HEPATOPROTECTIVE EFFECT OF FORSKOLIN IN CARBON TETRACHLORIDE-INDUCED MODEL OF ACUTE LIVER INJURY

BY

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ABSTRACT

Liver disease is one of the most common causes of death in the world. Nowadays, research studies have been focused on the development of new drugs for treatment of liver damage. Some natural products from medicinal plants have been found as potent agents for protection against liver injury induced by chemicals. Thus, it is interesting to find more effective natural products for protection against liver injury. Accordingly, the present study was designed to assess the hepatoprotective potential of a diterpenoid forskolin, isolated from the Indian plant Coleus forskohlii, at different doses in rat model with acute liver injury induced by carbon tetrachloride (CCl_4) at a dose of 1ml/kg intraperitoneally as a mixture with corn oil. Forkolin was administered in doses of 5,10,20,40 mg/kg intraperitoneally for 7 days. Its protective effect was assessed via liver function tests and histopathological liver sections. Significant reduction in the hepatic enzymes levels was found in animals treated with forskolin at a dose of 10mg/kg as well as restoration of hepatocellular architecture. Therefore, treatment with forskolin showed preventive effect against CCl_4-induced liver damage.

Keywords: Forskolin; Carbon tetrachloride; Hepatoprotective; Rats.

INTRODUCTION

Liver disease is a serious health problem throughout the world. A special interest in lowering liver damage arises since liver is a vital organ that plays a pivotal role in metabolism and detoxification of various endogenous and exogenous harmful substances **(Yang et al., 2010)**. Many hepatotoxicants are known to cause liver injury in humans, such as viruses, fungal products, bacterial metabolites, minerals, environmental pollutants and chemotherapeutic agents **(Wang et al., 2008)**. Despite new advances in hepatology, there is a lack of effective therapeutic strategies or specific medicines for the protection against hepatic disorders **(Wu et al., 2007)**. Therefore, herbal medicines that possess hepatoprotective effects have attracted the attention of many researchers in recent years **(Hermenean et al., 2012; Kang et al., 2013; Tipoe et al., 2010; Zhang et al., 2013)**.

Carbon tetrachloride (CCl_4), a potent hepatotoxin, is widely used as a chemical inducer of experimental liver injury **(Domitrovic and Jakovac, 2010)**. CCl_4 metabolism begins with the highly reactive trichloromethyl free radicals ($CCl_3\cdot$) by the action of the liver reduced nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P450 enzyme system **(McCay et al., 1984)**. These free radicals are believed to cause lipid peroxidation, and the breakdown of cellular membranes **(Manibusan et al., 2007)**.

Forskolin, a labdane diterpene is the main active ingredient in the ayurvedic herb Coleus forskohlii (Labatiae) that has been used in India since ancient times. The root portion of the plant has been traditionally used for medicinal purposes and contains the active constituent, forskolin. Historically, it has been used to treat hypertension, congestive heart failure, eczema, colic, respiratory disorders, painful urination, insomnia, and convulsions. Clinical studies have justified these traditional uses and indicate its therapeutic potential in asthma, angina, glaucoma, psoriasis, and prevention of cancer metastases **(Patel, 2010; Wagh et al., 2012)**. These properties encouraged us to investigate its hepatoprotective effect in a CCl_4-induced acute hepatotoxicity model.

MATERIALS AND METHODS

Materials

Forskolin was purchased from LC Laboratories (Woburn, MA, USA). It was dissolved in a mixture of dimethyl sulfoxide (DMSO) and saline. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals and experimental protocol

The studies were conducted in accordance with ethical guidelines of Ain Shams University, Egypt. Male albino rats weighing 150-250 g rats were used in this study. They were supplied by Nile Co. for Pharmaceutical and Chemical industries, Egypt and housed in an air-conditioned atmosphere at 22±2°C, under a 12 h light–dark cycle and provided with rodent chow and water ad libitum.

Forty-eight animals were randomly divided into six groups of eight rats each. Group I served as normal control, receiving vehicles only for 7 days. Groups II-V were given forskolin (5, 10, 20 and 40 mg/kg IP, respectively) dissolved in a DMSO/saline solution for 7 days and on the 7th day; after one hour of forskolin injection, a single dose of CCl_4 (1ml/kg,IP,1:1 mixture with corn oil) was given. Group VI served as

disease group, receiving vehicles for 7 days and on the 7th day; a single dose of CCl_4 (1ml/kg, IP, 1:1 mixture with corn oil) was given. 24 h after CCl_4 injection, Blood samples were collected from the retro-orbital plexus and allowed to clot. Serum was separated by centrifugation at 3000 rpm for 10 min, then stored at -20°C. Rats were sacrificed and liver tissues were dissected, washed and placed in 10% formalin for histopathological examination.

Assessment of liver function tests

The levels of ALT and AST were measured following the commercial kit's instructions (Spectrum diagnostics, Egypt).

