Regulatory Toxicology and Pharmacology 98 (2018) 184–189

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Screening of acute and sub-chronic dermal toxicity of *Calendula officinalis* L essential oil

Arun K. Mishra[a,*], Amrita Mishra[a], Pragya[a], Pronobesh Chattopadhyay[b]

[a] Central Facility of Instrumentation, Faculty of Pharmacy, IFTM University, Lodipur-Rajput, Moradabad, 244001, India
[b] Pharmaceutical Technology Division, Defense Research Laboratory, DRDO, Tezpur, 784001, India

ARTICLE INFO

Keywords:
Acute toxicity
Dermal toxicity
OECD
Calendula officinalis essential oil

ABSTRACT

The objective of the study is to access the safety of Calendula essential oil by studying acute and sub-chronic dermal toxicity. The dermal toxicities of Calendula essential oil were evaluated in accordance with OECD guidelines number 402 and 411 respectively. The animals were exposed to *Calendula officinalis* (CO) essential oil dose of 20 mL/kg body weight for acute dermal toxicity, whereas for dermal sub-chronic toxicity study, rats were exposed to CO oil 2.5, 5 and 10 mL/kg body weight, respectively, for 7 times in a week for 90 days. The parameters studies included CNS stimulation, depression, hematological parameters (RBC, WBC, Hb, Lymphocyte % etc), biochemical parameters (total protein, albumin, total bilirubin, ALP, AST, etc), relative organ weight, necropsy and histopathology. In toxicity studies, all animals exhibited normal behavior without any change in hematology, blood biochemistry, necroscopical and histopathology. The no observed effect level (NOEL) and no observed adverse effect level (NOAEL) of CO oil were 2.5 and 10 mg/kg/day, respectively. CO oil is under the herbal medicinal product according to the European Medicines Agency with the claim of an LD_{50} value of 20 mL/kg body weight. The result indicates that CO essential oil did not produce any significant toxic effects.

1. Introduction

Herbal treatment for skin related disorders has been used on the basis of traditional knowledge since ancient times. Even our biologically close relatives, the great apes, use herbal medicines for treatment of various ailments (Huffman, 2001). In recent years, there has been a resurgence of the use of herbs due to the some reasons, which includes the side effects of chemical drugs etc, thus there was need to come back to nature and therefore natural remedies became a part of the green revolution. Herbal treatments based on traditional knowledge, including those for skin disorders, are currently gaining popularity among patients and to some extent among physicians (Eisenburg et al., 1993).

India and various European countries have a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health *viz*. Ayurveda, Unani, Siddha, Homeopathy and Naturopathy. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health supplements as well as over-the-counter (OTC) drugs as self-medication. Herbal drugs are also prescribed in the non-allopathic systems (Gautam et al., 2003).

Many herbal medicines have been used since a long time, which have proved the record of good results. The use of herbal therapies for the treatment of dermatologic disorders has exhibited significant effect. There is need of standardization of herbal preparations and integrative medicines. For such preparations, specific recommendations about how to use and about the efficacy of herbs in the treatment of disease are to be fixed (Bedi and Shenefelt, 2002). According to a study on the attitude of modern medicine practitioners towards herbal products, general practitioners are relatively unfamiliar with herbal products even though some products are prescribed very commonly. Such practioners are willing to try an herbal product if its efficacy is scientifically proven, and would try herbal products if no modern medicinal remedies were available (Ahluwalia, 2014). People use self-medication for minor ailments such as cough, cold, diarrhoea and stomach problems using herbal medicines. Now a day's most of the natural products and herbal medicines are administered in most of the disease conditions for a prolong period without focusing proper dosage regimen, regulatory aspects and consideration of toxic implications. Therefore, regulatory enforcement is required for safe use of any herbal products. The regulatory aspects of herbal medicine changed considerably over the past century. Whereas many herbals once were recognized officially with compendium status, unlimited consumer access to currently marketed herbal products and accompanying information exists without

* Corresponding author. Central Facility of Instrumentation, Faculty of Pharmacy, IFTM University, Lodipur-Rajput, Moradabad, 244001, India.
E-mail address: arun_azam@rediffmail.com (A.K. Mishra).

https://doi.org/10.1016/j.yrtph.2018.07.027
Received 22 February 2017; Received in revised form 29 January 2018; Accepted 30 July 2018
Available online 31 July 2018
0273-2300/

recognized assurance for safety and quality based on official compendia. Therefore, toxicology data are required for prolonging the use of herbs and herbal products (Zhang, 1998).

Calendula officinalis L. (Asteraceae) is an important plant of genus Calendula (marigolds), having several medicinal application in India and all over the world. Calendula is a fast growing annual herb, easy to germinate and simple to care (Basch et al., 2006). The calendula plant can be cultivated by seed plantation in early outdoors (Arora et al., 2013). Calendula flower is often used in skin care products because of assistance in cell rejuvenation, wound healing, reducing inflammation, soothing and softening the skin. Oil of calendula flower has shown marked presence of flavonoids, coumarines, quinones, volatile oil, carotenoids and amino acids (Mishra et al., 2012a). Various terpenoids have been reported including sitosterols, stigmasterols, 3- monoesters of taraxasterol, erythrodiol, brein, ursadiol, faradiol-3-O-palmitate, faradiol- 3-O-myristate, faradiol-3-O-laurate, arnidiol-3-O-palmitate, arnidiol-3-O-myristate, arnidiol-3-O-laurate, oleanolic acid saponins: calenduloside AH, oleanane triterpene glycoside: Calendula glycoside A, Calendula glycoside B, Calendula glycoside C, glucosides of oleanolic acid (mainly found in roots of grown and senescing plants) and glucuronides (mainly found in flowers and green parts). Apart from this, various flavonoids have been isolated from the ethanol extract of the inflorescence of *C. officinalis* which includes quercetin, isorhamnetin, isorhamnetin-3-O-β-D-glycoside, calendoflavoside, calendoflavobioside, rutin, and isoquercitrin (Mishra et al., 2012b). The calendula oil is having great potential to quench the free radical reactions; hence, its application in area of antioxidant as cosmetics cannot be ignored. The flowers and the leaves are the chief parts, which are of medicinal and commercial significance. The extract of flowers and essential oil from flowers is used in treatments of several ailments as skin diseases (Guinot et al., 2008). In earlier research by author, the chemical composition of Calendula essential oil was studied by Gas chromatography-Mass spectroscopy (GC-MS) and 22 compounds were identified (Mishra et al., 2012a). Out of these 22 compounds, alpha pinene and 1,8 cineole were further quantified as these compounds are very vital in combating against ROS. The quantification of alpha pinene and 1,8 cineole were done by High performance liquid chromatography (HPLC) and later on, effects of Calendula essential oil-based cream on biochemical parameters of skin of albino rats against Ultraviolet B radiation were studied (Mishra et al., 2012b). In the study, physical stability parameters were also studied and Calendula essential oil-based formulations were found stable (Mishra et al., 2012c).

