Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(5):130-135

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Hepatoprotective effects of *Coleus forskohlii* (wild.) Briq. against carbon tetrachloride-induced hepatotoxicity in mice

Di Geng[#], Cong Li[#], Li-tao Yi, Lian-jin Weng[*] and Yuan-yuan Han[**]

Department of Chemical and Pharmaceutical Engineering, College of Chemical Engineering, Huaqiao University, Xiamen, Fujian province, PR China

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ABSTRACT

Our present study investigated the hepatoprotective effect of ethanol extract of Coleus forskohlii (wild.) Briq. (CFBE) in mice with CCl_4-induced liver damage. Seven days administration with CFBE decreased the serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL) and the indexes of organs, also along with the remission of hepatocellular degeneration/necrosis and inflammatory cell infiltration in histopathological examinations. Our results suggested that Coleus forskohlii (wild.) Briq. and its extract may have a potent hepatoprotective effects so as to be a hepatoprotective agent.

Keywords: *Coleus forskohlii*; Hepatoprotection; Carbon tetrachloride; Histopathology

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INTRODUCTION

Coleus forskohlii (Wild.) Briq. (Lamiaceae) is one of the most important species of the Coleus Herit genus [1] and found or cultivated throughout India, Ceylon and Moluccas [2]. According to previous researches its metabolic pattern is heavily characterized by a series of complex diterpenes with 8, 13-epoxy-labd-14-en-11-one skeleton [3]. It showed several pharmacological properties, such as abortifacient, contraceptive [4], and antipain property [5]. The plant was also used to treat gastritis and intestinal spasms [6], nausea [7], stomach ache, and as a purgative [8]. The roots of *C. forskohlii* are the rich sources of both enzymatic and non-enzymatic antioxidants besides their medicinal properties [9]. Previous studies showed that aqueous extract was able to partially inhibit the increase of wet weight, fat content and cholesterol level in liver [10]. However, the physiological effects of *C. forskohlii* aganist chemically induced liver damage have not been reported. Besides chemical medicine, herbal therapy is another effective alternative to treat liver injuries. Therefore, the study was aimed at evaluating the protective effects of *C. forskohlii* on CCl_4-induced hepatotoxicity.

EXPERIMENTAL SECTION

2.1. Animals

Male Kunming mice, weighing 20 ± 2 g, were purchased from Laboratory Animal Centre, Fujian Medical University (Fujian, China). Animals were housed 8 per cage (320 × 180 × 160 cm) under a normal 12-h/12-h light/dark schedule. Ambient temperature and relative humidity were maintained at 22 ± 2 °C and at 55 ± 5 %, and given a standard pellet diet and water *ad libitum*. The animals were allowed 1 week to acclimatize to the laboratory conditions prior to the experiments. All procedures were performed in accordance with the published guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals, approved by the State Council on October 31, 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on November 14, 1988).

2.2. Preparation of Extracts

The whole plants of *C. forskohlii* collected in Yunnan (China) were authenticated by Prof. Xuehua Song, China Pharmaceutical University (Nanjing, China). The Voucher specimen (NO. 11-101707) was deposited in the Department of Natural Chemistry, China Pharmaceutical University (Nanjing, China).

The air-dried herbs were pulverized, and the powder was extracted with ethanol three times for 2h under reflux followed by vacuum distillation at 60 °C. After that, the extract was freeze dried and stored in a vacuum desecrator. Prior to experiment, CFBE and SL were dissolved in 0.5% sodium carboxymethylcellulose (CMC).

2.3. Chemicals and reagents

CCl_4 was purchased from Merck (Shanghai, China). Silymarin was purchased from Shanghai Crystal Pure Co., Ltd (Shanghai, China). Olive oil, picric acid and CMC are from Shanghai Chemical Reagent Co., Ltd (Shanghai, China). Hematoxylin and eosin (H&E) was purchased from Sigma-Aldrich Co., Ltd (St. Louis, USA).

