group ($P < 0.01$), and the liver index was enormously increased ($P < 0.01$). Liver indexes of the rats in DMCS and DHCS groups were significantly lower than that in DOX group ($P < 0.05$). In DLCS group, fermented CS also decreased liver index but was not statistically significant. In CSC group, BW and liver index were not changed significantly when compared to that in NC group.

Evaluation of the blood biomarkers about hepatic injury

As shown in Figure 2, compared to NC, the contents of TP and ALB were enormously decreased ($P < 0.01$) in DOX group, and the activity of AST in DOX was statistically increased ($P < 0.05$). Compared to that in DOX group, the contents of TP and ALB were increased in DLCS and DHCS groups ($P < 0.05$), and the activities of AST and ALT in DMCS and DHCS groups were significantly decreased ($P < 0.01$). Compared to that in NC group, the activity of AST in CSC group was statistically decreased ($P < 0.01$).

Histopathological analysis

HE staining was used to investigate the pathological injury of liver tissue in this study. In DOX group, liver tissue appeared with obvious edema, degeneration, and lymphocyte infiltration. Moreover the lesions were relieved in DLCS, DMCS, and DHCS groups. Histopathological photos are shown in Figure 3.

Energy metabolites in liver tissue

The results of energy metabolites in liver tissue are shown in [Figure 4]. In DOX group, the concentrations of PCr, ATP, ADP, AMP, and TAN were significantly decreased when compared to those in NC group ($P < 0.01$, $P < 0.05$). Compared to DOX group, the concentrations of ATP, ADP, and TAN were increased in DLCS ($P < 0.05$) and DMCS groups ($P < 0.05$), AMP content was increased significantly in DMCS group ($P < 0.05$), ADP and TAN contents were also increased in DHCS groups ($P < 0.01$), and ATP/ADP ratio in DMCS group was significantly decreased ($P < 0.05$). In CSC group, ATP/ADP ratio, contents of PCr, and ATP were statistically decreased ($P < 0.05$) when compared to those in NC group.

Contents of hepatic cyclic adenosine monophosphate

Compared to the NC group, the content of cAMP in DOX-treated rats shown no statistical difference ($P > 0.05$), the content of cAMP showed an increase in CSC group ($P < 0.01$). In CS-treated animals, the content of hepatic cAMP was significantly increased compared to that in DOX group ($P < 0.01$) [Figure 5].

DISCUSSION

DOX has potent anticancer activity and has been widely used as the anticancer drug.[15] The cumulative DOX could induce liver toxicity after the long-term use.[16] Hepatocytes will undergo degeneration

