Journal of Ethnopharmacology 148 (2013) 210–217

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

In vitro and *in vivo* aphrodisiac properties of *Corchorus depressus* Linn. on rabbit corpus cavernosum smooth muscle relaxation and sexual behavior of normal male rats

Sandeep Kataria [a,*], Dilsher Kaur [b], Shaival Kamalaksha Rao [c], Ravi K. Khajuria [d]

[a] *Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur, Rajasthan, India*
[b] *Department of Pharmaceutical Chemistry, I.S.F. College of Pharmacy, Moga, Punjab, India*
[c] *Department of Pharmacognosy, C.U. Shah College of Pharmacy & Research, Surendranagar, Gujarat, India*
[d] *Instrumentation Division, Indian Institute of Integrative Medicine, Jammu, J&K, India*

ARTICLE INFO

Article history:
Received 21 September 2012
Received in revised form
8 March 2013
Accepted 4 April 2013
Available online 21 April 2013

Keywords:
Aphrodisiac
Sexual behavior
Libido
Potency
Corchorus depressus Linn

ABSTRACT

Ethnopharmacological relevance: *Corchorus depressus* Linn. has been used as an aphrodisiac in traditional Indian medicine to treat male sexual dysfunction and impotency.
Aim of the study: The petroleum ether, chloroform, ethyl acetate, n-butanol and aqueous fractions of 95% methanol extract of *Corchorus depressus* were screened initially for their *in vitro* aphrodisiac activity on rabbit corpus cavernosum smooth muscle. The chloroform fraction (CDC) was found to be the most active and therefore investigated further on general mating behavior, libido and potency of normal male Wistar albino rats in comparison with the standard drug, Sildenafil citrate.
Materials and methods: Animals were divided into the following groups: Control, SC CDC 100, CDC 200, and CDC 400, who received saline, Sildenafil citrate or the chloroform fraction of *Corchorus depressus* at doses of 100, 200 or 400 mg/kg b.wt., respectively. The route of administration for all the groups was oral dosing, which was once in a day for 45 days. To analyze the mating behavior, female rats with estrus phase were used.
Results: The chloroform fraction of methanolic extract of *Corchorus depressus* significantly reduced ML, IL, PEI and III. There was a significant increase in the MF, IF and EL and serum testosterone levels throughout the study period. The potency test significantly increased erections, quick flips, long flips and total reflex. *In vitro* aphrodisiac activity was significantly higher in chloroform fraction at a concentration of 25.0 mg/ml, which induced 71.4% relaxation. The combined results of the above mentioned models indicate that the chloroform fraction of *Corchorus depressus* produces a significant increase in sexual activity as exhibited by 25 mg/ml *in vitro* and 400 mg/kg *in vivo*. In comparison with the control, all the drug-treated groups have shown drug-induced effects for a few parameters.
Conclusions: *In vitro* and *in vivo* studies provide valuable experimental evidence that the chloroform fraction of methanolic extract of *Corchorus depressus* possesses aphrodisiac property. This study further substantiates the ethnopharmacological claims of *Corchorus depressus* as a sexual stimulating agent and offers a significant potential for studying the effect on male sexual response and its dysfunctions.

1. Introduction

Aphrodisiacs are substances that stimulate or increase sexual desire and sexual performance, where the former is controlled and regulated by the central nervous system which integrates tactile, olfactory, auditory, and mental stimuli, while sexual performance, which is quite different and not always dependent on sexual desire, is also called erectile performance or capacity. Even if sexual desire remains strong erectile dysfunction can occur, wherein sexual performance depends on a neurovascular event via the hemodynamic mechanisms of penile erection (Zamble et al., 2008). Though sexual dysfunction can be treated by both medical and surgical treatment modalities, plant-derived and herbal remedies continue to be a popular alternative for men and women seeking to improve their sexual life despite the availability of effective conventional medical treatments.

Different varieties of plants have found their use as sexual stimulants in traditional medicine of many countries (Suresh et al., 2009). Ang et al. (2003) report the use of *Aristolochia indica, Crocus sativus, Alpinia galanga* and *Allium cepa* as potent aphrodisiacs in

* Corresponding author. Tel.: +91 9785120002; fax: +91 291 222151.
E-mail address: katariasandy@gmail.com (S. Kataria).

0378-8741/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2013.04.011

Hindu medicine. The continued search for new botanicals with aphrodisiac activity is still attractive because they are readily accessible, affordable and less toxic. *Corchorus depressus* is one such plant claimed to have sex enhancing effect in the folk medicine of Rajasthan, India, with no experimental or clinical data in the open scientific literature.