2.4. Acute oral toxicity study

Two groups of mice were respectively dosed with CFBE at 5000 mg/kg body weight [11] and 0.5% CMC. Then, mice were observed for any symptoms of toxicity for two weeks as per guideline OECD-425 (2001).

2.5. Experimental design

The control group (group 1) and the model group (group 2) were given 0.5% CMC once daily for 7 days, intragastrically (i.g.) and group 3 was administered by SL. CFBE was given i.g. at dose of 100, 200 and 500 mg/kg to group4 to group 6. On the third and fifth days, the group 1 was administered by olive oil, intraperitoneally (i.p.) after 30 min of i.g. and CCl_4 was dissolved in olive oil (1%, v/v),which was given i.p. to the rest groups after 30 min of i.g.

2.6. Autopsy and serum biochemistry

Following CFBE treatment for seven days, food, but not water was withdrawn from the animals 24 h prior to decapitation. Blood was collected and separated by centrifuge at 10, 000 × *g* at 4 °C for 10 min. Serum was stored at 4 °C until assays. The serum AST, ALT, ALP, TBIL, DBIL and IBIL were determined on an auto-analyzer (Beckmann, America).

2.7. Histopathology

After the experimental period animals were decapitated, livers and spleens removed immediately, washed in normal saline, blotted with filter papers, weighed, sliced and examined. The liver and spleen indices were calculated as the weight of per 10 g body weight. Liver and spleen pieces were preserved in 10% formalin for 24 h and embedded in paraffin wax. Sections were taken and stained with H&E and photographed [12].

2.8. Statistical analysis

All data were expressed as mean ± S.E.M. We used one-way analysis of variance (ANOVA), followed by *post-hoc* Dunnett's test using the SPSS software (SPSS Inc., Chicago, USA) 20.0. A value of $p < 0.05$ was considered statistically significant for analysis.

RESULTS AND DISCUSSION

There was no mortality in the mice administered with *C. forskohlii* ethanol extract (CFBE) at 5000 mg/kg. Physically, they appeared normal and no signs of changes were observed. In addition, administration with CFBE did not affect the added weight between pre-experiment and after treatment (Figure 1).

Compared with the normal group, CCl_4-induced group significantly increased in the organ indexes and serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), indirect bilirubin (IBIL), direct bilirubin (DBIL), total bilirubin (TBIL) levels ($p < 0.01$) (Table 1 & Figure 2).