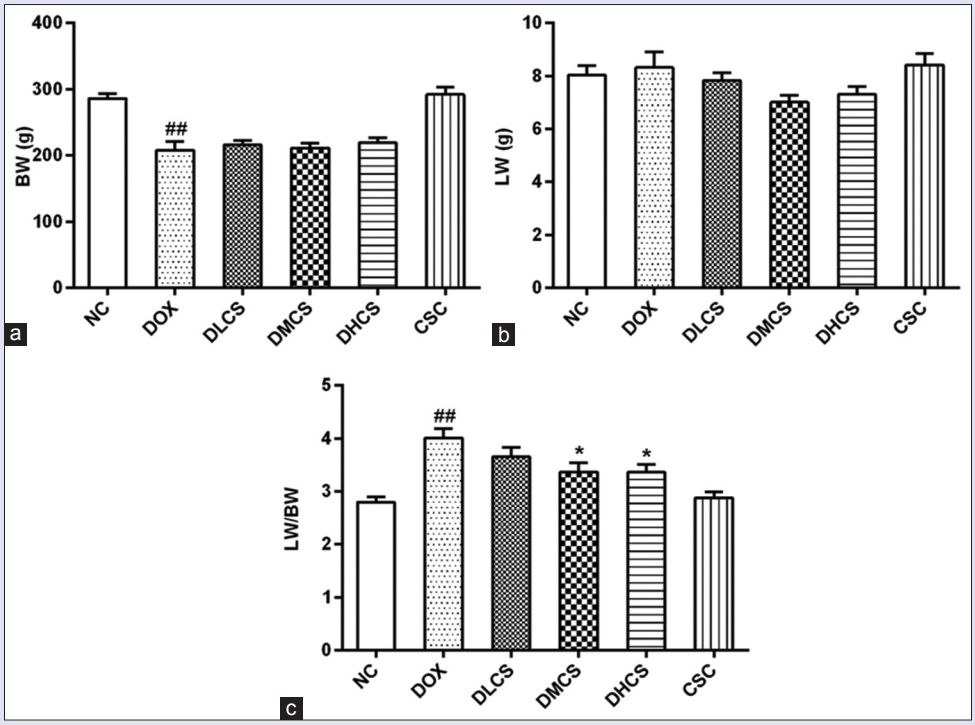

Figure 1: Effect of fermented *Cordyceps sinensis* on body weight (a), liver weight (b), and the ratio of liver weight to body weight (c) in rats treated with doxorubicin. Compared to normal control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to doxorubicin group, $^{*}P < 0.05$, $^{**}P < 0.01$. Data are presented as the mean ± standard error of the mean normal control group, $n = 10$. Cardiac stromal cell, *Cordyceps sinensis* (1.50 g/kg/d) control group, $n = 5$. DOX, doxorubicin control group, $n = 9$. DHCS, doxorubicin + higher dose (3.00 g/kg/d) of *Cordyceps sinensis* group, $n = 12$. DMCS, doxorubicin + middle dose (1.50 g/kg/d) of *Cordyceps sinensis* group, $n = 14$. DLCS, doxorubicin + lower dose (0.75 g/kg/d) of *Cordyceps sinensis* group, $n = 8$

