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Acute and sub-chronic toxicity studies of the aqueous extract from leaves of *Cistus ladaniferus L.* in mice and rats

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ABSTRACT

Ethnopharmacological relevance: *Cistus ladaniferus* L. (C.ladaniferus) (Cistaceae) is an aromatic shrub native to the Mediterranean region. The leaves are widely used in traditional medicine throughout Morocco for the treatment of various diseases including, diabetes, diarrhea, inflammation, and skin ailments. However, to the best of our knowledge, no systematic study concerning its toxicity profile has been reported.

Aim of the study: The study carried out evaluates the potential toxicity of the aqueous extract from leaves of the C.ladaniferus (CL extract) shrub, through the method of acute and sub-chronic oral administration in mice and rats.

Materials and methods: During the acute toxicity study, male and female mice were orally administrated with CL aqueous extract at single doses of 500, 1000, 2000, 3000 and 5000 mg/kg (n = 5/group/sex). Abnormal behavior, toxic symptoms, weight, and death were observed for 14 consecutive days to assess the acute toxicity. During the sub-chronic toxicity study, the aqueous extract was administered orally at doses of 500, 700 and 1000 mg/kg (n = 6/group) daily to Wistar rats of both sexes for 90 days. The general behavior of the rats was observed daily, and their body weight was recorded weekly. A urinalysis, biochemical analysis, hematological analysis, macroscopic examination and histopathological examination of several organs were conducted at the end of the treatment period.

Results: During the acute toxicity test, when mice were administered doses of 3000 and 5000 mg/kg, the CL extract produced a 10–30% mortality rate, respectively, and induced signs of toxicity. However, no mortality or adverse effect was noted at the doses of 1000 and 2000 mg/kg. The median lethal dose (LD50) of the extract was estimated to be more than 5000 mg/kg. In the subchronic study, the CL extract induced no mortality or treatment-related adverse effects with regard to body weight, general behavior, relative organ weights, urine, hematological, and biochemical parameters. Histopathological examination of vital organs showed normal architecture suggesting no morphological alterations. Moreover, the CL extracts improved lipid profile and exhibited a significant hypoglycemic effect in all doses tested in rats.

Conclusion: The results of the present study suggest that treatment with the CL extract for 13 weeks does not appear to produce significant toxicity, except at high dose. Therefore, the use of appropriate levels of the CL extract as a traditional medicine remedies should have a wide margin of safety for its therapeutic use.

1. Introduction

Traditional herbal medicines have been used to treat diseases and promote health for centuries worldwide (Yuan et al., 2016). The World Health Organization estimated that about 80% of the population, especially in developing countries, is referred to traditional herbal medicines, such as plant extracts or their derived products, for their primary health care needs (World Health Organization, 2008).

Despite the growing popularity and presumed safety of herbal medicines, adverse effects have become a major safety issue for natural products (Han et al., 2016). Consequently, in response to public health concerns, it is important to validate the safety of traditional herbal medicines before their use. Experimental data on the toxicity profile of medicinal plants and their extracts should be obtained to increase confidence in their safety for human use and in the development of pharmaceuticals (Yuet Ping et al., 2013). Therefore, systematic evaluation of medicinal plants for potential toxicity is a necessary step for the validation of their regular therapeutic use.

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Cistus ladaniferus L., also commonly known as rock rose, is a shrub species belonging to the Cistaceae family. This native and widespread aromatic plant is widely distributed in the Mediterranean region (Teixeira et al., 2007). *C. ladaniferus* L. and other species of Cistaceae are commonly used as traditional medicines in Mediterranean region for the treatment of several skin ailments, as anti-diarrhoeal, and as anti-inflammatory agents (Attaguile et al., 2000). This plant is known locally as "Touzal" in the north of Morocco or "Argale" in other regions throughout Morocco (Bellakhdar et al., 1991). In Moroccan traditional medicine, the decoction of the aerial parts of this plant is used as an antidiarrhoeal, antiacid, antidiabetic, and antispasmodic by the local population in northern Morocco (Bnouham et al., 2002; Merzouki et al., 2000).

Previous studies have reported various biological activities of *Cistus ladaniferus* extracts. For instance, the anti-inflammatory and analgesic effects of an aqueous extract of the plant have already been studied experimentally in rats (El Youbi et al., 2016). The aqueous extract of *Cistus* leaves was found to reduce systemic blood pressure in two animal models of hypertension, (Belmokhtar et al., 2009) and exhibited antispasmodic action on rodent isolated jejunum (Aziz et al., 2006). Moreover, the aqueous extract has been found to have platelet anti-aggregant property (Mekhfi et al., 2004) and vasodilator effect on isolated aortic rings (Belmokhtar et al., 2009). The phenolic extract of *C. ladaniferus* has also shown an antifungal potential against several *Candida* species (Barros et al., 2013). In vitro studies have demonstrated the antioxidant activity of *C.ladaniferus* extracts, which was investigated by several methods established for in vitro test (Amensour et al., 2010; Barrajón-Catalán et al., 2010). Aqueous and organic extracts of *C. ladaniferus* displayed antibacterial activity against Gram-positive bacteria (Barrajón-Catalán et al., 2010; Ferreira et al., 2012). Finally, cytotoxic activity against several human cancer cells was also reported (Andrade et al., 2009; Barrajón-Catalán et al., 2010).

