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Acute and sub-chronic toxicity of a lyophilised aqueous extract of *Centaurium erythraea* in rodents

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ABSTRACT

Ethnopharmacological relevance: An aqueous concoction made from centaury (*Centaurium erythraea* (L.) Rafn., (Gentianaceae) whole plant is used in the Moroccan traditional medicine for the treatment of diabetes, as well as a number of other diseases. No systematic study of the potential toxicity of the plant has been described.

Aim of the study: The present investigation was carried out to evaluate the safety of an aqueous extract of *Centaurium erythraea* whole plant (CE-extract) by determining its potential toxicity after acute and sub-chronic administration in rats and mice.

Materials and methods: For the acute study, the lyophilised CE-extract was administered to adult IOPS OFA mice in single oral doses of 1–15 g/kg given by gavage, and single intraperitoneal (i.p.) doses of 1–14 g/kg. General behavioral adverse effects, mortality, and latency of mortality were determined for up to 14 days. In the sub-chronic dose study, the CE-extract was administered orally at doses of 100, 600 and 1200 mg/kg daily for 90 days to Wistar rats. Body weight and selected biochemical and hematological parameters were determined every 30 days and at the end of 90 days of daily administration; sections of liver and kidney were examined histologically for any signs of organ damage at the end of the treatment.

Results: In the acute study in mice, there were no deaths or any signs of toxicity observed after oral administration of single doses of the CE-extract at any dose level up to the highest dose tested (15 g/kg), which was the no-observed-adverse-effect level (NOAEL). However, the mortality rate as well as the acute toxicity of the i.p. administered CE-extract increased progressively with increasing dose. The NOAEL for the i.p. dose was 6 g/kg while the lowest-observed-adverse-effect level (LOAEL) was 8 g/kg; the calculated acute toxicity (LD_{50}) of i.p. administered CE-extract in mice was 12.13 g/kg.

In sub-chronic studies in rats, the CE-extract (administered orally at daily doses of 100, 600 and 1200 mg/kg for 90 days), did not cause any changes in hematological and biochemical parameters, except a small reduction of mean corpuscular volume, and a decrease in serum glucose and triglyceride levels at the higher doses. Histopathological examination of the liver and kidneys at the end of the study showed normal architecture suggesting no morphological disturbances.

Conclusions: Because of the lack of toxicity of the CE-extract given by the oral route, and relatively high NOAEL values for the i.p. dose in the acute study in mice, as well as lack of mortality or clinically significant adverse changes in the biological and hematological parameters, and the morphology of liver and kidneys in rats after 90 days of daily dosing, it may be concluded that the CE-extract is relatively non-toxic. Also, in view of the doses consumed empirically in traditional medicine in Morocco, there is a wide margin of safety for the therapeutic use of *Centaurium erythraea.*

1. Introduction

Centaury [*Centaurium erythraea* (L.) Rafn. (Gentianaceae)], is known in Morocco as “Gosst l hayya” where it is considered as a medicinal herb. This plant grows abundantly in the Mediterranean zone (Fournier, 1961). The importance of centaury may be recognized from the fact that its use in traditional medicine has been described in the pharmacopoeia of 23 different countries (Hatjimanoli and Debelmas, 1977), and it was named, ‘The

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BW, body weight; CE, *Centaurium erythraea*; HCT, hematocrit; HGB, hemoglobin; LD_{50}, lethal dose 50; LOAEL, lowest-observed-adverse-effect level; MCH, mean RBC hemoglobin; MCHC, mean RBC hemoglobin concentration; MCV, mean RBC volume; NOAEL, no-observed-adverse-effect-level; RBC, red blood cells; WBC, white blood cells; S.E.M., standard error of mean.

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medicinal plant of the year 2004,' (Springefeld, 2004). Centaury is reputed to possess depurative, sedative, antipyretic, anthelmintic, and anti-inflammatory properties (Beth, 1995; Grieve, 1971). The plant has been used in the treatment of asthma, eczema, jaundice, intestinal parasitic infestation, rheumatism, wounds and sores, as well as to reduce blood pressure, gastrointestinal smooth muscle spasm, edema and digestive disorders (loss of appetite, stomach discomfort, bloating, indigestion), and as a liver and gall bladder stimulant (Grieve, 1971; Capasso et al., 1983; Bisset, 1994; Beth, 1995; Kültür, 2007). It has also been used as a tonic, blood purifier, and 'blood builder' (Schimmer and Mauthner, 1994). In addition, a decoction of the whole plant has been used in treating urine retention and abdominal colic (Claisse, 1989). In Moroccan folk medicine, centaury is used in the traditional therapy of many diseases including diabetes, fever, cardiac irregularity, abdominal colic, and as a diuretic (Bellakhdar et al., 1991; Bnouham et al., 2002; Jouad et al., 2001; Eddouks et al., 2007).

Studies in experimental animals have shown that the extracts of centaury have a variety of pharmacological activities, including diuretic (Flück, 1973; Haloui et al., 2000), antipyretic (Lacroix et al., 1973; Bellakhdar et al., 1991; Berkan et al., 1991), antibacterial (Kumarasamy et al., 2002), anti-inflammatory (Berkan et al., 1991; Valentão et al., 2002; Kumarasamy et al., 2003a), analgesic (Berkan et al., 1991; Valentão et al., 2002), free radical scavenging and antioxidant (Valentão et al., 2001; Kumarasamy et al., 2007), and hypoglycemic activity (Alaoui et al., 1992; Hamza et al., 2010). The plant extract also protected rats from acetaminophen-induced hepatotoxicity (Mroueh et al., 2004). The ethanolic extracts from dried plant material also displayed markedly antimutagenic properties in several *Salmonella typhimurium* strains (Schimmer and Mauthner, 1994).