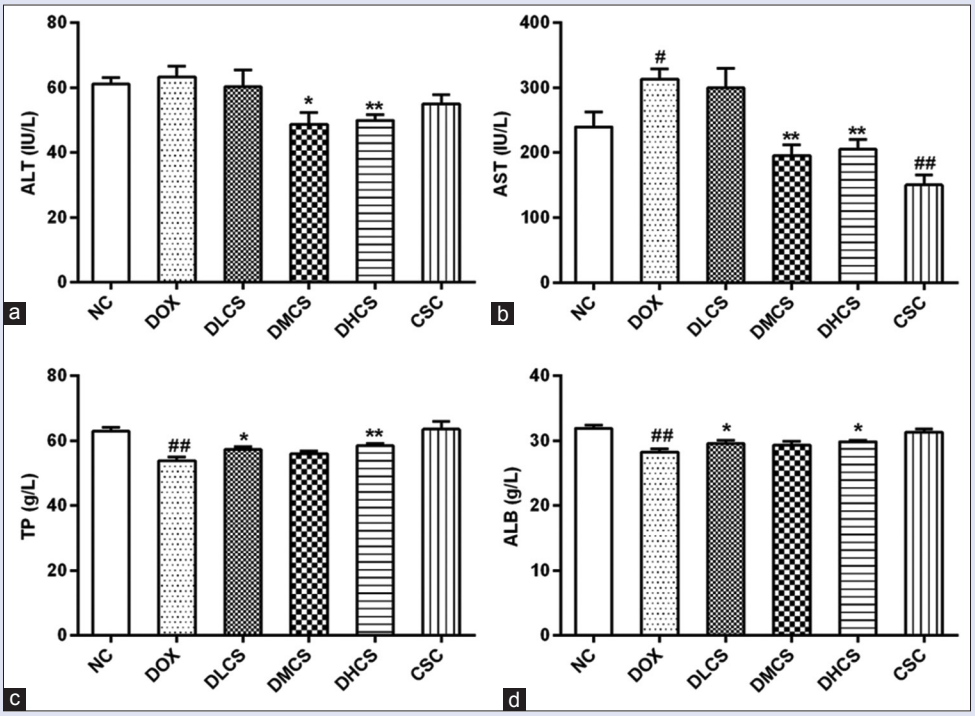

Figure 2: Effect of fermented *Cordyceps sinensis* on alanine aminotransferase (a), aspartate aminotransferase (b), total protein (c), and albumin (d) of rats treated with doxorubicin. Compared to normal control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to doxorubicin group, $^{*}P < 0.05$, $^{**}P < 0.01$. Data are presented as the mean ± standard error of the mean normal control group, $n = 10$. Cardiac stromal cell, *Cordyceps sinensis* (1.50 g/kg/d) control group, $n = 5$. DOX, doxorubicin control group, $n = 9$. DHCS, doxorubicin + higher dose (3.00 g/kg/d) of *Cordyceps sinensis* group, $n = 12$. DMCS, doxorubicin + middle dose (1.50 g/kg/d) of *Cordyceps sinensis* group, $n = 14$. DLCS, doxorubicin + lower dose (0.75 g/kg/d) of *Cordyceps sinensis* group, $n = 8$

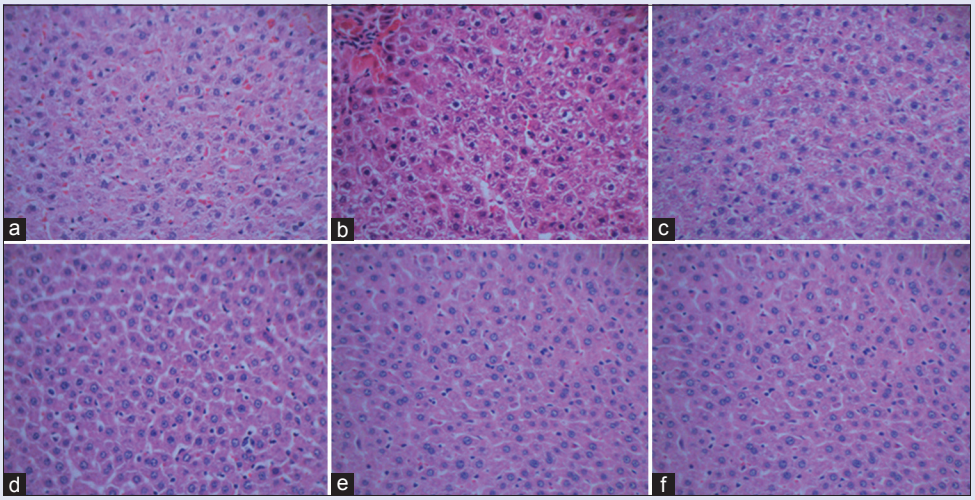

Figure 3: Effect of fermented *Cordyceps sinensis* on hepatic histopathology. (a) normal control group. (b) Doxorubicin control group. (c) Doxorubicin + lower dose (3.00 g/kg/d) of *Cordyceps sinensis* group. (d) Doxorubicin + middle dose (1.50 g/kg/d) of *Cordyceps sinensis* group. (e) Doxorubicin + higher dose (0.75 g/kg/d) of *Cordyceps sinensis* group. (f) *Cordyceps sinensis* (1.50 g/kg/d) control group (H and E, ×400)

and swelling, and hepatomegaly occurs when liver is injured, with the increase of liver index, and the mechanism is related to inflammatory pathway.[17] The increase of liver index is a key parameter in diagnosing hepatic disease. In this study, DOX-induced the loss of BW in rats was obvious, which may be related with the suppression of protein synthesis in part. Moreover, the morality was 40%, which was associated with the mucositis, cardiotoxicity, or nephropathy,[17,18] and other serious toxicity. The liver index in DOX group was higher than that in NC group, which could be related to edema, degeneration, and lymphocytic infiltration of hepatic tissue being proved by the result of histopathological examination. Therefore, it is obviously revealed that DOX-induced hepatic injury was successfully established. After treating with fermented CS for 23 days, there was an improvement in liver histopathology, including the decrease of edema and infiltration of inflammatory cells, and the decreases of liver index and mortality. One of the important characteristics of Chinese materia medica is that sometimes its dose-effect relationship is irregular,