The phytochemical composition of *C. ladaniferus* aqueous extract has been characterized in previous studies, showing that the extract was rich in phenolic compounds (Barrajón-Catalán et al; 2011; Fernández-Arroyo et al., 2010). In these studies, several groups of phenolic components were identified, such as Ellagic acid and derivatives (punicalagin isomers, cornusiin B, ellagic acid-7-xyloside, and methylated ellagic acid-7-xyloside), Hexahydroxydiphenoyl and derivatives (hexahydroxydiphenoyl-D-glucoside, pedunculagin), Phenolic acids and derivatives (gallic acid, glucogallin isomers, gentisoil glucoside, uralenneoside, mirciaphenone B), and Flavonoids (quercetin diglycosides, methylether and dimethylether kaempferol, apigenin).

The leaves of this plant also produce a fragrant oleoresin with a strong aromatic odor, known as *labdanum*, which is highly appreciated in the fragrance industry (Guimarães et al., 2009). Previous studies have reported the phytochemical composition of the resin and showed that is very rich in polyphenols (Gomes et al., 2005; Robles et al., 2003). Additionally, the presence of flavonoids in the exudate has been connected to the inhibitory activity of the resin on calcium transport in skeletal muscle (Sosa et al., 2004). In folk medicine, this resin has been used from ancient times to treat, dysentery, catarrh, diarrhea, and menstruation difficulties (Barrajón-Catalán et al., 2010).

C. ladaniferus L. provides an important source of valuable bee pollen in Mediterranean countries (Ortiz, 1994). This pollen and its extract, which are rich in flavonoids, have exhibited inflammatory and antioxidant activities (Maruyama et al., 2010). Furthermore, previous studies have shown that the pollen from *C. ladaniferus* has a preventive effect on bone loss in ovariectomised and streptozotocin diabetic rats (Yamaguchi et al., 2007a, 2007b).

We have previously demonstrated the hypoglycemic and hypolipidemic activities of *C. ladaniferus* in diabetic rats (El Kabbaoui et al., 2016). Despite these studies and the widespread use of this plant in Moroccan folk medicine, no study on the toxicological profile of extract from leaves has been reported. Thus, in order to obtain scientific information on its safety and potential toxicity, the present study was performed to assess the possible toxic effects of the aqueous extract of *C.ladaniferus* leaves after acute oral administration in mice and sub-chronic oral administration in rats.

2. Material and methods

2.1. Plant material and preparation of extract

Fresh leaves of *C. ladaniferus* (Cistaceae) were harvested from the Taounate region of northern Morocco in March 2016. A voucher specimen was taxonomically identified and deposited in the herbarium of the Faculty of Sciences and Techniques, Fes, Morocco (No. MA-FSTF 16). *C. ladaniferus* leaves were washed with water, air-dried, and ground into a fine powder using a mixer. The powder was then kept in a firmly closed container until extraction.

The plant extract was prepared following the standard traditional method described in Moroccan Pharmacopoeia (Lemhadri et al., 2006). 500 g of air-dried powder from the leaves of the plant was mixed with 5 L of hot distilled water. The mixture was kept under agitation for 6 h at room temperature. Thereafter, the infusion obtained was centrifuged, and the supernatant was filtered with Whatman no1 filter paper. The filtrate was then concentrated under vacuum at 50 °C using a rotary evaporator. The extract (yield = 26% w/w of the initial powder) was stored at −20 °C until use for toxicological investigations. For oral administration, the residue was freshly reconstituted on a daily basis in distilled water to the final concentrations required prior to the experiment.

2.2. Animals

Adult Swiss albino mice and Wistar rats were used for the acute and ninety-day toxicity studies, respectively. Wistar rats were obtained from the animal colony of the Biology Department of the Faculty of Sciences and Techniques (Fes, Morocco), while Swiss albino mice were purchased from the Pasteur Institute (Casablanca, Morocco). All animals were kept in polypropylene cages (males separated from females), and acclimatized for a period of 15 days prior to the start of the experiment. They were maintained under standard laboratory conditions of regular 12 h light/12 h dark cycle and temperature (24 ± 1 °C) throughout the experimental period. They also were fed clean tap water and commercial rodent chow ad libitum during the experimental period. All experimental procedures were conducted in accordance with the NIH general guidelines for the Care and Use of Laboratory Animals.

2.3. Acute oral toxicity study

The assay of acute toxicity was performed according to the Organization for Economic Cooperation and Development (OECD) guideline 423 (OECD, 2001a). A total of 60 mice weighing between 27 and 37 g were randomly divided into six experimental groups of 10 mice each (5 males and 5 females per group). After fasting overnight, *C. ladaniferus* aqueous extract was administered to each treatment group at single doses of 500, 1000, 2000, 3000, 5000 mg/kg, respectively, by oral gavage. The control groups were treated with the same volume of distilled water. After dosing, all animals were observed individually for mortality and changes in general behavior during the first 30 min, then at 2,4, 6, 10 and 24 h following treatment. Symptoms of toxicity such as hypo-activity, piloerection, breathing difficulty, tremors, and convulsion were evaluated after administration of the various extract doses. The LD 50 value was determined according to the method described by the OECD Guidelines 423 (OECD, 2001a). During the remaining experimental period, the animal observation was performed at least once per day for the post-dosing period of 14 days. Body weights were measured at the initiation of treatment, and on days 4, 7, 11 and 14 after administration. On the 14th day, the mice were sacrificed under