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SHORT COMMUNICATION

Effects of *Cimicifuga racemosa* extract on liver morphology and hepatic function indices

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Abstract

Cimicifuga racemosa (black cohosh) is a herbaceous perennial plant, that has been traditionally used for a variety of ailments (dyspepsia, climacteric complaints, muscular rheumatisms, menstrual cramps). From laboratory and clinical studies, black cohosh seems to have a relatively good safety profile, even if a number of case reports of hepatotoxicity were a matter of recent concern.

Aim: A number of case reports indicated that *C. racemosa* could induce hepatotoxicity. We evaluated the effects of black cohosh extract on liver morphology, and on levels of various hepatic function indices in rats.

Methods: Wistar rats received 300 mg/kg/day of *C. racemosa* extract by gavage, for 30 days. Biochemical analysis of serum was conducted by an automated, random-access clinical chemistry analyzer. Liver samples were used for hystomorphological and immunohistochemical examination, for the detection of apoptosis (TUNEL assay), and for the determination of GSH level (spectrophotometrical analysis).

Results: *C. racemosa* extract does not affect liver morphology and hepatic function indices, in rats.

Conclusions: On the basis of experimental data, the use of 300 mg/kg/day of black cohosh appears quite safe in rats. Nevertheless, in humans the safety of *C. racemosa* should be further monitored, in terms of patient-related factors.

Keywords: Black cohosh; *Cimicifuga racemosa*; Hepatotoxicity; Immunohistochemistry; Liver function indices; Liver histomorphology

Introduction

Cimicifuga racemosa (L.) Nutt (black cohosh) is a herbaceous perennial plant, belonging to the Ranunculaceae family. Firstly in North America, and later all over the world, the root and rhizome of black cohosh have been used for a variety of ailments, including the treatment of dyspepsia, climacteric complaints, epilepsy, malaria, cough, sleeping disorders, and for the relieve of muscular rheumatisms and menstrual cramps (Borrelli and Ernst, 2002; Bolle et al., 2007). Though laboratory and clinical studies indicate that *Cimicifuga racemosa* has a relatively good safety profile, a number of case reports of hepatotoxicity have raised some concern (European Medicines Agency, 2006a; Nisbet and O'Connor, 2007; Joy et al., 2008). The European Medicines Agency (EMEA) recently assessed 42 case reports of hepatotoxicity related to ingestion of black

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cohosh (EMEA, 2006a). The conclusions of EMEA were that "all discussed cases of the literature and pharmacovigilance reports are poorly documented" and "the connection of herbal medicinal products containing *Cimicifuga racemosa* root and hepatotoxicity should be seen as a signal" (EMEA, 2006b). Moreover, the Herbal Medicinal Product Committee (HMPC) recommends to Marketing Authorization Holders to investigate the risk of hepatotoxicity in products containing black cohosh. Present work was aimed to evaluate the effect of an extract of black cohosh on liver morphology and hepatic function indices in rats after oral administration for 30 days.

Materials and methods

Animals and treatments

Male Wistar rats were purchased from Charles River (Lecco, Italy). Upon arrival at the laboratory, animals were housed in an artificial 12 h light/dark cycle, at 22 ± 1 °C, with 50 ± 5% of humidity. Each rat was housed individually, and acclimated for 2 weeks. Rats were fed with pelleted food and water, available *ad libitum*. Twelve rats were randomly assigned to two experimental groups: control group and *Cimicifuga racemosa* extract group. The extract was suspended in carboxymethylcellulose 0.5%, and administered at the desired dosing level of 300 mg/kg/day in the volume of 5 ml/kg. Control animals received the vehicle. This dose was chosen as an intermediate dose, on the basis of preliminary experiments and as reported by Lüde et al. (2007). Rats were weighted daily, and were treated by gavage. After 30 days of treatment, animals were sacrificed by inhalation of ethylic ether. The experimental protocol of this work was performed in accordance with the Italian ethics legislation (Ministry of Health, D.L. 116/92).

Chemicals

Cimicifuga racemosa root dry extract (Batch no. 0601850) was kindly supplied by EPO (Milan, Italy). The extract was standardized in triterpene glycosides (2.5% calculated as 27-deoxyactein); the extraction solvent was ethanol 50% w/w. Alpha-SMA for hepatic stellate cells (HSCs, clone 1A4), and PCNA:PC10, were obtained from Santa Cruz Biotechnology Inc. (Heidelberg, Germany), and were diluted 1:100. TUNEL ApopTag Peroxidase *In situ* Apoptosis Detection Kit S7100 was from Chemicon International (Milan, Italy). Biotinylated antibody, and 3-3′ diaminobenzidine were obtained from Dako (Glostrup, Denmark). All other chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Biochemical analysis

The blood samples were obtained by intracardiac puncture. The clotted blood was centrifuged at 3000 rpm for 20 min in a refrigerated centrifuge (4 °C). The serum was separated and used to evaluate aminotransferases, alkaline phosphatase, gamma-glutamyl transpeptidase, total and direct bilirubin, by an automated, random-access clinical chemistry analyzer (Abbott Aeroset®) (Duly et al., 2001).

Determination of GSH levels

Reduced glutathione (GSH) was determined as described by Raja et al. (2007), with slight modifications. Briefly, the liver was dissected in pieces of 0.5 g and liver portions were homogenized in ice-cold KCl (0.15 M). Homogenate was deproteinized with 10% of trichloroacetic acid. After centrifugation, the presence of thiolic groups (index of reduced glutathione) was assessed by adding Ellman's reactive. The absorbance was determined using a microplate reader Biorad 3550 at 412 nm.

Light microscopy and immunohistochemistry

Liver fragments were obtained from the right lobe, fixed in buffered formalin and embedded in paraffin. For histomorphologic studies the sections were stained with haematoxylin–eosin and Masson's trichromic and observed at the magnification of 20× with a light microscope Leica DM 4500B. The microscope was connected to a Videocam (ProgRes C10 plus), and provided with a Image Analysis System, Delta Sistemi (Rome, Italy). Immunohistochemical analysis was performed according to Carpino et al. (2004), and Gaudio et al. (2006). For the detection of apoptosis the terminal deoxynucleotide transferase end labelling (TUNEL) method was used according to the instructions given by the supplier. In all immunoreactions negative controls were also included.

Statistical analysis

Rats' body weight was analyzed using a two-way (treatments × days) analysis of variance (ANOVA) for repeated measures. Liver:body weight ratio, GSH levels, and blood parameters were analyzed by parametric Student's *t*-test. The level of statistical significance was $p < 0.05$.

Results and discussion

The extract of *Cimicifuga racemosa* did not induce any effect on body weight gain and liver:body weight