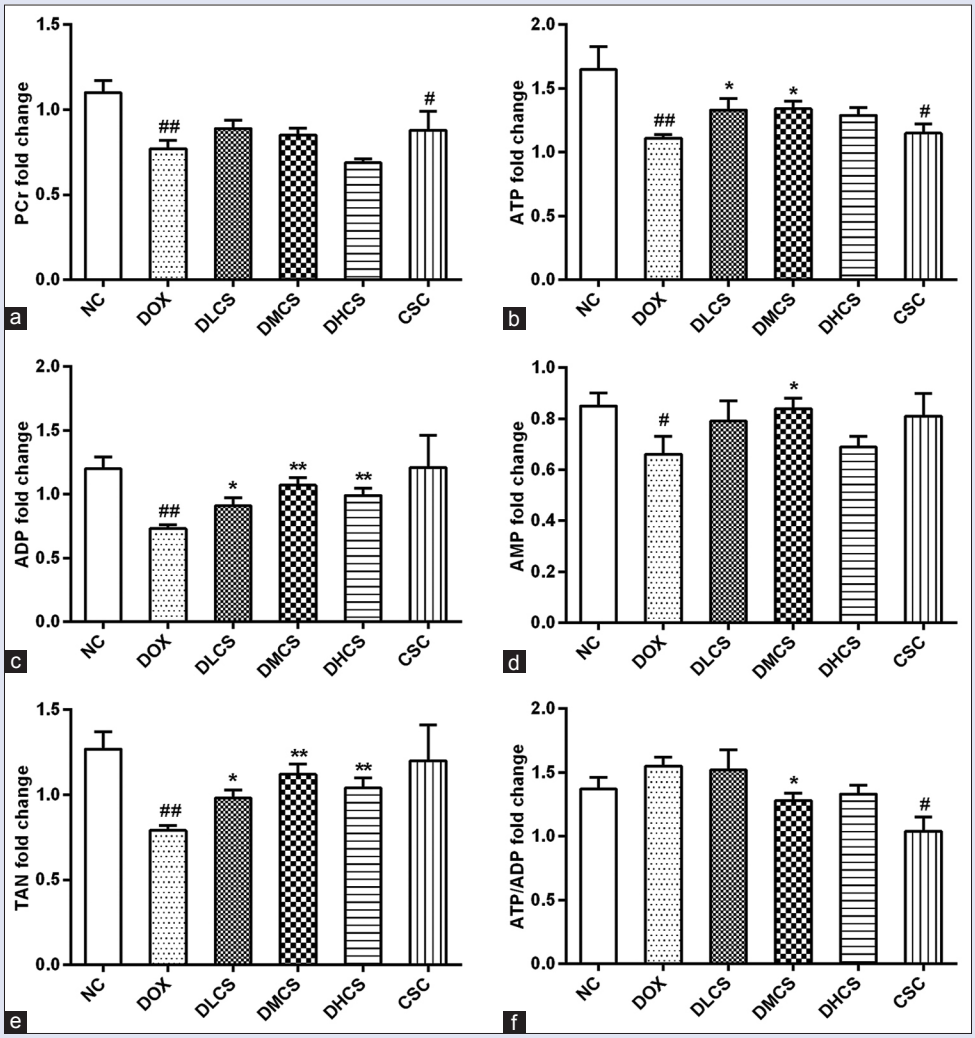

Figure 4: Effect of fermented *Cordyceps sinensis* on contents of phosphocreatine (a), adenosine triphosphate (b), adenosine diphosphate (c), adenosine monophosphate (d), total adenine nucleotides (e), and adenosine triphosphate/adenosine diphosphate ratio (f). Compared to normal control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to doxorubicin group, $^{*}P < 0.05$, $^{**}P < 0.01$. Data are presented as the mean ± standard error of the mean normal control group, $n = 10$. Cardiac stromal cell, *Cordyceps sinensis* (1.50 g/kg/d) control group $n = 5$. DOX, doxorubicin control group, $n = 9$. DHCS, doxorubicin + higher dose (3.00 g/kg/d) of *Cordyceps sinensis* group, $n = 12$. DMCS, doxorubicin + middle dose (1.50 g/kg/d) of *Cordyceps sinensis* group, $n = 14$. DLCS, doxorubicin + lower dose (0.75 g/kg/d) of *Cordyceps sinensis* group, $n = 8$. PCr, phosphocreatine. ATP, adenosine-triphosphate. ADP, adenosine diphosphate. AMP, adenosine monophosphate. TAN, total adenine nucleotides. The contents of phosphocreatine, adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate were expressed as fold of the normal control group

and sometimes even it has the opposite effect between small dose and large dose. The complexity of Chinese materia medica chemical and the different response on the target or system of different active ingredients is the main reason for the irregular in the dose-effect relationship relative. Within a certain range of the doses, the interaction effect between different active ingredients can be coordinated, and beyond this range will probably show the role of constrained. Interestingly, we found that the middle dose of fermented CS had a better effect than the high dose of fermented CS in mortality, which is consistent with reference[19] and in accordance with the characteristics of the irregularity of the Chinese materia medica.

