ELSEVIER

Contraception 79 (2009) 479–487

Contraception

Original research article

Reversible antifertility effect of aqueous rhizome extract of *Curcuma longa* L. in male laboratory mice

Raghav Kumar Mishra, Shio Kumar Singh*

Department of Zoology, Banaras Hindu University, Varanasi, Uttar Pradesh 221 005, India

Received 23 August 2008; revised 1 January 2009; accepted 4 January 2009

Abstract

Background: The purpose of the present study was to evaluate the antifertility potential of *Curcuma longa* L. in the male laboratory mouse.
Study Design: Mice of the Parkes (P) strain were orally administered aqueous rhizome extract of *C. longa* (600 mg/kg body weight per day for 56 and 84 days), and effect of the treatment on various male reproductive endpoints and fertility was evaluated. Recovery studies were also performed.
Results: Histologically, testes in mice treated with the plant extract showed nonuniform degenerative changes in the seminiferous tubules as both affected and normal tubules were observed in the same section; the affected tubules showed loosening of germinal epithelium, intraepithelial vacuolation and mixing of spermatids of different stages of spermatogenesis. Marked reductions in diameter of seminiferous tubules, height of germinal epithelium and number of germ cells in Stage VII tubules were also noted in testes of extract-treated mice. Epididymis and seminal vesicle also showed histological alterations. Furthermore, the treatment had adverse effects on motility, viability, morphology and number of spermatozoa in the cauda epididymidis, levels of sialic acid in the epididymis and fructose in the seminal vesicle, serum level of testosterone and on fertility and litter size. By 56 days of treatment withdrawal, however, the above parameters recovered to control levels.
Conclusions: The results show that in P mice *C. longa* treatment causes reversible suppression of spermatogenesis and fertility, thereby suggesting the potential of this plant in the regulation of male fertility.

Keywords: *Curcuma longa*; Mice; Seminiferous tubules; Spermatozoa; Testosterone; Fertility

1. Introduction

From times immemorial, humans have relied on plants and their products as sources of drugs and therapeutic agents. Thus, the quest for developing an antifertility agent from a plant source in the regulation of male fertility appears to be an attractive proposition. Further, such an approach is cost-effective and has relatively low toxicity [1,2]. There is therefore a need to explore plants for their antifertility potential in the male, with the hope of developing a contraceptive for use in men. We have previously carried out studies with gossypol tetra-acetic acid [3,4], *Azadirachta indica* [5], *Allamanda cathartica* [6] and *Bacopa monnieri* [7]. In this study, we report our findings with *Curcuma longa* L.

The plant *C. longa* L. (family Zingiberaceae) is a perennial herb and is widely cultivated in India. Dried rhizome of *C. longa* is the source of turmeric (a symbol of prosperity in Hindu culture). In Ayurveda, rhizome of *C. longa* has been used in the treatment of a variety of diseases such as those associated with skin, liver and pulmonary and gastrointestinal systems [8]. Furthermore, the plant has also been shown to possess antimutagenic and anticarcinogenic properties [9]. However, potential of this plant in the regulation of male fertility has not been well studied. The present study deals with the effect of the aqueous rhizome extract of *C. longa* on the male reproductive organs and fertility of the Parkes (P) strain mouse, which we have been using for an animal model [3–7]. We have evaluated various male reproductive end points such as organs weight, sperm

* Corresponding author. Tel.: +91 542 6702532; fax: +91 542 2368174.
E-mail address: shioks@rediffmail.com (S.K. Singh).

0010-7824/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.contraception.2009.01.001

parameters, histopathology, hormone assay and fertility indices; recovery studies were also performed.

2. Materials and methods

2.1. Plant material and preparation of extract

Fresh rhizomes of *C. longa* were locally collected from the field and were authenticated by experts from the Botany Department of the Banaras Hindu University. A voucher specimen (Z-ING-2008-21) was deposited in the herbarium of the Botany Department of the Banaras Hindu University. The shade-dried rhizomes were cut into small pieces and coarsely ground. The ground plant material was then processed for preparation of aqueous extract of the rhizomes with sterile distilled water [6,10]. Briefly, the ground plant material weighing 50 g was covered with sterile distilled water in a ratio of 1:20 (w/v) and boiled in a glass vessel for 1 h. It was then cooled to room temperature and filtered. The filtrate was evaporated to dryness in oven at 37°C, and the yield of the extract was approximately 4 g, i.e., about 8.0% of the raw material. The extract was suspended in sterile distilled water, and the dose was expressed as dry weight of the extract.

