Effect of *Cinnamomum cassia* Methanol Extract and Sildenafil on Arginase and Sexual Function of Young Male Wistar Rats

Sumanta K. Goswami, PhD,* Mohammed N. Inamdar, PhD,* Rohitash Jamwal, M.Tech,† and Shekhar Dethe, PhD†

*Department of Pharmacology, Al-Ameen College of Pharmacy, Bangalore, India; †Bioassay Lab, Research and Development Centre, Natural Remedies Private Limited, Bangalore, India

DOI: 10.1111/jsm.12535

ABSTRACT

Introduction. Herbs have been used as an aphrodisiac since ages. *Cinnamomum cassia* is an important ingredient of many Ayurvedic formulations to treat male sexual disorder including erectile dysfunction (ED).

Aim. The objective of the present study was to evaluate erectogenic and aphrodisiac activity of methanol extract of *C. cassia* bark in young male rats.

Methods. Methanol extract of *C. cassia* was screened in vitro for arginase inhibition potential and IC_{50} was determined. Effect of the extract was observed in vitro on phenylephrine pre-contracted isolated rat corpus cavernosum smooth muscle (CCSM) at 0.1, 1, 10, and 100 μg/mL. Young male Wistar rats were dosed with extract at 100 mg/kg body weight for 28 days and its effects on sexual behavior and penile smooth muscle : collagen level were observed.

Main Outcome Measure. Effect of *C. cassia* was studied on arginase activity in vitro and sexual behavior of young male rats.

Results. *C. cassia* inhibited arginase activity in vitro with an IC_{50} of 61.72 ± 2.20 μg/mL. The extract relaxed phenylephrine pre-contracted isolated rat CCSM up to 43% and significantly increased ($P < 0.05$) sexual function of young male rats. Treatment with the extract also increased smooth muscle level and decreased collagen level in rat penile tissue.

Conclusion. The study proves usefulness of methanol extract of *C. cassia* bark for increasing sexual function.

Goswami SK, Inamdar MN, Jamwal R, and Dethe S. Effect of *Cinnamomum cassia* methanol extract and sildenafil on arginase and sexual function of young male Wistar rats. J Sex Med 2014;11:1475–1483.

Key Words. *Cinnamomum cassia*; Arginase; Enzymes and Enzyme Inhibitors Regulating Corporal Smooth Muscle Relaxation; Sexual Function; Sildenafil; NOHA

Introduction

Penile erection is an integral part of male sexual function, and many neurotransmitters, ion channels, and enzymes are involved in the control of erectile mechanisms, centrally and peripherally [1]. Cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 inhibitors (PDE5is) like sildenafil, vardenafil, and tadalafil are commonly used for the management of erectile dysfunction (ED), a common male sexual dysfunction [2,3]. PDE5is act by relaxing corpus cavernosum smooth muscle (CCSM) (penile tissue smooth muscle) and increasing penile blood flow. Other enzymes and their inhibitors, which have been implicated in the management of ED, include arginase [4] and Rho kinase 2 (ROCK-II) [5].

Indian medicinal plants described as Vajikaran herbs/aphrodisiacs have been used for the management of male sexual disorders including ED [6–11]. Because of a lack of literature, it is difficult to comment on the mechanism of action of these plants. However, the efficacy of these extracts

might be attributed to inhibition of one or more of the above mentioned enzymes. One such plant with an unknown mechanism of action is *Cinnamomum cassia*.

Methanol extract of the *C. cassia* (CCME) was previously reported by authors of this article to inhibit ROCK-II in vitro with an IC_{50} of 9.40 ± 1.93 μg/mL [12]. In this study, we evaluated the effect of CCME on in vitro arginase activity and isolated rat CCSM. Effect of the extract was also studied on sexual function of young male rats in addition to its effect on smooth muscle : collagen level in penile tissue of these rats.

