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Phytochemical analysis and effects on ingestive behaviour of a *Caralluma fimbriata* extract

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ABSTRACT

Caralluma fimbriata Wall. is currently used as a "natural slimming" food supplement, likely due to its content in pregnane glycosides. In the present study, a commercially available *Caralluma fimbriata* extract (Slimaluma®; CFE, 100 mg/kg) has been evaluated for its ability to affect the ingestive behaviour in female rats, also with reference to the modulation of the brain neuropeptides NPY and ORX.The interference of CFE with α-amylase and lipase enzymes has been investigated *in vitro*, as possible peripheral mechanism of action. Also, the chemical composition of CFE has been assessed by NMR and spectrophotometric analysis.

Results from *in vivo* study showed that CFE induced effects neither on blood parameters, nor on liver and gut histomorphology. Interestingly, a reduction in body weight gain with an increase in water intake and hypothalamic levels of NPY and ORX peptides were found. Phytochemical analysis, showed CFE contained about 12% of pregnane glycosides and 1.3% of polyphenols.

Present results suggest possible effects of *C. fimbriata* on ingestive behaviour, likely mediated by central and peripheral mechanisms.

1. Introduction

Obesity and overweight are growing public health problems and their management is becoming of great clinical importance (Karatsoreos et al., 2013). Preliminary approaches in the management of overweight patients are preventive measures (diet, eating habits, etc.) and lifestyle changes (e.g., physical activity, specific exercises). The pharmacological approaches intended to counteract overweight include two main classes of drugs: the appetite suppressants (sibutramine, amphetamine derivatives, etc.) and the inhibitors of nutrient absorption (i.e., orlistat). However, pharmacological treatments are often accompanied by serious adverse effects (Astell et al., 2013), so that it is not surprising that consumers commonly turn to herbal dietary supplements.

Among natural agents used as appetite suppressants, *Caralluma fimbriata* Wall. [syn. *Caralluma adscendens* var. *fimbriata* (Wall.) Gravely & Mayur. – Fam. Apocynaceae] is one of the less studied. It is an edible succulent cactus used for centuries by Western Indians as both a food and an appetite suppressant (Yuliana et al., 2011; Gooda Sahib et al., 2012). Nowadays it is used as a "natural" slimming food supplement, and a standardized extract of *C. fimbriata* (Slimaluma®) has been patented (Yuliana et al., 2011; Dutt et al., 2012). Pregnane glycosides seem to be responsible for the slimming properties of this plant (Kuriyan et al., 2007; Astell et al., 201). These compounds were also found in other medicinal plants such as *Hoodia gordonii*, that are reported to affect food intake (Gooda Sahib et al., 2012). Moreover, other phytochemicals, including flavonoids, saponins etc. have been found in *C. fimbriata* (Astell et al., 2013).

In spite of its traditional use, the scientific evidence on the *C. fimbriata* efficacy is poor, and the majority of the available

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literature should be examined critically since a high proportion of studies are sponsorized and/or funded just by the company involved in preparation and/or marketing of the *C. fimbriata* commercial extracts (Kuriyan et al., 2007; Odendaal et al., 2013; Lakshmi et al., 2014; Rajendran et al., 2014; Sudhakara et al., 2014).

Furthermore, taking into account the well-known problems of standardization of the herbal preparations marketed as food supplements (Wolsko et al., 2005; Garg et al., 2012), and considering that a poor quality can affect the safety and efficacy of herbal drugs (Chan, 2003), ascertaining the true composition of end products represents an important goal.

Based on the above considerations and without any conflict of interest, present study was aimed to characterize the chemical composition of a commercially available extract of *C. fimbriata* (CFE), by modern analytical techniques as suggested by EMA (EMEA/HMPC/253629/2007). Furthermore, in order to investigate the activity of this extract in feeding behaviour, we tested the effect of CFE on food and water intake in a non-obese rat model, after a sub-chronic treatment. Being pregnane glycosides recognized to have appetite-suppressant effects, probably via enhanced hypothalamic signaling (MacLean and Luo, 2004), and considering the key role of hypothalamus on the regulation of energy homeostasis (Williams et al., 2001), we also evaluated the effect of *C. fimbriata* on the hypothalamic expression of neuropetide Y (NPY) and orexin (ORX), two factors playing a crucial role on feeding attitude (Fick and Belsham, 2010). Finally, we assessed the capability of *C. fimbriata* extract to inhibit the α-amylase and lipase enzymes, as possible peripheral mechanisms of action.

2. Material and methods

2.1. Herbal extract

CFE (a dry ethanolic extract from the aerial parts of *C. fimbriata* named Slimaluma®, lot no. FAIT120503613) was purchased by the FAGRON Company (Bologna, Italy). It appears as a brown and water-soluble powder. According to the technical data sheet, the extract is reported to contain 27.5% of total pregnane glycosides. The quality assessment of the product, carried out by the Company, excluded the presence of germs (e.g., *Escherichia coli*, *Salmonella* spp, *Pseudomonas aeruginosa*, *Staphylococcus aureus*), molds, fungi and pesticides, while the amount of unavoidable heavy metals (i.e., Pb, Cd, As, Hg) was lower than the limits allowed.