Hepatic injury is always reflected in the abnormal levels of ALT, AST, TP, and ALB in serum. ALT and AST are abundant in liver tissues. When liver cells get damaged, such as inflammation[20] or poisoning,[21] AST and ALT contained in hepatocyte may be released into blood; therefore, the serum activities of AST and ALT are generally used to evaluate the damage of the liver. TP and ALB are mostly synthesized by the liver, whose contents in serum will decrease when liver is injured. Hence, the contents of TP, ALB can reflect the liver function. In this study, DOX induced the significant increase of serum AST activity and the significant decrease of the serum contents of TP and ALB. Those show the damage of hepatocytes and the weakening of liver function. Pretreatment with fermented CS effectively protected rats against DOX-induced hepatic damage, as evidenced by the decreased activities of serum AST and ALT and the increased contents of serum TP and ALB.

Hepatotoxicity induced by DOX is associated with altering hepatic mitochondrial function.[22] DOX treatment can impair mitochondrial respiration and production of adenine nucleotides.[23] The decomposition of ATP into ADP is the key way for providing energy. ADP and AMP transmute into ATP by fatty acid (FA) oxidation and carbohydrate oxidation when ATP is decreased. Hence, adenine nucleotides (ATP, ADP, and AMP) play an important role in hepatic energy metabolism.[24] In this study, DOX decreased significantly the contents of adenine nucleotides of the liver, including ATP, ADP, and AMP. This shows that rats with