2.2. Animals

Adult (age 12–15 weeks) male laboratory mice of the P strain weighing 32–34 g were used in the investigations. Mice were from closed and randomly bred colony maintained in the animal house of the Department of Zoology, Banaras Hindu University. Animals were housed in a well-ventilated room at 23±2°C with 12-h photoperiod and relative humidity of 50±20% and were maintained on pellet food (Mona Laboratory Animal Feeds, Varanasi) and drinking water ad libitum. Animals in each group were housed separately in polypropylene cages (450 mm× 270 mm×150 mm) with dry rice husk as the bedding material. General health condition and body weight of the animals were monitored throughout the experimental period. Animals were maintained according to the Guidelines of Institutional Animal Ethics Committee.

2.3. Treatments

Mice were randomly allocated to six groups (nine animals per group) and treated as follows:

Groups	Treatment (dose and duration)	Necropsy (time after last treatment)
1	Untreated controls	
2	Distilled water-treated controls	24 h
3	*C. longa* at 600 mg/kg BW for 56 days	24 h
4	*C. longa* at 600 mg/kg BW for 84 days	24 h
5	*C. longa* at 600 mg/kg BW for 84 days	56 days
6	Distilled water-treated controls	56 days

BW, body weight.

Aqueous rhizome extract of *C. longa* was suspended in sterile distilled water and administered orally, with the help of an oral feeding needle. The dosing material was made fresh each day and administered after stirring. Controls (Groups 2 and 6) were administered an equivalent volume of sterile distilled water (0.5 mL/100 g body weight per day) in a similar manner. The dose of *C. longa* was selected on the basis of a pilot study conducted in our laboratory in P mice. We used 56- and 84-day durations in order to assess the effect of the treatment for longer periods.

2.4. Necropsy

After recording final body weights at the end of the treatment schedule, animals were sacrificed by decapitation. The testis, epididymis and seminal vesicle were dissected out, blotted free of blood and weighed.

2.5. Sperm analyses

At the time of euthanasia, spermatozoa were obtained from cauda epididymidis of six mice in each group in physiological saline maintained at 37°C [11]. Motility, viability and number were assessed [12]. The preparations used for assessing sperm viability were also used for assessing sperm morphology. The criteria of Wyrobek and Bruce [13] and Zaneveld and Polakoski [14] were employed for evaluation of sperm abnormalities.

2.6. Histological studies

For histological examination, the testis, epididymis and seminal vesicle were randomly excised from left or right sides of six mice in each group, fixed in Bouin's fluid, dehydrated in graded ethanol series, cleared in benzene and embedded in paraffin. Tissues were sectioned at 6 μm, and the sections were then stained with periodic acid-Schiff (PAS) and counterstained with hematoxylin.

Stages of spermatogenesis in mouse testis were identified according to the criteria of Russell et al. [15]. For evaluation of the quantitative changes in spermatogenesis caused by *C. longa* treatment, germ cell number at Stage VII of the spermatogenic cycle was determined; Stage VII is the most frequent stage of spermatogenesis and contains spermatogonia A, preleptotene spermatocytes, pachytene spermatocytes and spermatids. For this purpose, 10 seminiferous tubules at Stage VII of the spermatogenic cycle were randomly selected from a section of the testis from six mice in each group. The crude count of different germ cells was corrected using Abercombie's formula [16]. The diameter of the seminiferous tubules and height of the germinal epithelium were also measured in Stage VII round or slightly oblique seminiferous tubules (n=10) [7]. Percentage of affected seminiferous tubules was also determined [3].

2.7. Testosterone assay

Serum level of testosterone was measured by radioimmunoassay using commercial kit, as per manufacturer's instruction (Immunotech, Marseille, France). The sensitivity of the assay was 0.025 ng/mL. All samples were quantified