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ScienceDirect
Experimental and Toxicologic Pathology 61 (2009) 443–451
EXPERIMENTAL AND TOXICOLOGIC PATHOLOGY
www.elsevier.de/etp

Aquilegia vulgaris extract attenuates carbon tetrachloride-induced liver fibrosis in rats

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Received 16 February 2006; accepted 23 October 2008

Abstract

Six groups of male Wistar rats were treated as follows: in groups II, III and V liver damage was induced by CCl_4 (*per os*, 1590 mg/kg b.w. day) given 2 days a week for 6 weeks; group III was treated simultaneously with ethanol extract of A*quilegia vulgaris* (100 mg/kg b.w. day) for 6 weeks; group V with silymarin, positive control, at a dose of 100mg/kg b.w. day for 6 weeks; and groups IV and VI received only the extract or silymarin, respectively. Microsomal lipid peroxidation in the liver increased following CCl_4 treatment by 61–213% and was not changed significantly by the extract. The effect of silymarin was more pronounced, 19–52% decrease in the lipid peroxidation level. Hepatic glutathione was depleted by 22% in CCl_4-treated rats. The extract tested did not change this parameter. The activity of antioxidant enzymes was significantly reduced after CCl_4 administration, by 42–63%. Co-administration of the extract or silymarin resulted in significant increase in these enzymes activity; however, the basal level was not reached. Hepatic hydroxyproline concentration was elevated over 5-fold in comparison with controls. Co-administration of the extract or silymarin decreased the level of hydroxyproline by 66% and 55%, respectively. Activity of serum hepatic enzymes was elevated in rats treated with CCl_4 by 47–8700%. Both the extract and silymarin reduced significantly these enzymes' activity. The extract caused a fall in bilirubin and cholesterol level in rats treated with CCl_4 by 42% and 17%, respectively. Histopathological examination revealed less-severe fibrosis in rats co-administered the extract or silymarin when compared to animals treated with CCl_4 alone.

Keywords: *Aquilegia vulgaris*; Rat; Liver fibrosis; Liver cirrhosis; Antioxidant enzymes; Lipid peroxidation; Hydroxyproline

Introduction

Liver fibrosis is a very complex and dynamic process in which Kupffer cells (resident liver macrophages) and hepatic stellate cells (HSC) are known to play a major role. Macrophages stimulate the progression of the fibrogenic process, while stellate cells synthesize most of the matrix protein (Poli and Parola, 1997). It became

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CA, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; GGT, γ-glutamyltransferase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione S-transferase; HSC, hepatic stellate cells; LPO, lipid peroxidation; SDH, sorbitol dehydrogenase; SOD, superoxide dismutase.

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doi:10.1016/j.etp.2008.10.007

increasingly evident that oxidative stress is associated with various cellular reactions during the development of fibrosis (Gebhardt, 2002). Lipid peroxidation (LPO) and certain LPO products induce genetic overexpression of fibrogenic cytokines, the key molecules in the pathomechanism of fibrosis, as well as increased transcription and synthesis of collagen. Both these events can be downregulated at least in experimental models by the use of antioxidants (Poli and Parola, 1997).

Prevention as the most effective approach toward cirrhosis often fails; therefore, antifibrotic treatment that halts the progression of cirrhosis is important. Several plant-derived substances such as colchicine, silymarin, *trans*-resveratrol, Gingko biloba composita and Sho-saiko-to (extract of seven herbs in chinese folk medicine) have been proposed as antifibrotic agents (Stickel et al., 2002).

It was shown that some flavonoids interfere with fibrogenic functions of HSC and Kupffer cells *in vitro* and are inhibitors of different protein kinases involved in signal transduction. Since protein kinases also play a role in the activation of HSC; such an inhibitory effect of flavonoids may be of significance for interrupting the pathogenic process (Gebhardt, 2002).

