ELSEVIER

Contraception 75 (2007) 71–78

Contraception

Original research article

Chenopodium album seed extract: a potent sperm-immobilizing agent both in vitro and in vivo

Shrabanti Kumar[a], Shampa Biswas[b], Debayan Mandal[a], Heramba Nandan Roy[b], Smritinath Chakraborty[b], Syed N. Kabir[b], Sukdeb Banerjee[a], Nirup B. Mondal[a,*]

[a]*Steroid and Terpenoid Chemistry Department, Indian Institute of Chemical Biology, Jadavpur, Kolkata 700 032, India*
[b]*Department of Reproductive Biology Research, Indian Institute of Chemical Biology, Jadavpur, Kolkata 700 032, India*

Received 11 November 2005; revised 30 May 2006; accepted 21 July 2006

Abstract

Purpose: Aqueous decoction of *Chenopodium album* seeds (CAD) was assessed for its sperm-immobilizing and contraceptive efficacy in laboratory mammals.

Method: Spermicidal efficacy was evaluated in vitro by a modified Sander–Cramer test. The mode of spermicidal action was assessed by (a) supravital and double fluoroprobe staining of sperm, (b) hypoosmotic swelling tests and (c) transmission electron microscopy. Contraceptive efficacy was evaluated by intrauterine and vaginal application of CAD in rats and rabbits, respectively, followed by their mating and evaluation of pregnancy outcomes.

Results: The minimum effective concentration of CAD that induced instantaneous immobilization of rat spermatozoa in vitro was 2 mg/mL. The mechanism of CAD action involved disintegration of sperm plasma membrane and dissolution of acrosomal cap causing sperm death. Fertilization of oocytes and establishment of implantation were prevented in the uterine horn that was administered with CAD, while these events occurred unhindered in the untreated contralateral side. In rabbit, intravaginal application of CAD significantly blocked the establishment of pregnancy.

Conclusion: CAD possesses appreciable spermicidal potential, which may be explored as an effector constituent of vaginal contraceptive.

Keywords: *Chenopodium album*; Chenopodiaceae; Sperm immobilization; Sperm viability; Spermicide viability; Spermicide

1. Introduction

The population explosion is a global problem that poses significant threat to the quality of life, more particularly in the underdeveloped and developing countries. The key requirement to combat this grave situation is the availability of suitable contraceptive devices that people would adopt to control birth rate. A number of contraceptive methods are available; however, acceptability of these methods has quite often been limited by their associated untoward side effects, failure rate or irreversibility. This prevailing situation demands the development of newer contraceptive options that should be simple, safe, reversible and cost-effective and, overall, that would be acceptable to the majority of the world population irrespective of culture, religion and race. Of the contraceptive strategies available, the barrier methods with spermicide are the second most commonly used option that has gained recent surge of interest because of its temporal use with possible avoidance of systemic effects, over-the-counter availability and easy usage [1]. The currently available spermicidal contraceptive formulations are effective, but their repeated use is associated with vaginal/cervical irritation or even ulceration and disturbance of the normal vaginal microflora that facilitates microbial infection and renders the subject susceptible to sexually transmitted diseases [2]. Therefore, development of safer spermicidal preparations is, at present, greatly needed.

The contemporary trends of drug discovery including reproductive health care emphasize investigation of the terrestrial and marine environments for potent molecules, particularly in developed countries [3]. India, the center of mega-biodiversity with her varied climatic, altitudinal and

* Corresponding author. Tel.: +91 33 2473 3491x121; fax: +91 33 2473 5197.
E-mail address: nirup@iicb.res.in (N.B. Mondal).

0010-7824/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.contraception.2006.07.015

soil conditions, possesses 3500 medicinal plants [4]. Efforts are being paid to look into the practicability of employing these herbs for fertility regulation [5,6]. Saponins and terpenoids of plant origin have a variety of biological effects including immobilization of spermatozoa [7]. Saponins represent the effective components of the majority of the spermicidal preparations that are currently available in the form of vaginal jelly, cream, or gels or as a part of a dual protection device, such as lubricated condom [8].