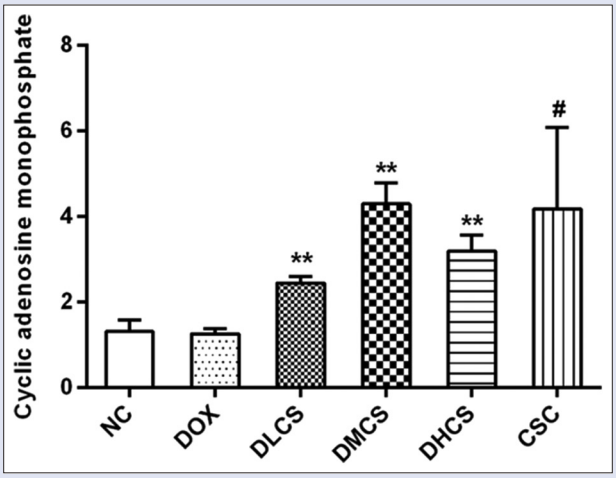

Figure 5: Effect of fermented *Cordyceps sinensis* on contents of cyclic adenosine monophosphate. Compared to normal control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to doxorubicin group, $^{*}P < 0.05$, $^{**}P < 0.01$. Data are presented as the mean ± standard error of the mean normal control group, $n = 10$. Cardiac stromal cell, *Cordyceps sinensis* (1.50 g/kg/d) control group $n = 5$. DOX, doxorubicin control group, $n = 9$. DHCS, doxorubicin + higher dose (3.00 g/kg/d) of *Cordyceps sinensis* group, $n = 12$. DMCS, doxorubicin + middle dose (1.50 g/kg/d) of *Cordyceps sinensis* group, $n = 14$. DLCS, doxorubicin + lower dose (0.75 g/kg/d) of *Cordyceps sinensis* group, $n = 8$. cAMP, cyclic adenosine monophosphate. The content of cyclic adenosine monophosphate was expressed as fold of the normal control group

DOX-treated hepatic injury suffer from hepatic energy impairment and fermented CS can attenuate this impairment, being revealed by the changed concentrations of ATP, ADP, AMP, and TAN. However, with the increase of dose, when fermentation CS dosage increased to more than clinical equivalent dose (middle dose), its role in the improvement of energy metabolism did not show a better effect than the middle dose fermented CS, and it may be related to the complexity of the active components of CS.

As an important predictor of the respiration rate of mitochondria, ATP/ADP ratio plays a key role in the evaluation of energy metabolism.[25] Some studies find that hepatic injury can lead a decrease of ATP/ADP ratio and the mechanism is associated with an increase of glucose utilization and FA utilization to makeup for the reduction of ATP.[26,27] On the other hand, some studies have also found that when the energy metabolism of the liver is injured, the ratio of ATP/ADP will rise, which may be related to the inhibition of respiratory rate and the reduction of glucose utilization.[25,28] In this study, DOX increased slightly ATP/ADP ratio but without significant and the pretreatment of fermented CS could regulate ATP/ADP ratio to the normal level. The result shows that fermented CS can rectify the DOX-induced abnormalities of hepatic adenine nucleotides.

PCr, the high-energy storage compound, participates in the ATP production and its depletion can promote the decrease of ATP. In this study, PCr content was decreased in DOX-treated rats. That shows the impairment of hepatic energy metabolism. Interestingly, fermented CS could slightly attenuate the decrease of PCr content induced by DOX, though the difference was not very significant. As mentioned above, it shows that fermented CS can attenuate the DOX-induced impairment of hepatic high-energy storage.

In CSC group, the concentrations of ATP and PCr and ATP/ADP ratio were decreased, but the levels of ADP, AMP, and TAN were not changed. Fermented CS contains carbohydrate, protein, amino acid, fat, moisture are some of these,[13] which can strengthen the body to enhance the overall function of the body after using a long time, including the enhancement of hepatic energy metabolism function. In this experiment, animals were stopped giving the medicine 24 h and fasted for 12 h before the end of the experiment. Compared to the normal rats, the liver energy metabolism function of rats in CSC group was stronger, which may lead to the decreased concentrations of ATP and PCr. Fermented CS did not show significant adverse effects on normal rats, in this study, and there are no reports on the toxicity of fermented CS in energy metabolism. Whether the effects of fermented CS on ATP and PCr to normal rats cause other adverse reactions, we will further attention in the later research.

As an intracellular second messenger, cAMP has been used as an important indicator of energy metabolism.[29] The imbalance of energy is usually accompanied by a decrease in cAMP concentration, and drug regulation of the energy metabolism is accompanied by an increase in the concentration of cAMP.[30] The increase of cAMP content can activate the cAMP signal pathway. Multiple processes are regulated by cAMP. With the production of cAMP, mitochondrial function can be enhanced to produce ATP.[31] In this study, the content of hepatic cAMP was increased significantly in CS-treated rats. Meanwhile, CS control rats also experienced a significant increase in hepatic cAMP content. The result shows that fermented CS can increase hepatic cAMP to upregulate the hepatic energy metabolism and ameliorate hepatic injury.