Aquilegia vulgaris (L.) (Ranunculaceae), syn.: columbine, is a perennial herb indigenous in central and southern Europe. Decoction from leaves and stems of *A. vulgaris* has been used in folk medicine against liver and bile duct disorders, especially for the treatment of jaundice, and chronic skin inflammation. The herb is a component of the immunostimulating preparation Padma 28 and homeopathic drugs (PDR for Herbal Medicines, 2000).

We have isolated and identified several flavonoids (Bylka and Matławska, 1997a, b; Bylka, 2001; Bylka et al., 2002) and phenolic acids (Drost-Karbowska et al., 1996) in aerial parts of the plant as well as alkaloids in roots (Szaufer-Hajdrych et al., 1998). The predominant compound was 4′-methoxy-5,7-dihydroxyflavone 6-C-glucopyranoside (isocytisoside) (Bylka and Matławska, 1997a).

Our previous investigation has demonstrated that extracts of *A. vulgaris* and isocytisoside could protect against acute hepatotoxicity induced by carbon tetrachloride (Adamska et al., 2003) as well as paracetamol (Jodynis-Liebert et al., 2005).

In the light of the above findings it could be expected that the extract of *A. vulgaris* rich in flavonoids may improve liver function also in chronic liver disease. Thus, the purpose of the present study was to examine whether treatment with *A. vulgaris* ethanol extract exerts any beneficial effect on liver function, antioxidant defense system and histopathology in experimental liver cirrhosis induced by carbon tetrachloride in rat.

Materials and methods

Chemicals and plant material

The chemicals used were purchased from Sigma Chemical Co. *A. vulgaris* stems and leaves were collected in the Botanical Garden of A. Mickiewicz University, Poznań, Poland, in June 2002. A voucher specimen is deposited in the authors' laboratory (No. KF 12612002).

Ethanol extract of *A. vulgaris* was prepared as described before (Adamska et al., 2003). The extract contained mainly isocytisoside, as well as isocytisoside 7-O-glucoside, isoorientin, orientin, isovitexin 4′-O-glucoside, apigenin 7-O-rutinoside, apigenin 7-O-glucoside and apigenin (Bylka and Matławska, 1997a, b; Bylka, 2001). Additionally, the phenolic acids, caffeic, ferulic, *p*-cumaric, resorcylic, *p*-hydroxybenzoic, vanilic, sinapic and chlorogenic, were identified (Drost-Karbowska et al., 1996). Quantitative analysis of isocytisoside was performed by the HPLC method. The Lachom-Merck chromatograph equipped with a DAD detector and a Zorbax SB-C18 column (250×4.6 mm; 5 μm) was used. The mobile phase was methanol–water–formic acid (40:60:1) at a flow rate of 1 ml/min. The standard curve was made in the range 2–12 μg. The content of isocytisoside was 1.5%.

Experimental design

Male Wistar rats (200 ± 10 g) (bred in the Department of Toxicology Poznań University of Medical Sciences) were divided randomly into six groups, eight animals each. The rats were housed in plastic cages (Techniplast), four rats per cage, and maintained in a temperature-, humidity-, ventilation- and light- (12 h light/dark cycle) controlled environment. They were given free access to commercial rat chow and tap water. In groups II, III and V, liver damage was induced by CCl_4 given *per os* at a dose of 1590 mg/kg b.w., 2 days a week for 6 weeks. Group III was treated simultaneously with ethanol extract at a dose of 100 mg/kg b.w. day for 6 weeks. According to the same protocol, group V was given silymarin (100 mg/kg b.w. day) used as a positive control. Groups IV and VI received only the extract and silymarin, respectively, for 6 weeks at a dose of 100 mg/kg b.w. day. Group I served as control and was given vehicle. All substances were administered intragastrically in a mixture of water–olive oil (1:1) with a drop of Tween 20. Blood was collected by intracardiac puncture under terminal halothan anaesthesia. Livers were removed, perfused with ice-cold 1.15% KCl and homogenized in buffered sucrose solution (Tris, pH = 7.55). Microsomal and cytosol fractions were prepared by differential centrifugation according