Chenopodium album L. is a small herb that grows all over India as a common agricultural weed. The aerial parts of the leafy young plants are edible and, hence, are consumed in some parts of the world: as cooked vegetable throughout the Indian subcontinent and as a salad in Western countries. Ground seeds and fruits are used as poultry feed [9], and the plant possesses diuretic, laxative and sedative properties [10]. A literature survey revealed a few reports with regard to its chemical composition [11-15] and biological evaluation [16,17], but no investigation has yet been carried out regarding its fertility-related activity. The present communication reports on the in vitro spermicidal activity of *C. album* seed extract. It has been observed that an aqueous decoction of *C. album* seed effectively inhibits sperm motility and viability in a dose-dependent manner.

2. Materials and methods

2.1. Plant material

Matured fruits of *C. album* (Linn.) were collected from the medicinal plant garden of R.K. Mission, Narendrapur, Kolkata, during May 2005 and identified by Dr. Debjani Basu, Asst. Director, Botanical Survey of India, Howrah, West Bengal, India. A voucher specimen (No. 786) was deposited in the Steroid and Terpenoid Chemistry Department, Indian Institute of Chemical Biology. The tiny black seeds were segregated from the pericarp of the fruits and then ground in an industrial blender.

2.2. Preparation of extracts

The powdered seeds were extracted with water (3×5 L) at ambient temperature. The combined extract was concentrated under reduced pressure using a rotary evaporator (Eyela, Tokyo Rikakikai, Tokyo, Japan) at 45–50°C. The concentrated extract was divided into two parts. One part was transferred into an amber-colored glass container and kept in the refrigerator for future use. The other part [prepared *C. album* seed decoction (CAD), 40 g] was partitioned between *n*-BuOH (GR grade, Merck India Ltd., Mumbai) and water to produce a decoction free of inorganic salts. The organic layer was evaporated to dryness under reduced pressure, resulting in a greenish-white amorphous residue (22.5 g).

2.3. Purification of the decoction

The residue obtained after the removal of the solvent was subjected for detection of pesticides in CAD. One gram of residue (CAD) was soaked in 100 mL of dry petroleum ether (40–60°C), diethyl ether and acetone, respectively, stirred over a magnetic stirrer for 5 min, filtered and concentrated at boiling water temperature. The decoctions (petroleum ether, diethyl ether and acetone) were subjected to thin-layer chromatography (TLC; silica-coated aluminum sheet, silica gel $60F_{254}$, Merck India Ltd.), with standard solutions of common pesticides, endosulfan, phorate, permethrin, BHC and carbofuran (Bayer India Ltd., Mumbai), and developed in a solvent mixture of benzene/chloroform/ethyl acetate (60:30:10). After development, the TLC plate was dried in air. The spots were visualized under UV torch (254 and 365 nm) as well as by exposure to iodine vapor. Comparison of retardation factor (R_f) values substantiated that no traces of the common pesticides, that is, endosulfan, phorate, permethrin, BHC and carbofuran, were found to be present in the decoction.

2.4. Chemical composition of the decoction

The CAD residue was passed through a Diaion HP-20 (ion exchange resin, Nippon Rensui Co., Tokyo, Japan) column and washed with water. The fractions eluted with water were evaporated under reduced pressure. TLC studies revealed an intimate mixture of two compounds. On acid hydrolysis [18], the mixture yielded the aglycon as a single product. ^{1}H- and ^{13}C-NMR spectral analysis indicated the aglycon to be oleanolic acid, which was also confirmed by comparison with an authentic sample and spectral data [19]. The aqueous part was worked up [20] and tested for sugars. Glucuronic acid and glucose were identified as monosaccharides by paper chromatography upon comparison with authentic specimens. Isolation of oleanolic acid indicated that both glycosides have the common genin, oleanolic acid, but only differ in the interglycosidic linkage/aglycon–sugar linkage. The complete chemical structure (intersugar–sugar and aglycon linkage) of the glycosides is yet to be established.

2.5. Chemicals

Chemicals and solvents were of analytical grade and were procured from Merck India Ltd.; eosin–nigrosin was from Sisco Research Laboratory India; fluorescinisothiocyanate (FITC), ethidiumbromide (EtBr), PMSG and HCG were from Sigma (St. Louis, MO, USA).

2.6. Animals

Adult Sprague–Dawley rats (180–220 g) were maintained under light and temperature control (complying with standard husbandry conditions) with food and water ad-libitum and were acquired from our institute's animal facility. All experiments were performed in accordance with the guidelines formulated by the animal ethics committee of Indian Institute of Chemical Biology.

2.7. Isolation of caudal rat sperm exudates

Sperm were collected from adult male rats, which have been proven to be fertile, after sexual abstinence for at least