CONCLUSION

DOX induces hepatic injury accompanying hepatic energy metabolism disorders. Artificial fermented CS can attenuate DOX-caused hepatic toxicity by regulating energy metabolism, and the effect may be associated with the production of hepatic cAMP content to improve the hepatic energy metabolism and hepatic injury. Moreover, this study can be one indication for the further research on CS.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gibson NM, Greufe SE, Hydock DS, Hayward R. Doxorubicin-induced vascular dysfunction and its attenuation by exercise preconditioning. J Cardiovasc Pharmacol 2013;62:355-60.
2. Nagai K, Oda A, Konishi H. Theanine prevents doxorubicin-induced acute hepatotoxicity by reducing intrinsic apoptotic response. Food Chem Toxicol 2015;78:147-52.
3. Nagai K, Fukuno S, Oda A, Konishi H. Protective effects of taurine on doxorubicin-induced acute hepatotoxicity through suppression of oxidative stress and apoptotic responses. Anticancer Drugs 2016;27:17-23.
4. Mete R, Oran M, Topcu B, Oznur M, Seber ES, Gedikbasi A, *et al.* Protective effects of onion (*Allium cepa*) extract against doxorubicin-induced hepatotoxicity in rats. Toxicol Ind Health 2016;32:551-7.
5. Yen HC, Oberley TD, Vichitbandha S, Ho YS, St. Clair DK. The protective role of manganese superoxide dismutase against adriamycin-induced acute cardiac toxicity in transgenic mice. J Clin Invest 1996;98:1253-60.
6. Parker MA, King V, Howard KP. Nuclear magnetic resonance study of doxorubicin binding to cardiolipin containing magnetically oriented phospholipid bilayers. Biochim Biophys Acta 2001;1514:206-16.
7. Syed M, Skonberg C, Hansen SH. Mitochondrial toxicity of diclofenac and its metabolites via inhibition of oxidative phosphorylation (ATP synthesis) in rat liver mitochondria: Possible role in drug induced liver injury (DILI). Toxicol *In Vitro* 2016;31:93-102.

