Phytomedicine 18 (2011) 1161–1169

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Spilanthes acmella ethanolic flower extract: LC–MS alkylamide profiling and its effects on sexual behavior in male rats

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ARTICLE INFO

Keywords:
Aphrodisiac
Male sexual behavior
N-Alkylamides
Nitric oxide
Spilanthes acmella
Testosterone

ABSTRACT

According to Indian Systems of Medicine, *Spilanthes acmella* (L.) Murr. (Family – Asteraceae), is considered effective in the treatment of sexual deficiencies especially due to ageing. In the present study, characterization of ethanolic extracts of the *Spilanthes acmella* flower and its effect on general mating pattern, penile erection and serum hormone levels of normal male Wistar albino rats were investigated and compared with sildenafil citrate. *In vitro* nitric oxide release was also investigated in human corpus cavernosum cell line. As *N*-alkylamides are a promising group, their profiling was performed using a gradient reversed phase high performance liquid chromatography/electrospray ionization ion trap mass spectrometry (HPLC/ESI-MS) method on an embedded polar column. MS^1 and MS^2 fragmentation data were used for identification purposes. For assessment of sexual behavior, animals were divided into five groups of eight male rats. The extracts (50, 100 and 150 mg/kg body weight/day) and sildenafil citrate (5 mg/kg body weight/day) (positive control) were administered orally for 28 days. The behavioral and sexual parameters were observed at days 0, 15, 28 and after a lapse of 7 and 14 days of discontinuance of drug treatment. Five *N*-isobutylamides, one 2-methylbutylamide and one 2-phenylethylamide were identified. The orally administered extract had a dose dependent positive effect on mounting frequency, intromission frequency and ejaculation frequency and the most significant effects ($p < 0.05$) were observed at 150 mg/kg treatment, even after a lapse of 7 and 14 days of discontinuance of drug treatment. A dose dependent effect was also observed on the FSH, LH and testosterone serum levels. With 150 mg/kg of ethanolic extract the values for FSH, LH and testosterone were 3.10 ± 0.25 mIU/ml, 6.87 ± 0.18 mIU/ml and 3.72 ± 0.12 ng/ml, respectively. *In vitro* nitric oxide release was 21.7 ± 2.9 μM, which was significantly higher compared to the control group ($p < 0.01$). Sildenafil citrate exhibited also a significant effect on NO release, but no effect on hormone levels of rats was observed. The aphrodisiac potential of an ethanolic *Spilanthes acmella* extract was demonstrated *in vitro* and *in vivo*. *N*-Alkylamides might attribute to the improved sexual potential. Study lends support to the traditional utilization of *S. acmella* as a sexual stimulating agent.

Introduction

Human search for sexual enhancers from natural substances is as old as civilization itself. Ancient history in most cultures helped society in its desire to improve the sexual experience as evident by writings in holy texts and sculptures in Hindu temples. For many years, people have searched for ways to achieve sexual desire, sexual health and sexual techniques (Jain et al. 2010).

Sexual function is an important component of quality of life and subjective well-being in humans. Sexual problems are widespread and adversely affect mood, and interpersonal functioning. The main problems are related to sexual desire and male erectile dysfunction. Successful treatment of sexual dysfunction may improve not only sexual relationships, but also the overall quality of life (Shin et al. 2010).

Sexual dysfunctions increase with ageing and etiological factors, including degenerative diseases, increase in injuries and stress associated with industrialized lifestyles. It can be treated by both medical and surgical modalities. However, plant-derived and herbal remedies continue to be a popular alternative (Rowland and Tai 2003). For several hundred years, people around the world have

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doi:10.1016/j.phymed.2011.06.001

used locally grown plants as supplements to energize, vitalize, and eventually to improve male sexual functions.

The availability of the large number of sex-improving drugs in the traditional Ayurvedic System of Medicine is a unique and distinctive feature of this system. In this system, Rasayana drugs constitute a special category, the uses of which are advocated for rejuvenation, revitalization and longevity. A special class of Rasayana drugs is known as Vrishya or Vajikarana. They are associated with an improvement of male sexual potency and thereby ensure a *supraja*, or better progeny. Traditionally, the main aim of using Vajikaran was to achieve successful copulation for healthy reproduction, along with an improvement in sexual pleasure as an additional benefit. Vajikaran drugs are specially recommended to people suffering from sexual insufficiency and people in advanced age losing interest in sexual act or failing in sexual performance (Sharma et al. 2010). Besides having many specific drugs for enhancing sexual functions, the most commonly used is "akarkara". Different plants are being referred to as akarkara, but the most prominent one is *Spilanthes acmella* (*S. acmella*). The akarkara plants are empirically used as powerful aphrodisiac in traditional medicine practice in cases of sexual debility or depressed desire.

S. acmella has long been used in the traditional Indian Systems of Medicine for the treatment of various sexual inadequacies and is claimed to improve sexual functions in man. Other ethnopharmacological claims associated with *S. acmella* include its usage in treatment of rheumatism, inflammation, stimulant and as sialagogue for stammering, tongue paralysis, stomatitis, toothache, headache and treatment of asthma, rheumatism, fever, sore throat and hemorrhoids and gum infections (Prachayasittikul et al. 2009; Vijeyaanandhi et al. 2007). In addition, its extract is traditionally added to nutritional supplement and cosmetics to accelerate repair of wrinkles which are caused due to vasoconstriction of skin. Pharmacologically, *S. acmella* is a potential vasodilator, antioxidant (Wongsawatkul et al. 2008) and immunomodulator (Savadi et al. 2010). Previous studies have demonstrated its diuretic, antibacterial, and anti-inflammatory activities (Ratnasooriya et al. 2004). Recently, it has also been demonstrated that spilanthol in *S. acmella* extracts permeates the skin and buccal mucosa (Boonen et al. 2010a,b).