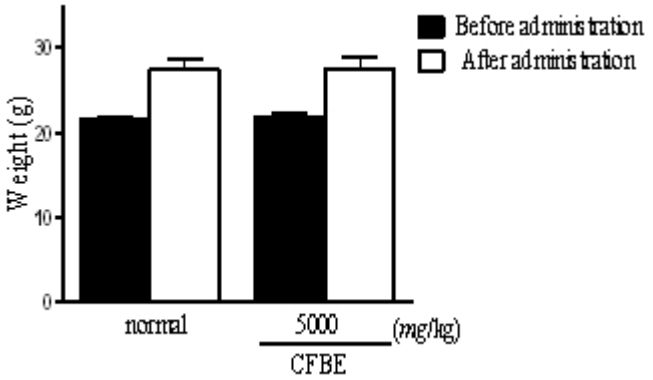


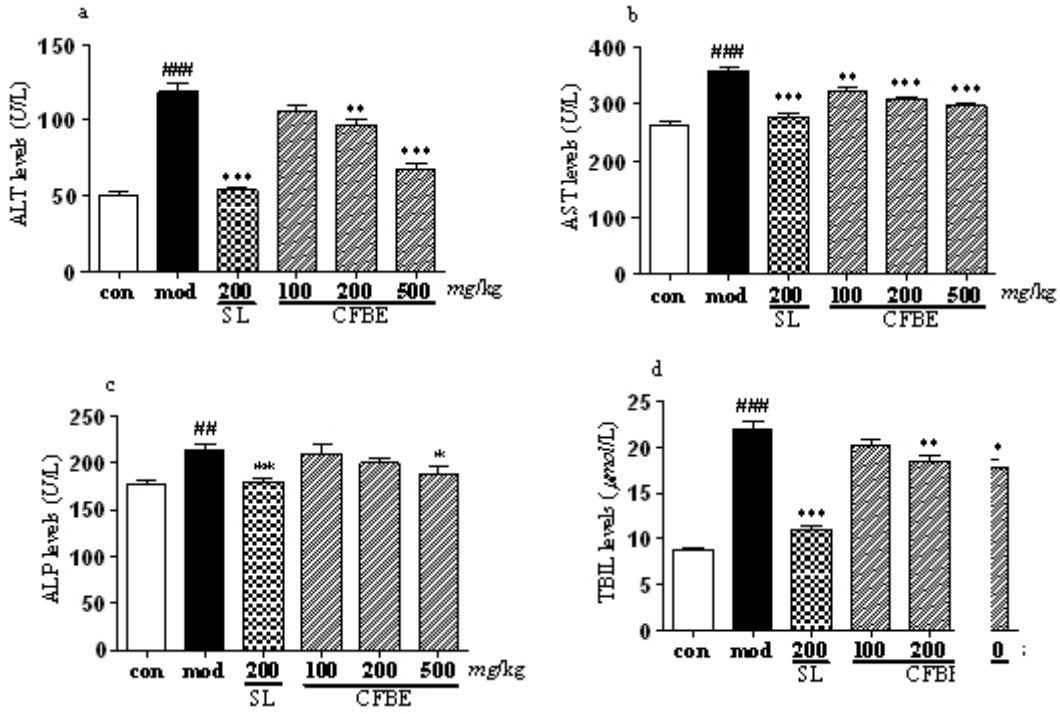
Figure 1. Weights of mice before and after administration with CFBE

Table 1. Livers and spleens indexes

Groups	Body wt. (g)	Liver index (g/10 g body wt.)	Spleen index (g/10 g body wt.)
Normal	28.8 ± 1.24	0.390 ± 0.036	0.024 ± 0.005
Model	26.5 ± 1.98	0.481 ± 0.023$^{\#}$	0.033 ± 0.007$^{\#}$
Silymarin/(200 mg/kg)	28.0 ± 1.32	0.403 ± 0.022*	0.026 ± 0.004*
CFBE/(100 mg/kg)	26.8 ± 1.42	0.467 ± 0.041	0.034 ± 0.002
CFBE/(200 mg/kg)	27.1 ± 1.56	0.455 ± 0.034	0.033 ± 0.005
CFBE/(500 mg/kg)	27.5 ± 1.56	0.412 ± 0.024*	0.027 ± 0.007*

Note: mean ± s, n=8. $^{\#}p < 0.05$ vs with normal group, $^{*}p < 0.05$ vs model group

Mice treated with 200 mg/kg body weight of Silymarin (SL) showed a significant restoration of all serum parameters as compared with the CCl_4-indueced group ($p < 0.01$). The mice administrated with the CFBE extract at dose of 500mg/kg almost produced similar activities to those with SL (Figure 2). However, the mice administrated with SL and the CFBE (500 mg/kg) lost in the liver and spleen indexes (Table 1).