CO oil is commonly used as such and in cosmetic formulations, especially in baby care products. There are many creams, lotions and baby oils available in market, containing varying amount of CO oil like WELEDA™ Calendula baby oil, Deep steep™ baby diaper cream, Baby & Eve™ Calendula baby cream and Curash™ baby care healing cream. Till date no data is available on the toxicity of the CO oil. In this reference, we evaluated the acute and sub-chronic dermal toxicities of the same in Wister rats.

2. Material and methods

2.1. Animals

Adult Wistar rats of either sex weighing from 200 to 250 g were used in the present study and were procured from institutional animal house, Pharmacy Department, IFTM University, Moradabad, India. All the animals were kept in temperature controlled room conditions, with 12 h alternating light and dark cycles. The animals were given adequate nutrition and water *ad libitum*. The protocols were followed as per "Guidelines for the Care and Use of Laboratory Animals" and approved by the Institutional Animal Ethics Committee (IAEC) (Res. Number, 2011/837ac/PhD/02; Dated 14.08.2011) (Guide for the Care and Us, 2011).

2.2. Extraction of CO oil

The CO oil was extracted in Clevenger apparatus by hydro distillation. The fresh flowers were packed in the apparatus with the sufficient amount of water. After 3 h of distillation, the oil layer was separated and dried by Na_2SO_4. The yield was found to be 1.25% v/w. The obtained oil was subjected to GC-MS (Mishra et al., 2012d) and quantitative HPLC analysis. By employing HPLC method, the concentration of 1,8-cineol and α-pinene in CO oil were found to be 8.12 ± 0.7 and 22.53 ± 0.2% respectively (Mishra et al., 2012e).

2.3. Acute dermal toxicity study

The study was performed in compliance with OECD guidelines number 402 (Prado-Ochoa et al., 2014; Zhai et al., 2007). Twenty-four animals were selected in the present study and each group was having six animals (n = 6). All the rats were nulliparous and non-pregnant. Treatment groups in the study received CO oil 2.5 mL/kg (Group-2), 5 mL/kg (Group-3), 10 mL/kg (Group-4). Group 1 served as control. The rats having intact skin were considered in the present study and cautiously experiments were performed to avoid any skin damage. 24 h before the test, hairs were clipped at the dorsal side of the trunk of the rats. CO oil (flowers of CO collected from botanical garden, Moradabad, after due authentication, extracted by Clevenger method) was applied at a dose of 20 mL/kg body weight on the test area and afterward, same was covered with Cotton Crepe Bandage (K. S. Surgical, Modinagar, India) for 24 h. The residual CO oil was removed from test area by washing with distilled water, after the end of exposure periods and test area was made dried. The animals were kept under observation to record the signs of toxicity at 30 min, 4 and 24 h after the removal of films. The observation was continued upto 14 d. Mortality and morbidity of the animals was recorded during the experimental observation period. Body weight was recorded prior to treatment and afterward on each day till end day of the experiment. On day 14, surviving rats were euthanized and dissections of the animals were performed.

2.4. Sub-chronic dermal toxicity studies

For sub-chronic dermal toxicity study, OECD guidelines as per 411 were followed (Abdel-Rahman et al., 1987; Leal Parente et al., 2012; Subchronic Dermal Toxicit, 1981). Forty adult male and 40 female Wistar rats were randomly divided into four groups (n = 20; 10 male & 10 female for each group). The back of test animals in area (5 × 5cm^2) was shaved prior to treatment. The animals were divided into following four groups: Group-1 indicates control, Group-2 indicates CO oil treated with 2.5 mL/kg, Group-3 indicates CO oil treated with 5 mL/kg, Group-4 indicates CO oil treated with 10 mL/kg. The test animals were shaved 2 times/week throughout the experimental period. The shaved skin was rubbed once daily for seven times/week with CO oil over a period of 13 weeks.

2.5. Mortality and clinical signs

Clinical signs of all test animals were recorded. Animals body weight was recorded twice daily in subchronic and once in a day in acute dermal toxicity study (DeLorme et al., 2005).

2.6. Hematological parameters and coagulation

The blood samples were collected from the retro-orbital plexus by using a capillary tube and collected in an ependroff having anticoagulant under light anesthesia (diethyl ether, CDH, New Delhi, India) (Van Herck et al., 2001). Hematological auto-analyzer (Beckman Coulter Ltd., Mumbai, India) was used to measure hematology parameters including white blood cell (WBC) count, % lymphocytes, hemoglobin (Hb) concentration, total red blood cell (RBC) count,