Phytochemical analysis of ethanolic extract of *S. acmella* revealed that it is rich in *N*-alkylamide. The main *N*-alkylamide of *Lepidium meyenii* (Maca), known as macamide, and of *Anacyclus pyrethrum*, known as pellitorine, have been found effective in improvement of sexual behavior (Cicero et al. 2001; Sharma et al. 2010). Keeping in view the growing popularity and market interest in herbs for sexual problems, and lack of scientific studies on *S. acmella*, present investigation was undertaken to evaluate the scientific foundation for the concept of Vajikaran Rasayana.

Materials and methods

Animal stock

The protocol for experimentation was approved by Institutional Animal Ethics Committee of Dr. Hari Singh Gour University, Sagar, India (Animal Eths Comm/IE/98/Reg No379/01/ab/CPCSEA) and was in accordance with international standard on the care and use of experimental animals. Inbred, 40 sexually active Wistar strain male albino rats, weighing 150–180 g were used for the present study. Female rats from the same strain rats, used as stimulus for evaluation of sexual behavior, were prepared for experimentation, using the method reported by Agmo (2003). In brief, before all testing sessions, female estrus was induced by administration of estradiol benzoate (25 μg/rat), followed by progesterone (250 μg/rat), 48 h later. Females were used between 4 and 8 h after the progesterone administration. Both steroids were purchased from Sigma (St. Louis, MO, USA). They were dissolved in arachis oil (Kriti, India) and injected subcutaneously in a volume of 0.1 ml/rat. The rats were housed at room temperature (24 ± 2 °C) on a reversed day-night cycle (dark from 06:00 to 18:00) and relative humidity of 50–55%. They were fed with a standard pellet diet and water *ad libitum*.

Preparation of extracts

The plant *S. acmella* was identified and authenticated at Department of Botany, Dr. H.S. Gour University Sagar (M.P.) India and a herbarium has been deposited there as well (Herbarium No. Bot/02-8/2008). The flowers of the plant were collected from the area in vicinity of the campus and were dried at room temperature (25–35 °C). Next, the flowers were reduced to powder and passed through a sieve (60 mesh), fed in a soxhlet extractor and extracted with ethanol (95%) till complete exhaustion. The extract was collected and dried under vacuum by using a rota vapor (Heidolph, Germany). The yield of the ethanolic extract was found to be 6.1% (w/w). Before oral administration, the *S. acmella* extract was suspended in 1% sodium CMC.

HPLC/ESI-MS analytical N-alkylamide profiling

Analytical samples were prepared by dissolving the extract and reference material in a acetonitrile:water mixture (50/50, v/v), followed by filtration over a 0.45 μm nylon HPLC filter (Whatman, Dassel, Germany). The LC/MS apparatus consisted of a Spectra System SN4000 interface, SCM1000 degasser, P1000XR pump, AS3000 autosampler and was equipped with a Finnigan LCQ Classic ion trap mass spectrometer in positive ion mode (all Thermo, San José, CA, USA). Data were acquired using Xcalibur 2.0 software (Thermo, San José, CA, USA). A Prevail RP C_{18} column (250 mm × 4.6 mm, 5 μm) with suitable guard cartridge (4.6 mm × 7.5 mm, 5 μm) (both from Grace, Lokeren, Belgium) was used (Boonen et al. 2010b). The injection volume was 25 μl. The flow rate was set to 1.0 mL/min and the linear gradient used was as follows (where A = 1% acetic acid in ultrapure water and B = HPLC grade acetonitrile): t = 0 min, A:B (80:20, v/v); t = 0–150 min, A:B (10:90, v/v); t = 150–151 min, A:B (80:20, v/v); t = 151–166 min, A:B (80:20, v/v). ESI was conducted using a capillary voltage of 3 kV. Nitrogen was used as the sheath and auxiliary gas with the heated capillary set at 275 °C. MS–MS spectra were obtained by collision induced dissociation (CID) of the parent m/z, with the relative collision energy set at 35%. Identification was based on the m/z values and fragmentation ions, while quantification was performed using a laboratory reference material ("A. Vogel *Spilanthes*", Biohorma, Lummen, Belgium) with spilanthol (0.11%, w/w) as the reference biomarker.

Treatment

Male rats were divided into five equally spaced groups. Group I served as blank and received only vehicle, *i.e.* normal saline. Groups II, III and IV were given an oral daily dose of 50, 100 and 150 mg/kg *S. acmella* extract, respectively. Group V rats were administered daily and orally with 5 mg/kg sildenafil citrate (a generous gift of Sun Pharma, India). The course of the treatment was 28 days.

Toxicity study

Two groups, containing six male and six female rats were used. The test group received an oral dose of 2 g/kg *S. acmella* extract. The rats in the control group received 1 ml of tap water. Behavioral parameters such as convulsions, sedation, and hyperactivity, grooming and accelerated breathing were observed. The animals