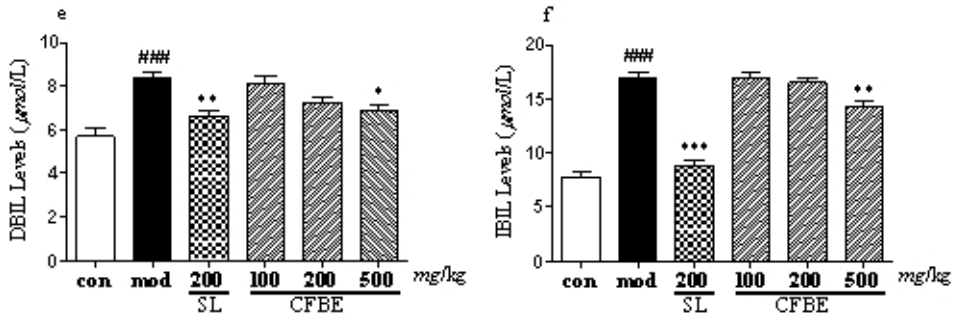


Figure 2. Effects of CFBE on the serum ALT[(a)], AST[(b)], ALP[(c)], TBIL [(d)], DBIL[(e)] and IBIL[(f)]
$^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs vehicle-control group (Control)； $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs vehicle with CCl_4 group (Model).

In addition, the histological pattern of the livers in mice which were treated with CFBE (500 mg/kg) exhibited regeneration of hepatocytes, normalization of inflammatory hepatic and necrosis (Figure 3F). While, CCl_4-induced mice characterized by the loss of cellular boundaries and nuclear dissolution in comparison with normal mice (Figure 3A & Figure 3B).

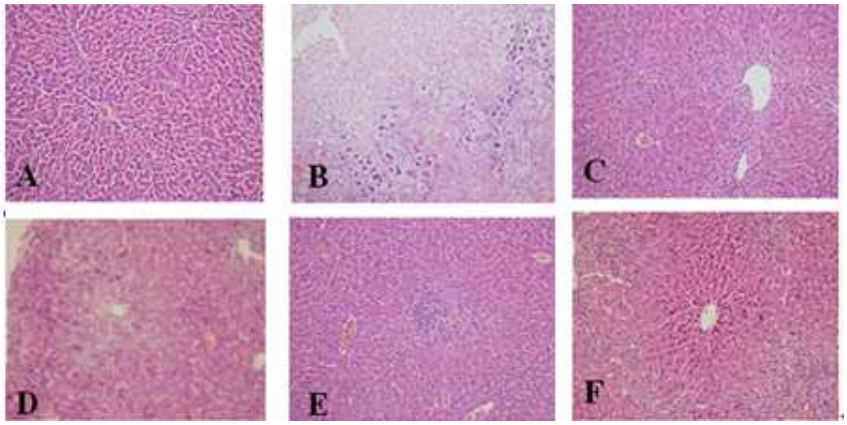


Figure 3. Effects of CFBE on hepatic morphological analysis (× 200 H&E): control group (A), CCl_4-model group (B), Silymarin + CCl_4 group (C), CFBE 100 mg/kg + CCl_4 group (D), CFBE 200 mg/kg + CCl_4 group (E), CFBE 500 mg/kg + CCl_4 group (F), (* inflammatory infiltration; # dilated sinusoidal spaces)

Herbal products have long been used in traditional folk medicine to maintain health or as remedies for various human diseases [13]. There are numerous natural products, plants and polyherbal formulations claimed to have hepatoactivity. Previous study found that the leaves of *Plectranthus amboinicus* (Lour) Spreng, another herb of family Lamiaceae, possessed hepatoprotective activities in rats [14]. However, the effects of *C. forskohlii* in the context of chemically induced liver injury have not been reported. Our study investigated that the hepatoprotective of ethonal extract of *C. forskohlii* (CFBE) on CCl_4-induced acute hepatic injury in mice.

Liver plays a major role in protecting and detoxifying the body from foreign substances [15]. Liver injury is a major disease associated with various pathological processes including progressive fibrosis, portal hypertension and carcinoma [16]. Free radical generation, mitochondrial dysfunction and depletion of antioxidants lead to the progression of fibrosis and cirrhosis [17].