Histopathological examination

For light microscopy, liver specimens were fixed in 10% formalin and embedded in paraffin. Sections of 4 um thickness were cut, stained with hematoxylin and eosin and subjected to photomicroscopic examination.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer as a post hoc test. The 0.05 level of probability was used as the criterion for significance. All statistical analyses were performed using Instat software package (version 3.06).

RESULTS

Effect of Forskolin on ALT and AST in serum

Compared to the control group, CCl_4 induced a significant increase in serum ALT and AST levels reaching 160 and 21.9 fold respectively. Concurrent administration of 5, 20 and 40 mg/kg Forskolin with CCl_4 did not show any significantly decrease in ALT and AST levels as compared to CCl_4 group. However, the 10 mg/kg dose of forskolin showed a significant decrease in ALT and AST reaching 18.6 % and 25.2 % respectively as compared to the CCl_4 group (Table 1).

Table 1. Effect of different doses of Forskolin on serum ALT and AST levels.

Groups	ALT (U/L)	AST (U/L)
Control	8.56 ± 1.55^{b}	58.63 ± 3.02^{b}
CCl_4	1372.8 ± 256.35^{a}	1282.08 ± 115.42^{a}
CCl_4+Forskolin (5mg/kg)	1250.67 ± 319.91^{a}	870.2 ± 213.25^{a}
CCl_4+Forskolin(10mg/kg)	254.67 ± 37.166^{b}	323.6 ± 89.46^{b}
CCl_4+Forskolin (20mg/kg)	1763.33 ± 157.38^{a}	1405.25 ± 391.88^{a}
CCl_4+Forskolin (40mg/kg)	936 ± 173.94^{a}	1033.17 ± 165.05^{a}

*** Data are the mean ± SD (n=6). a or b: Significantly different from control or CCl_4 group, respectively at $P < 0.001$ using ANOVA followed by Tukey-Kramer as a post-hoc test.**

Histopathological findings

Liver sections from control group stained with H&E showed normal hepatic architecture **(Figure 1A)**. CCl_4 group showed centrilobular necrosis with ballooning degeneration in the hepatocytes associated with dilatation in the central vein **(Figure 1B)**. Pretreatment with 5mg/kg, 20mg/kg and 40mg/kg Forskolin failed to reduce the extensive hepatocellular damage caused by CCl_4. Liver sections from those 3 groups showed centrilobular necrosis with ballooning degeneration in diffuse manner all over the hepatocytes in association with congestion in the portal vein **(Figure 1 C,E,F)**. However, pretreatment with 10mg/kg Forskolin dose significantly reduced these alterations as liver sections only showed ballooning degeneration in some of the hepatocytes with dilatation in the central vein **(Figure 1 D)**. A histopathologist graded these histopathological findings **(Table 2)**.

Table 2. Histopathological grading.

Groups	Histopathological Alteration		
	Centrilobular necrosis	**Ballooning degeneration**	**Dilatation of central vein**
Control	-	-	-
CCl_4	+++	++	++
CCl_4+Forskolin (5 mg/kg)	++	++	+
CCl_4+Forskolin (10 mg/kg)	-	++	++
CCl_4+Forskolin (20 mg/kg)	+++	++	+
CCl_4+Forskolin (40 mg/kg)	+++	+++	++

+++ (Severe), ++ (Moderate), + (Mild), - (Nil)