Materials and Methods

Plant Material and Extraction

Bark of *C. cassia* (500 g) was procured from a local market in Bangalore, India, during January 2011. The raw material was identified and authenticated by comparison with the crude drug reference standard by Dr. P. Santhan, Taxonomist, Natural Remedies Pvt. Ltd. The voucher specimen was stored in the natural product library of Natural Remedies Pvt. Ltd., and methanol extract of remaining dried bark was prepared as described elsewhere [12].

Chemicals and Materials

L-Arginine, $MnCl_2$, N^{ω}-hydroxy-L-arginine (NOHA, an arginase inhibitor), and Sirius red (direct red 80) from Sigma-Aldrich, Co. (St. Louis, MO, USA), dimethylaminobenzaldehyde, bovine serum albumin (BSA), potassium sodium tartrate, picric acid, xylene, and Tween 20 from HiMedia Labs (Mumbai, India), and Folin–Ciocalteu reagent from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India) were collected. Diethylstilbestrol from Penta Pharmaceuticals (Mumbai, India) and progesterone from Sun Pharmaceutical Ind. Ltd. (Mumbai, India) were procured. Watson Pharma India Ltd. (Mumbai, India) provided sildenafil as a gift sample. All other reagents used in the study were of analytical grade.

Animals

The animal experimentation was performed after review and approval of the study protocol by our institutional animal ethics committee. Young Wistar rats of either sex weighing 200–250 g were fed with normal rat chow, had free access to drinking water, and were maintained in 12-hour light and dark cycles at 25°C.

Isolation of Arginase Enzyme

Crude arginase enzyme was isolated from liver of rat as described by the method of Schimke [13] with some modifications. Briefly, rat weighing 250 g was anaesthetized and sacrificed by cervical dislocation. Thoracic cavity was opened up and liver was carefully removed. The liver was washed twice in 0.01 M Tris-HCl buffer containing 0.05 M $MnCl_2$ (pH 7.5). Further, liver was cut into pieces and homogenized in three volumes of ice-cold buffer. The homogenate was centrifuged at 4°C, 15,000 *g* for 15 minutes, and supernatant was separated. To the supernatant, 1.5 volumes of chilled acetone (–10°C) was added, mixed, and centrifuged at –10°C, 15,000 *g* for 5 minutes. The precipitate was homogenized in five volumes of buffer and centrifuged at 4°C, 15,000 *g* for 10 minutes. The supernatant was collected and dialyzed for 20 minutes against buffer in an ice bath using a magnetic stirrer. The dialyzed solution was heated at 60°C for 20 minutes on water bath while continuously stirring it with glass rod. The resulting solution was cooled on ice bath and centrifuged again at 4°C, 15,000 *g* for 10 minutes. The supernatant was cooled to 0°C followed by addition of equal volume of chilled ethanol (–10°C) containing 0.05 M $MnCl_2$. The mixture was centrifuged at –10°C, 15,000 *g* for 10 minutes. Three volumes of ethanol (4°C) were added to the above supernatant and centrifuged at 4°C, 15,000 *g* for 10 minutes. The precipitate was finally suspended and homogenized in 3 mL of 0.01 M Tris-HCl buffer containing 0.05 M $MnCl_2$ (pH 7.5) and stored at –80°C till use.

Arginase Inhibition Assay

Protein content of crude arginase enzyme was estimated by the method of Lowry et al. using BSA as a standard [14].

In arginase (inhibition) assay, specific activity of arginase can be determined by the amount of arginine consumed or the amount of urea formed. We measured the amount of urea generated in arginase (inhibition) assay to establish the specific activity of arginase used. Absorbance of urea-Ehlrich's reagent (*p*-dimethylaminobenzaldehyde in 3.6 N H_2SO_4) complex of the assay was plotted against the standard curve of different concentration of urea and a fixed concentration of Ehlrich's reagent [15].

Arginase inhibition assay was performed as per the method of Hagan and Dallam [16]. In brief, a solution containing crude enzyme (protein