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

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**ABSTRACT**

Our present study investigated the hepatoprotective effect of ethanol extract of *Coleus forskohlii* (wild.) Briq. (CFBE) in mice with CCl₄-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

**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 CCl₄-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 desiccator. Prior to experiment, CFBE and SL were dissolved in 0.5% sodium carboxymethylcellulose (CMC).

2.3. Chemicals and reagents
CCl₄ 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 CCl₄ 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, CCl₄-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).
Table 1. Livers and spleens indexes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body wt. (g)</th>
<th>Liver index (g/10 g body wt.)</th>
<th>Spleen index (g/10 g body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>28.8 ± 1.24</td>
<td>0.390 ± 0.036</td>
<td>0.024 ± 0.005</td>
</tr>
<tr>
<td>Model</td>
<td>26.5 ± 1.98</td>
<td>0.481 ± 0.023*</td>
<td>0.033 ± 0.007*</td>
</tr>
<tr>
<td>Silymarin/(200 mg/kg)</td>
<td>28.0 ± 1.32</td>
<td>0.403 ± 0.022*</td>
<td>0.026 ± 0.004*</td>
</tr>
<tr>
<td>CFBE/(100 mg/kg)</td>
<td>26.8 ± 1.42</td>
<td>0.467 ± 0.041</td>
<td>0.034 ± 0.002</td>
</tr>
<tr>
<td>CFBE/(200 mg/kg)</td>
<td>27.1 ± 1.56</td>
<td>0.455 ± 0.034</td>
<td>0.033 ± 0.005</td>
</tr>
<tr>
<td>CFBE/(500 mg/kg)</td>
<td>27.5 ± 1.56</td>
<td>0.412 ± 0.024*</td>
<td>0.027 ± 0.007*</td>
</tr>
</tbody>
</table>

Note: mean ± s, n=8. *p < 0.05 vs 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 CCl₄-induced 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).
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, CCl₄-induced mice characterized by the loss of cellular boundaries and nuclear dissolution in comparison with normal mice (Figure 3A & Figure 3B).

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 hepatopoactivity. 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 CCl₄-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 (CCl₄) is a hepatotoxic agent, which induces hepatotoxicity by metabolic activation. Therefore, it selectively causes toxicity in liver cells, maintaining semi-normal metabolic functions [18]. CCl₄ is metabolically activated by the cytochrome P₄₅₀-dependent mixed oxidase in the endoplasmic reticulum to form a trichloromethyl free radical (CCl₃•), 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 CCl₄ 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 CCl₄ 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 CCl₄-induced mice lacked in gaining body
In short, the results of this study demonstrated that CFBE can effectively prevent the CCl4-induced toxicity is likely to be the recorded increase in the liver, spleen weights and serum markers.

According to the literature [23], CCl4 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, LD50 was estimated to be > 5000 mg/kg. Hence, 1/10th of the LD50, 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 CCl4. 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 CCl4-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).

REFERENCES

[12] O Coskun; B Yakan; E Oztas; A Sezen. Turkish Journal of Medical Sciences, 2000, 30(2), 27–29.