Regulatory Toxicology and Pharmacology 86 (2017) 167–176

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Safety and tolerability of a dried aqueous spearmint extract

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ARTICLE INFO

Article history:
Received 14 December 2016
Received in revised form
28 February 2017
Accepted 3 March 2017
Available online 7 March 2017

ABSTRACT

Spearmint (*Mentha spicata* L.) and spearmint extracts are Generally Recognized as Safe (GRAS) for use as flavoring in beverages, pharmaceuticals, and confectionaries. Studies of spearmint extracts in humans and animals have reported conflicting results with respect to toxicity. Since the chemical composition of these extracts was not reported and the spearmint source material was different, the relevance of these existing data to evaluating the risks associated with ingestion of a dried aqueous spearmint extract standardized to rosmarinic acid is not clear. Hence, the safety and tolerability of the dried aqueous spearmint extract was evaluated as part of a double-blind, randomized, placebo-controlled trial in healthy adults with age-associated memory impairment. Ingestion of both 600 and 900 mg/day for 90 days had no effect on plasma levels of follicular stimulating hormone, luteinizing hormone, or thyroid stimulating hormone, or other safety parameters including vital signs, plasma chemistry or whole blood hematology values. Additionally, there were no reported severe adverse events, no significant between-group differences in the number of subjects reporting adverse effects and the adverse events reported could not be attributed to ingestion of the extract. These results therefore show that ingestion of the aqueous dried spearmint extract is safe and well-tolerated.

1. Introduction

Spearmint (*Mentha spicata* L.) and spearmint extracts are Generally Recognized as Safe (GRAS) for use as flavoring agents in beverages, pharmaceuticals, and confectionaries (21 CFR 182, 21 CFR 182.20). Spearmint and spearmint-derived products have also been used to treat gastrointestinal and respiratory conditions (Ulbricht et al., 2010), improve memory and learning (Wilkinson et al., 2002; Tucha et al., 2004; Johnson and Miles, 2008; Farr et al., 2016; Reagan-Shaw et al., 2008; Nieman et al., 2014), reduce joint inflammation and pain (Connelly et al., 2014; Pearson et al., 2012), and as a potential treatment for hirsutism (Akdoğan et al., 2007). Importantly, a variety of adverse effects have been reported in rodents following the ingestion of spearmint and spearmint extracts, including increases in oxidative stress, lipid peroxidation, uterine damage, kidney and liver malformations, follicular stimulating hormone (FSH) and luteinizing hormone (LH), decreased testosterone levels, and pituitary and thyroid hypertrophy (Akdogan et al., 2003, 2004a, 2004b; Güney et al., 2006; Lasrado et al., 2015; Grant, 2010).

Spearmint contains a wide variety of compounds. Carvone is the most notable compound because it gives the plant its distinctive aroma. Another is rosmarinic acid (RA), which is a polyphenolic antioxidant with immunosuppressant, hepato- and neuro-protective, anti-inflammatory, antibacterial and antiviral activities (Petersen and Simmonds, 2003; Iuvone et al., 2006; Renzulli et al., 2004; Yun et al., 2003; Tewtrakul et al., 2003). Recently, two proprietary non-genetically modified lines of spearmint that produce naturally higher levels of RA relative to commercial spearmint, were developed using conventional breeding practices (Narasimhamoorthy et al., 2015). Compositional studies of oil extracts derived from these two lines of spearmint show that they produce detectable levels of borneol, mintlactone, and γ-elemene. These compounds were not detectable in oil extracts made from commercially-available spearmint. In addition, they contain lower levels of α-terpinene, limonene, and γ-terpinene, and higher levels of myrcene, 1,8-cineole, β-caryophyllene, and α-cubebene compared to commercial spearmint, and do not contain carvone and β-bourbonene (Narasimhamoorthy et al., 2015).

The toxicity of the dried aqueous extract derived from the two

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http://dx.doi.org/10.1016/j.yrtph.2017.03.005
0273-2300/

proprietary lines of spearmint, also tested herein, was previously evaluated in an Organization for Economic Cooperation and Development (OECD)-compliant 90-day rat toxicology study conducted in Sprague-Dawley rats (Lasrado et al., 2015). The extract contained 15.4% RA and a variety of other water soluble phenolic constituents, but no detectable levels of camphor, eucalyptol, limonine, carvone, pulegone, menthofuran, and carvacrol. Although there were no treatment-related clinical signs or adverse effects in body weight, feed consumption, neurological parameters, hematology, clinical chemistries, gross pathology, or histopathology at any of the doses of extract tested (0, 422, 844, and 1948 mg/kg bw/day), the ingestion of greater than 844 mg/kg bw/day caused significant and dose-dependent increases in the absolute and relative weights of the pituitary glands in male rats and thyroid glands in male and female rats. Significant, but not dose-dependent increases were also found in the relative weights of the hearts and livers of male rats and the absolute and relative weights of the salivary glands in female rats treated with 1948 mg/kg bw/day. Importantly, because no treatment-related histopathological changes were found and it is known that rats are more sensitive to thyroid toxicants than humans (Dohler et al., 1979; Miller et al., 2009), the increases in pituitary and thyroid weights were considered non-adverse and the no observed adverse effect level (NOAEL) was established at the highest dose tested, 1948 mg/kg bw/day. Utilization of a 100-fold safety factor to the NOAEL, an acceptable daily intake (ADI) for the dried aqueous spearmint extract can be established at 19.48 mg/kg bw/day or 1363 mg/day for a 70 kg human, based on this study. An intake at this level would expose consumers to approximately 3 mg/kg bw/day of RA.