Corchorus depressus Linn., (CD) known as *Baphuli,* is a multipurpose herb of Tiliaceae family that is used extensively for the treatment of sexual dysfunction (Jain et al., 2004) when crushed with sugar candy and taken orally with goat's milk. The plant is rich in flavonoids such as apigenin, luteolin, depressonol A, depressonol B (Zahid et al., 2002), quercetin and kaempferol (Harsh and Nag, 1988). Triterpenes and sterols (Depressoside A and B, β-sitosterol, etc.) were found in the whole plant, root and fruit of CD (Nag and Harsh, 1982; Ahmad et al., 1998). Over the years, pharmacological evaluations of this plant showed that it exhibited antimalarial (Simonsen et al., 2001), antifungal, antibacterial (Harsh et al., 1983; Harsh and Nag, 1988), analgesic and antipyretic (Vohora et al., 1981) activities. Keeping in view the growing popularity and market interest in herbs for sexual problems, and lack of scientific studies on *Corchorus depressus*, the present study was designed to address these issues to lend support to the existing information pertaining to the beneficial effect of this plant in treating sexual disorder. The methanol extract of *Corchorus depressus* was fractionated into petroleum ether, chloroform, ethyl acetate, n-butanol and aqueous fractions and these were screened for their *in vitro* aphrodisiac activity. This study was conducted to investigate whether these fractions of *Corchorus depressus* have a direct relaxing effect on rabbit corpus cavernosum smooth muscle. In this study, the chloroform fraction of *Corchorus depressus* was found to be the most active one and was further investigated in sexual behavior, potency and libido, acute toxicity, organ morphology, testosterone and sperm analysis with its probable gastric ulceration and adverse effects in sexually normal male albino rats.

2. Materials and methods

2.1. Chemical

Estradiol benzoate and progesterone were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sildenafil citrate was obtained as a gift sample from Zydus Cadila (Ahmedabad, India). All other chemicals used were of analytical grade (Sigma-Aldrich or Merck).

2.2. Plant material

The whole plant of *Corchorus depressus* L. was collected from the area in the vicinity of the Dausa district, Rajasthan, India, in September 2010. A voucher specimen (JNU/JODHPUR/CD/SK-1), identified by Mr. P.M. Padhye, was deposited at Department of Botany, Botanical Survey of India (BSI), Jodhpur, Rajasthan, India. Plant material was washed with distilled water to remove epiphytes and dirt particles and dried at room temperature (25–35 °C). The dried plant material was manually ground to a fine powder.

2.3. Extraction of solvent fractions

Thirty one hundred grams of shade dried whole plant powder was extracted with 95% methanol by Soxhletion process. The yield of methanol extract (ME) was 248 g (8%). The ME was partitioned between petroleum ether and water (6:1) using a separating funnel. This mixture was thoroughly mixed for 15 min and after 6 h the petroleum ether fraction (PE) was collected. The aqueous layer was further fractionated with chloroform (CF), ethyl acetate (EA) and then with n-butanol (NB) as described by Arumugam et al. (2008) with a slight modification. All fractions were concentrated in a rotary evaporator. The yield of these fractions was 37.295, 26.3, 43.4 and 77 g, correspondingly and constituted about 15%, 11%, 17.5% and 31% of the methanol extract. The aqueous fraction (AF) was lyophilized and found to weigh 59.4 g (24% of ME).

2.4. Preliminary phytochemical screening

A preliminary phytochemical screening was carried out using various tests for identification of the main constituents of the active chloroform fraction of *Corchorus depressus* such as alkaloids, steroids, saponins, flavonoids, and tannins. Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) analysis of the chloroform fraction were performed in order to characterize the fraction. For HPTLC analysis, silica gel GF_{254} precoated plates from E-Merck were used with toluene:ethyl acetate:glacial acetic acid:formic acid (2:1:1:0.75 v/v) as the solvent system. The sample was applied on a thin layer chromatography (TLC) plate using a Linomat V applicator. The length of the chromatogram run was 8 cm. After developing the TLC plates were dried by air blowing. The densitometric analysis was performed on a Camag TLC scanner in the absorbance mode at 194 nm (Kataria et al., 2011).

2.5. Animals

Twelve-week-old female (body weights around 175–200 g) and male (body weights around 225–250 g) albino rats of Wistar strain were used for the present study. All animal experiments were performed as per the protocols and recommendation of the Institutional Animal Ethics Committee of Pinnacle Biomedical Research Institute, Bhopal, India (Animal Eths Comm/IE/Reg no. 1283/c/09/CPCSEA, Protocol Approval Ref. no, PBRI/11/IAEC/PN-159A) and were in accordance with international standards on the care and use of experimental animals. Female rats from the same strain, used as stimulus for evaluation of sexual behavior, were prepared for experimentation, using the method of Anders (1997).

2.6. Preparation of drug solution and route of administration

Both steroids, estradiol benzoate and progesterone, were dissolved in arachis oil and injected subcutaneously in a volume of 0.1 ml/rat. In brief, before all testing sessions, female estrus was induced by successive administration of estradiol benzoate (25 mg/rat) followed by progesterone (250 mg/rat), within a 48 h gap. Females were used between 4 h and 8 h after progesterone administration. The rats were housed singly in separate standard cages and maintained under standard laboratory conditions (temperature 24–28 °C, relative humidity 60%–70%, and 12 h light–dark cycle) with free access to solid pellet diet and water *ad libitum* throughout the study.

2.7. Smooth muscle relaxation bioassay

Strips of rabbit corpus carvernosal smooth muscle were dissected and mounted in an organ-bath chamber containing Krebs-PSS solution with the following composition: NaCl = 7.01 g/l, KCl = 0.34 g/l, KH_2PO_4 = 0.1 g/l, $NaHCO_3$ = 1.99 g/l, $CaCl_2$ = 0.2 g/l, $MgSO_4$ = 0.3 g/l and glucose = 1.8 g/l. One end of the muscle was secured to the inside case of the perfusion bath and the other end to the thin wire connected to a Harvard isotonic force transducer for isotonic tension measurements. Changes in isotonic tension