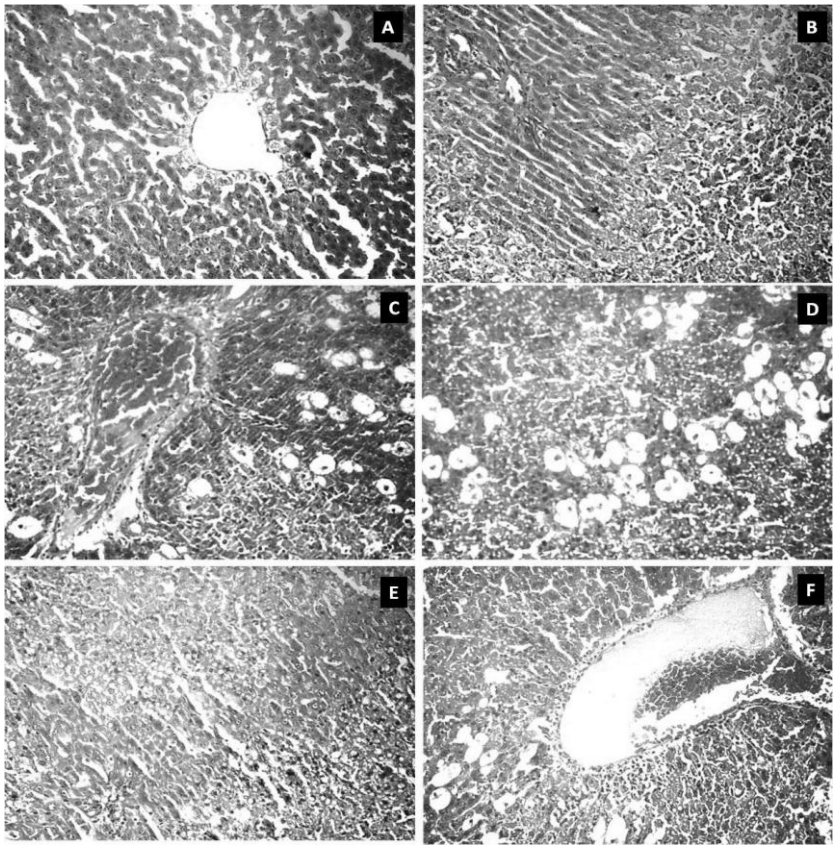

Figure 1. Histopathological analysis of rat liver sections using H&E (x40):

A: Control group. B: CCl_4 group. C: Group pretreated with 5 mg/kg Forskolin. D: Group pretreated with 10 mg/kg Forskolin. E: Group pretreated with 20 mg/kg Forskolin. F: Group pretreated with 40 mg/kg Forskolin.

DISCUSSION

Acute and chronic liver diseases constitute a global concern, and it is mostly induced by viral hepatitis, alcoholism, iron overload or drug toxicity. Among these types of liver injuries, there is consistent evidence of enhanced production of free radicals and/or a significant decrease in antioxidant defense mechanisms **(Hoek and Pastorino, 2002)**. Many drugs have been used in the treatment of liver damage. However, the medical treatments for acute and chronic liver diseases are often difficult to handle and have limited efficacy. Thus, there is a need to explore new medicines with high efficacy for the protection against liver diseases.

CCl_4 has been widely used for experimental induction of hepatic fibrosis in rats **(Hernandez-Munoz et al., 1990; Pierce et al., 1987)**. CCl_4 undergo metabolism by cytochrome P450 2E1 leading to production of free radicals which cause lipid peroxidation of the hepatocellular membrane, followed by inflammatory cytokines release and, eventually, hepatocellular damage **(Recknagel et al., 1989; Weber et al., 2003)**. The later was evidenced by the significant elevation in the serum levels of the aminotransferases enzymes ALT and AST, because these enzymes are cytoplasmic and are released into the blood after cellular damage **(Recknagel et al., 1989)**.

Results showed that a significant increase in the level of ALT and AST in CCl_4-treated rats. The level of ALT and AST decreased significantly in administration of 10 mg/kg dose of forskolin only. However, other doses failed to protect the liver against CCl_4-induced toxicity as evidenced by the high serum levels of ALT and AST. These biochemical results were confirmed by the histopathological findings. Our results were in accordance with a previous study which showed that forskolin reduced serum ALT levels and preserved hepatic architecture in a mouse model of liver ischemia/reperfusion injury **(Ji et al., 2012)**.

Based on the previous findings, this study confirms that forskolin, indeed, has a hepatoprotective effect against CCl_4-induced acute hepatotoxicity. The anti-oxidant and anti-inflammatory effects reported in previous studies on forskolin might be the reason for its hepatoprotective effect **(Irie et al., 2001; Kamata et al., 1996; Niaz and Singh, 1999)**.

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تأثير الكبد الوقائي لعقار "فورسكولين" في نموذج تجريبي لإصابة الكبد الحادة الناجمة عن رابع كلوريد الكربون

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تعد أمراض الكبد من إحدى الأمراض الشائعة المسببة للوفاة في العالم. في الوقت الحاضر، ركزت الدراسات البحثية على تطوير أدوية جديدة لعلاج أمراض الكبد. و قد تم العثور على بعض المركبات المستخلصة من النباتات الطبيعية و التي أظهرت فاعلية قوية لعلاج إصابة الكبد الناجمة عن المواد الكيميائية. ولذلك، فمن المشجع العثور على المزيد من المنتجات الطبيعية الفعالة لعلاج إصابة الكبد. و بناءاً على ذلك، فقد تم تصميم هذه الدراسة لتقييم فاعلية عقار "فورسكولين" ، المستخلص من النبات الهندي كوليوس فورسكولي، بجرعات مختلفة في نموذج تجريبي لإصابة الكبد الحادة الناجمة عن رابع كلوريد الكربون (CCl_4) بجرعة ١ مل/كغ كخليط مع زيت الذرة و التي تم حقنها في الغشاء البريتوني للجرذان. تم حقن عقار "فورسكولين" في الغشاء البريتوني بجرعات ٥ و ١٠ و ٢٠ و ٤٠ مغ/كغ لمدة ٧ أيام. وقد تم التحقق من تأثير الكبد الوقائي عن طريق اختبارات وظائف الكبد و التشريح المرضي لأجزاء من نسيج الكبد. نجح عقار "فورسكولين" بجرعة ١٠ مغ/كغ في تقليل انزيمات الكبد و استعادة بنية الكبد. و بالتالي، فإن عقار "فورسكولين" يقي من إصابة الكبد الناتجة عن CCl_4.