Centaurium erythraea has been the subject of several phytochemical investigations, which have resulted in the isolation and identification of a variety of compounds, including, centauroside, centapicrin, flavonoids, gentiopicrin, gentiopicroside, isocoumarin, phenolic acids, swertiamarin, triterpenes, wertiamarine, and xanthones (Van Der Sluis and Labadie, 1981; Takagi et al., 1982; Kaouadji et al., 1986; Hatjimanoli et al., 1988; Schimmer and Mauthner, 1994; Jovanović et al., 2009; Valentão et al., 2000; Valentão et al., 2002; Kumarasamy et al., 2003a; Valentão et al., 2003a; Van Wyk and Wink, 2004; Schimmer and Mauthner, 1996; Bellakhdar, 2006). In addition, several steroids (β-sitosterol, stigmasterol, campesterol, brassicasterol and δ^7-stigmasterol), and essential amino acids (alanine, leucine, phenylalanine and tryptophan) have also been identified in centaury (Aquino et al., 1985). Many of these compounds are known to exhibit important biological activities, such as antimicrobial, antipyretic, anti-inflammatory, antimutagenic, antitumorigenic and antioxidative properties and as appetite stimulant (Schimmer and Mauthner, 1994; Kumarasamy et al., 2003a,b; Valentão et al., 2003b; Van Wyk and Wink, 2004).

In spite of the popular use of centaury in traditional medicine, no systematic evaluation of its toxic effects has been carried out. Therefore, the aim of the present study was to investigate the acute and sub-chronic toxic effects of an aqueous extract of whole *Centaurium erythraea* plant in rats and mice, and establish the safety of the plant in traditional medicine.

2. Materials and methods

2.1. Plant material

Mature whole *Centaurium erythraea* plants were collected in North Morocco between May and June (2006) and stored at room temperature in a dry place prior to use. The plants were authenticated as *Centaurium erythraea* by Professor M. Fennan of the Department of Botany, Scientific National Institute (Rabat), and a voucher specimen (H-44) was deposited in the Institute.

2.2. Preparation of the aqueous extract of Centaurium erythraea

The whole *Centaurium erythraea* plants were washed quickly in running water, dried in an oven at 40 °C and then powdered in a Willey mill. An aqueous extract of the plant was prepared by boiling the powder (50 g) in 500 mL distilled water under reflux for 20 min. The decoction obtained was centrifuged, filtered, frozen at −20 °C and then lyophilised (FreeZone® Dry 4.5, USA) to yield approximately 12% (w/w) of the residue, which was stored at 20 °C until used.

The residue was dissolved in distilled water (CE-extract) prior to the experiment every day.

2.3. Acute toxicity study of CE-extract in mice

Healthy IOPS OFA strain mice of either sex (obtained from our animal colony; raised from the original strain procured from Iffa-Credo, l'Arbresle, France), weighing between 25 and 35 g were divided in groups of 10 (5 males and 5 females). Animals, segregated according to the gender and housed five per plastic cage, were maintained in a room at a temperature of 25 ± 1 °C, with photoperiod of 12 h (from 06:00 h to 18:00 h), and frequent air changes. Mice had free access to tap water and regular rodent food, except for a short fasting period before the treatment with single doses of the lyophilised CE-extract. The CE-extract (dissolved in distilled water adjusted to 10 mL/kg per dose) was administered by gavage at doses of 0, 1, 3, 5, 7, 9, 11, 13, and 15 g/kg body weight (BW) or by the intraperitoneal route at doses of 0, 2, 4, 6, 8, 10, 12, 13 and 14 g/kg BW.

The animals were observed for general behavioral changes, signs of toxicity and mortality continuously for 1 h after treatment, then intermittently for 4 h, and thereafter over a period of 24 h (Twaij et al., 1983). The mice were further observed for up to 14 days following treatment (Silva et al., 2007) for behavioral changes and signs of toxicity and/or death, and the latency of death. The LD_{50} values were determined according to the method of Litchfield and Wilcoxon (1949).

2.4. Study of sub-chronic toxicity of CE-extract in rats

Wistar albino rats of either sex, weighing 200–240 g, were housed in plastic cages (three to a cage, segregated by gender) under the same conditions as described above for mice. The animals were divided into four Groups (I, II, III and IV) of 10 rats each (5 females and 5 males), and their weights were recorded. The CE-extract (dissolved in distilled water adjusted to 10 mL/kg per dose) was administered orally, daily for 90 days to Groups I–IV at doses of 0, 100, 600 and 1200 mg/kg BW, respectively. The doses were selected based upon the recommendations by the U.S. Department of Commerce (Cohrsson and Covello, 1999), human dose in traditional medicine (see later), and the results of the acute (single dose) study in mice. Toxic manifestations and mortality were monitored daily. At the end of each 30-day period, body weights were recorded. Blood samples were obtained (collected in capillary tubes) by retro-orbital sinus puncture (Waynforth, 1980) under light diethyl ether anaesthesia, and collected with anticoagulant (200 μL blood and 50 μL of 4 mM ethylenediamine tetraacetate or heparin). Blood with the ethylenediamine tetraacetate was used immediately for the determination of hematological parameters, while heparinized blood was centrifuged at 4000 rpm for 10 min at 4 °C; plasma obtained was stored at −20 °C until analysed for biochemical parameters.