The pituitary gland is composed of the posterior and anterior pituitary glands. The anterior pituitary is often referred to as the "master gland" because, together with the hypothalamus, it secretes a variety of hormones that regulate the activity of cells in distant endocrine glands. In contrast, the posterior pituitary acts as a distribution site for the hypothalamus, releasing the hormones oxytocin and vasopressin into the blood stream. The hormones produced by the anterior pituitary gland include prolactin (PRL), growth hormone (GH), adrenocorticotropin (ACTH), LH, FSH, and thyroid-stimulating hormone (TSH). PRL stimulates the mammary glands to produce milk, GH stimulates growth, ACTH stimulates cortisol release by the adrenal gland, TSH regulates the growth and proliferation of cells in the thyroid gland, and LH and FSH both regulate menstruation and ovulation. Because it is well known that physiological differences in the pituitary-thyroid axis, also known as thyroid homeostasis, exist between rats and humans (Dohler et al., 1979; Miller et al., 2009), there is significant uncertainty when setting human health exposure criteria for extracts or compounds that are potentially thyroid active chemicals based on the results of rodent toxicology studies. To understand the safety and tolerability of the dried aqueous spearmint extract, and its effect on pituitary, thyroid, and cognitive function, safety outcomes were included as part of a randomized, double-blind, placebo-controlled, parallel group clinical trial conducted in healthy subjects with age-associated memory impairment. Hence, only the safety and tolerability results, including those evaluating pituitary and thyroid function, are reported herein.

2. Materials and methods

2.1. Materials

The dried aqueous spearmint extract (standardized to 14.5–17.5% rosmarinic acid) was prepared from two proprietary spearmint lines established by selective-breeding (Narasimhamoorthy et al., 2015) and supplied by Kemin Foods L.C. (Des Moines, IA). Briefly, the proprietary spearmint crop is grown in the United States in accordance with Good Agricultural Practices (GAP). The harvested leaves are dried via a proprietary process, milled and extracted under heat with water and phosphoric acid. The extract is subsequently filtered and dried to obtain the dry spearmint extract, which is manufactured in compliance with current food Good Manufacturing Practices (cGMP). Previously conducted compositional analyses of this extract revealed the presence of polyphenols and their derivatives, including but not limited to rosmarinic, salvianolic, lithospermic, and caftaric acids (Cirlini et al., 2016), which are present at levels ranging from 24 to 32% total phenolics when expressed as gallic acid equivalents (GAE) using the Folin-Coicalteu Reagent (FCR) method (Singleton et al., 1999). In addition, analyses of the monoterpene constituents in the extract revealed that carvacrol is present at approximately 20 ppm and the levels of camphor, eucalyptol, limonene, carvone, menthofuran and pulegone are undetectable (limit of detection of 5 ppm) (Narasimhamoorthy et al., 2015; Lasrado et al., 2015). Proximate analyses have shown that the extract contains approximately 64% carbohydrate, 7% protein, 1% fat, 5% moisture and 23% ash (Kemin Foods, unpublished data). Importantly, this dried aqueous spearmint extract was manufactured from the same proprietary spearmint lines, using the same production methods, and adhered to the same product specifications as the dried spearmint extract used by Lasrado et al., 2015, and Nieman et al., 2014.

To conduct this trial, the extract meeting, was packaged into 300 and 450 mg capsules by Five-Star Pharmacy (Clive, IA). The placebo capsules were prepared from microcrystalline cellulose. The cognitive assessments administered during the study were the Memory Assessment Clinic Scale Questionnaire (MAC-Q) (Crook et al., 1992; Dunbar et al., 2007), the Cognitive Drug Research (CDR) test battery (Simpson et al., 1991), the Verbal Paired Associates (VPA) I and II portions of the Wechsler Memory Scale (WMS) IV (Wechsler, 2009; Dunbar et al., 2007), the Mini Mental State Examination (MMSE) (Folstein et al., 1975; Mitrushina and Satz, 1991), the Profile of Mood States (POMS) questionnaire (McNair et al., 1992, 2003), the Bond-Lader Visual Analog Scale (VAS) of Mood and Alertness (Bond and Lader, 1974), the Leeds Sleep Evaluation questionnaire (LSEQ) (Parrott and Hindmarch, 1978), and the Subject Global Improvement (SGI) questionnaire (Dunbar et al., 2011; Lieberman et al., 2013).

2.2. Study design

The study was conducted as a randomized, double-blind, placebo-controlled study designed to evaluate the effects of the dried aqueous spearmint extract. Healthy men and women aged 50–70 yrs, with Age-Associated Memory Impairment (AAMI) (defined as a score of $\geq$25 on the MAC-Q, a score of $\leq$29 and $\leq$ 9 on the VPA I and II portion of the WMS-IV, and a score of $\geq$24 on the MMSE, a body mass index (BMI) of 18.5–35 kg/m^2, at least a high school diploma, and who were willing to maintain their habitual diet, exercise routines, comfortably abstain from tobacco products for at least 1 h prior to and throughout the duration of the test visits, willing to eat breakfast at test visits and at home on a daily basis throughout the study period, comfortably abstain from caffeine prior to and throughout the duration of all clinic visits, abstain from alcohol consumption and avoid vigorous physical activity for 24 h prior to all clinic visits and obtain consistent sleep duration the evening before study visits throughout the study period were enrolled. Subjects having uncontrolled hypertension (systolic blood pressure $\geq$ 160 mm Hg or diastolic blood pressure $\geq$ 100 mm Hg), abnormal laboratory test results of clinical significance (at the discretion of the Investigator), a history or presence of clinically important cardiac (including coronary heart