2.2. Chemicals

Tannic acid (CAS 1401-55-4; 99.9% purity), sodium carbonate (Na_2CO_3; CAS 497-19-8; 99.9% purity), Folin-Ciocalteu's phenol reagent, and aluminum chloride hexahydrate ($AlCl_3$ x 6 H_2O; CAS 7784-13-6; Ph. Eur. purity) were purchased from Merck (Darmstadt, Germany), while α-amylase, α-glucosidase, lipase, potato starch, 4-nitrophenyl a-D-glucopyranoside (PNG), *p*-nitrophenylpalmitate (PNP), polyvinylpyrrolidone (PVPP), 3,5-dinitrosalicylic acid (DNSA), acarbose, orlistat, quercetin, 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (TSP), ethanol (EtOH), deuterium dioxide (DO2), deuterated methanol (CD3OD), the polyclonal rabbit IgG antibodies against NPY and ORX, the biotinilated goat IgG and all other chemicals, included those for histomorphological study, were provided by Sigma-Aldrich (Milan, Italy). Deuterium oxide (D-99%; Cat. No. DLM4-100) was from Cambridge Isotope Laboratorie Inc., Andover, MA 01810 USA. Chemicals for ultrastructural study were: glutaraldehyde, uranyl acetate and lead citrate (SIC, Rome, Italy) osmium tetroxide (Agar Scientific, Stansted, UK), propylene oxide (BDH Italia, Milan, Italy) and epoxy resin (Electron Microscopy Sciences, Hatfield, PA, USA). Products for clinical chemistry, including GLUC3 4483/190, GGT2 2721/122, ASTLP 7493/190, ALTLP 7388/190, ALP2 3752/190, CHOL2 9773/190, HDLC3 9803/190, TRIGL 7107/322, AMYL2 3742/122, LIPC 9590/322, TP2 3734/190 and LDHI2 4732/122 were provided by Roche Diagnostics, CH. Insulin rat and streptavidin peroxidase conjugate were from Demeditec Diagnostics (Kiel, Germany) and GE Healthcare (UK), respectively.

2.3. Phytochemical analysis

Owing to the variability of commercial preparations, the first step of the present study was to chemically characterize the extract. At this aim, nuclear magnetic resonance (NMR) spectroscopy was employed to evaluate the total pregnane (Tomassini et al., 2014) content, while colorimetric assays were performed to determine the amount of polyphenol compounds.

2.3.1. NMR spectroscopy

10 mg of CFE extract were dissolved in 600 μl D_2O/CD_3OD mixture (2: 1 ratio, respectively) containing TSP at the final concentration of 2 mM, as internal standard for chemical shift. All the NMR experiments were performed on a Bruker Avance III spectrometer operating at a Larmor frequency of 400,13 MHz for ^1H and 100 MHz for ^{13}C. Signal assignments were achieved by standard homonuclear 1H−1H TOCSY and heteronuclear 1H−13C HSQC and HMBC bidimensional experiments. 1D (one-dimensional) ^1H NMR spectrum was acquired with a *presat* pulse sequence for solvent suppression, a spectral width of 15 ppm, 64 k data points, 5.5 s of acquisition time, 128 scans and a repetition delay of 9.5 s in order to achieve full relaxation for all protons. 2D (two-dimensional) $^1H-^1H$ TOCSY (Total Correlation Spectroscopy) spectrum was acquired with a data matrix of 8 k x 256 data points, a spectral width of 15 ppm in both dimensions, 80 scans and a mixing time of 110 ms. 2D $^1H-^{13}C$ HSQC (Heteronuclear Single Quantum Correlation) spectrum was acquired with a data matrix of 8 k x 256 data points for hydrogen and carbon respectively, a spectral width of 15 ppm for hydrogen dimension and 200 for the carbon one, 128 scans and an average coupling constant of 145 Hz. Spectra processing was performed using Bruker software TOPSPIN for 2D experiments and ACD 12.0 for 1D ones.

2.3.2. Colorimetric determination of total polyphenols, tannins and flavonoids

Total polyphenols were determined according to Rababah et al. (2005), with minor changes. Each sample (20 μl of a solution 1 mg/ml of *C. fimbriata* extract in EtOH 50% v/v) was mixed with 100 μl of the Folin-Ciocalteau reagent (10% v/v) and incubated for 5 min. After addition of 80 μl of a sodium carbonate solution (7.5% w/v), the mixture was shaken and incubated for 2 h, then the absorbance was measured at 765 nm by a microplate reader (Bio-Rad, Hercules, CA, USA). The content of total polyphenols was expressed as tannic acid equivalents (TAE) per milligram of sample.

To determine the amount of tannins, 100 mg PVPP were added to 1 ml of an extract solution (1 mg/ml in EtOH 50% v/v). PVPP induces the formation of an insoluble complex with tannins, which precipitate. 20 μl of supernatant were used to determine the polyphenol content, as described above, while the tannin amount was obtained by the difference between total polyphenols and the polyphenols contained in the supernatant, and was expressed as TAE per milligram of sample.

Flavonoids were measured by the spectrophotometric method described by Meda et al. (2005) with minor changes. Briefly, the extract (50 μl of a solution 1 mg/ml), aluminum trichloride (20 μl of a solution 10% w/v in methanol), $NaNO_2$ (10 μl of a solution 5% w/v in deionized water) and NaOH (60 μl of a solution 1 M) were added