Carbon tetrachloride (CCl_4) is a hepatotoxic agent, which induces hepatotoxicity by metabolic activation. Therefore, it selectively causes toxicity in liver cells, maintaining semi-normal metabolic functions [18]. CCl_4 is metabolically activated by the cytochrome P_{450}-dependent mixed oxidase in the endoplasmic reticulum to form a trichloromethyl free radical ($CCl_3^{\bullet}$), which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation. These result in lossing of metabolic enzyme activation, reduction of protein synthesis and lossing of glucose-6 phosphatase activation, finally lead to liver damage (degeneration, necrosis and fibrosis of liver cell) [19].

In our study, the administration of CCl_4 to mice caused visual and quantifiable responses, which were recognizable by the alterations in the body, liver, spleen weights, histopathology, and levels of serum molecular markers. All these indications in line with the previous reports that CCl_4 contributes to the development of liver injury via multiple mechanisms [18] such as the oxidation of its metabolic products, oxidative stress, decreased antioxidant defenses and lipid peroxidation [20,21]. Compared with the normal group the CCl_4-induced mice lacked in gaining body

weights (Table 1), which may be given reasons to the lower levels of nutrient absorption, energy utilization, and metabolic efficiency [18]. The hepatocyte proliferation is a critical determinant for the survival of liver from an injury [22]. Based on this, the upregulation of the hepatocyte activity in response to the CCl_4-induced toxicity is likely to be the recorded increase in the liver, spleen weights and serum markers.

According to the literature [23], CCl_4 treatment causes the increase of serum enzymatic levels. In the hepatotoxic mice, we detected elevated levels of serum ALT, AST, ALP, DBIL, IBIL, and TBIL (Figure 2), which were typically measured for assessing the liver injuries. The results were in line with the earlier reports. The increase in serum enzymatic activities is related to hepatic parenchymal damage since ALT is released from mitochondrial and cytosolic localization, which are from membranal sites. And cellular rupture allows the enzyme to escape into the blood [24]. The increase of ALT, AST, and ALP levels in intoxicated mice indicated hepatocytes degeneration/necrosis, a possible cholestasis and the damage in the integrity of the hepatocytes [25].

There was no mortality in the mice administered with CFBE at 5000 mg/kg. Physically, behavior patterns were similar to the normal group. These findings provided sufficient evidence to conclude that the orally administered CFBE were safe and did not cause any extract-related toxicity. In acute toxicity, LD_{50} was estimated to be > 5000 mg/kg. Hence, $1/10^{th}$ of the LD_{50}, i.e. 500 mg/kg was selected for the study.

Pretreatment with CFBE (500 mg/kg) in mice has been proven to be an efficient strategy against liver injuries induced by CCl_4. CFBE can significantly decrease the serum ALT, AST, ALP, DBIL, IBIL, and TBIL levels and alleviate hepatocellular degeneration/necrosis, inflammatory cell infiltration, congestion, and sinusoidal dilatation. The biochemical parameters levels of serum and histopathological analysis indicated that CFBE has stabilized the hepatocytes membranes and interrupted the release of enzymes from liver into blood. The lowered bilirubin levels supported this action since it implied more stable erythrocyte plasma membranes. These findings are consistent with those of Roshan Patel [14] who found that aqueous and methanolic extracts of *P. amboinicus* inhibited membrane lipid peroxidation, increased levels of liver enzymes and decreased hepatocellular necrosis as well as inflammatory cell infiltration in mice.

CONCLUSION

In short, the results of this study demonstrated that CFBE can effectively prevent the CCl_4-induced hepatic damage in mice and therefore it may be potential developed as an hepatoprotective agent. This is the primary report about the hepatoprotective of CFBE, further studies with individual active compounds existed in *C. forskohlii* are in proceeding, which will enable us to understand the exact mechanism of hepatoprotective action by CFBE.

Acknowledgements
The project was supported by the Natural Science foundation of China (NO. 81202940) and the Fundamental Research Funds for the Central Universities of Huaqiao University of China (NOs. JB-ZR1152 and JB-ZR1226).

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