8. de Medeiros HC, Constantin J, Ishii-Iwamoto EL, Mingatto FE. Effect of fipronil on energy metabolism in the perfused rat liver. Toxicol Lett 2015;236:34-42.
9. Liu X, Zhong F, Tang XL, Lian FL, Zhou Q, Guo SM, *et al. Cordyceps sinensis* protects against liver and heart injuries in a rat model of chronic kidney disease: A metabolomic analysis. Acta Pharmacol Sin 2014;35:697-706.
10. Wang XB, Liu P, Tang ZP. Intervening and therapeutic effect of cordyceps mycelia extract on liver cirrhosis induced by dimethylnitrosamine in rats. Zhongguo Zhong Xi Yi Jie He Za Zhi 2008;28:617-22.
11. Wu R, Gao JP, Wang HL, Gao Y, Wu Q, Cui XH, *et al.* Effects of fermented *Cordyceps sinensis* on oxidative stress in doxorubicin treated rats. Pharmacogn Mag 2015;11:724-31.
12. National Pharmacopoeia Committee. Pharmacopoeia of the People's Republic of China. Beijing: China Medical Science Press; 2015. p. 832-3.
13. Hsu TH, Shiao LH, Hsieh C, Chang DM. A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom DongChongXiaCao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. Food Chem 2002;78:463.
14. Vagnozzi R, Marmarou A, Tavazzi B, Signoretti S, Di Pierro D, del Bolgia F, *et al.* Changes of cerebral energy metabolism and lipid peroxidation in rats leading to mitochondrial dysfunction after diffuse brain injury. J Neurotrauma 1999;16:903-13.
15. Chakravarty G, Mathur A, Mallade P, Gerlach S, Willis J, Datta A, *et al.* Nelfinavir targets multiple drug resistance mechanisms to increase the efficacy of doxorubicin in MCF-7/Dox breast cancer cells. Biochimie 2016;124:53-64.
16. Kumral A, Giriş M, Soluk-Tekkeşin M, Olgaç V, Doğru-Abbasoğlu S, Türkoğlu Ü, *et al.* Beneficial effects of carnosine and carnosine plus Vitamin E treatments on doxorubicin-induced oxidative stress and cardiac, hepatic, and renal toxicity in rats. Hum Exp Toxicol 2016;35:635-43.
17. Wat E, Ng CF, Wong EC, Koon CM, Lau CP, Cheung DW, *et al.* The hepatoprotective effect of the combination use of fructus schisandrae with statin – A preclinical evaluation. J Ethnopharmacol 2016;178:104-14.
18. Alishahi A, Roshan VD, Hedayyati M. Pretreatment effects of regular aerobic training on the IGF system and hepatotoxicity induced by doxorubicin in rats. Asian Pac J Cancer Prev 2013;14:7427-31.
19. Liu Z, Li P, Zhao D, Tang H, Guo J. Protective effect of extract of *Cordyceps sinensis* in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. Behav Brain Funct 2010;6:61.
20. Kubes P, Mehal WZ. Sterile inflammation in the liver. Gastroenterology 2012;143:1158-72.
21. Jeon GJ, Park JH, Kim MS, Yu JW, Park JH, Kim MS, *et al.* A case of lead poisoning with drug-induced liver injury after ingestion of herbal medicine. Korean J Gastroenterol 2015;65:375-8.
22. Dirks-Naylor AJ, Kouzi SA, Bero JD, Phan DT, Taylor HN, Whitt SD, *et al.* Doxorubicin alters the mitochondrial dynamics machinery and mitophagy in the liver of treated animals. Fundam Clin Pharmacol 2014;28:633-42.
23. Wided K, Hassiba R, Mesbah L. Polyphenolic fraction of algerian propolis reverses doxorubicin induced oxidative stress in liver cells and mitochondria. Pak J Pharm Sci 2014;27:1891-7.
24. Satoh S, Tanaka A, Hatano E, Inomoto T, Iwata S, Kitai T, *et al.* Energy metabolism and regeneration in transgenic mouse liver expressing creatine kinase after major hepatectomy. Gastroenterology 1996;110:1166-74.
25. Kunz W, Bohnensack R, Böhme G, Küster U, Letko G, Schönfeld P, *et al.* Relations between extramitochondrial and intramitochondrial adenine nucleotide systems. Arch Biochem Biophys 1981;209:219-29.
26. de Souza CO, Kurauti MA, Silva Fde F, de Morais H, Curi R, Hirabara SM, *et al.* Celecoxib and ibuprofen restore the ATP content and the gluconeogenesis activity in the liver of walker-256 tumor-bearing rats. Cell Physiol Biochem 2015;36:1659-69.
27. Nishikawa T, Bellance N, Damm A, Bing H, Zhu Z, Handa K, *et al.* A switch in the source of ATP production and a loss in capacity to perform glycolysis are hallmarks of hepatocyte failure in advance liver disease. J Hepatol 2014;60:1203-11.
28. Kato T, Niizuma S, Inuzuka Y, Kawashima T, Okuda J, Tamaki Y, *et al.* Analysis of metabolic remodeling in compensated left ventricular hypertrophy and heart failure. Circ Heart Fail 2010;3:420-30.
29. Huynh TT, McDougald D, Klebensberger J, Al Qarni B, Barraud N, Rice SA, *et al.* Glucose starvation-induced dispersal of pseudomonas aeruginosa biofilms is cAMP and energy dependent. PLoS One 2012;7:e42874.
30. Magata S, Taniguchi M, Suzuki T, Shimamura T, Fukai M, Furukawa H, *et al.* The effect of antagonism of adenosine A1 receptor against ischemia and reperfusion injury of the liver. J Surg Res 2007;139:7-14.
31. Wang Z, Liu D, Varin A, Nicolas V, Courilleau D, Mateo P, *et al.* A cardiac mitochondrial cAMP signaling pathway regulates calcium accumulation, permeability transition and cell death. Cell Death Dis 2016;7:e2198.