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Original article

Efficacy of *Cinnamomum cassia* Blume. in age induced sexual dysfunction of rats

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ABSTRACT

Objective: *Cinnamomum cassia* has been suggested in Ayurveda for the management of sexual dysfunction. This research work was conducted to shed some light on the mechanism of action of the extract, and evaluate the efficacy of its methanol extract in age induced sexual dysfunction in male Wistar rats. Secondary objective of the project was to study the effect of treatment on sperm parameters and smooth muscle:collagen level in rat penile tissue.
Methods: Young and aged male rats were treated with methanol extract of *Cinnamomum cassia* at a dose of 100 mg/kg and sexual behavior was observed on 28th day in presence of female rats in estrous phase. Sildenafil was used as standard medicine. Effect of treatment was studied on epididymal sperm parameters, and Massons trichrome staining of rat penile tissues was performed to know the level of smooth muscle:collagen.
Results: The treatment significantly increased sexual function in aged rats that had decreased in comparison to young rats, but did not have any significant effect on sperm count, live and defective sperm percentage. However, treatment induced an increase in smooth muscle level and a decrease in collagen level in the aged rat penile tissue in comparison to that of age matched control.
Conclusion: Based on our studies, we found that *Cinnamomum cassia* extract was effective in management of sexual dysfunction in aged rats and hence we propose a possible mechanism of action for *Cinnamomum cassia* which could be responsible for restoring sexual activity in aged rat.

1. Introduction

Erectile dysfunction (ED), a male sexual dysfunction is the 'inability of the male to attain and maintain erection of the penis sufficient to permit satisfactory sexual intercourse'.[1] It is a marker of cardiovascular disorder, decreases quality of life, affects elderly males, smokers, and those with diabetes and high blood pressure.[2,3]

Among the many treatment options available, a cGMP specific phosphodiesterase inhibitor, sildenafil, is commonly used.[3] Others include dopamine receptor agonist (apomorphine), selective serotonine re-uptake inhibitor (trazodone), alpha-2 receptor blocker (yohimbine), and non-specific phosphodiesterases inhibitor (papaverine). Sildenafil is a first line of medicine for increasing erectile function and was used as standard medicine in our studies. It prevents degradation of cGMP, which regulates blood flow in the penis. Many other enzymes and their inhibitors have been implicated in the management of ED including Rho-kinase 2 (ROCK-II).[4,5] Methanol and successive aqueous extract of *Cinnamomum cassia* have been reported to inhibit ROCK-II where methanol extract was found to be more potent.[6]

Ayurveda, an ancient Indian system of alternative medicine has suggested use of herbs for the management of sexual dysfunction.[7] A number of studies on Indian herbal extracts have reported an increase in sexual function in normal, castrated and diabetic rats.[8–12] However, to best of our knowledge, no modern scientific literature is available for the efficacy of *Cinnamomum cassia* in age induced sexual dysfunction. The current research work was carried out to study the efficacy of *Cinnamomum cassia* methanol extract (CCME) in age induced sexual dysfunction and understand the possible mechanism of its action by which the extract ameliorates the condition. In addition, the effect of CCME was studied on sperm parameters and smooth muscle:collagen level in aged rat penile tissue.

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2. Materials and methods

2.1. Plant material and extraction

Dried bark of *Cinnamomum cassia* was purchased from Amrutha Keshari Ayurvedic store, Bangalore and authenticated by Dr. P. Santhan (Taxonomist at Natural Remedies Private Limited/NRPL, Bangalore). Specimen sample was stored at NRPL repository with specimen number NPL/CD/169. Methanol extract of *Cinnamomum cassia* (CCME) was prepared as described previously.[13]

2.2. Chemicals and materials

Tween 20 and Sodium chloride (HiMedia Labs, India); Diethyl stilbestrol (Penta Pharmaceuticals, India); progesterone (Sun Pharmaceutical Ind. Ltd., India); Triton™ X-100 and merthiolate (Sigma Aldrich Co., USA); and Sildenafil (Watson Pharma India Ltd., India) were procured. All other reagents used in the study were of analytical grade.

2.3. Animals

Young (6 month old) male and female Wistar rats weighing 200–250 g and aged (24 months old) male rats weighing 300–350 g were used. The animals had free access to food and drinking water, and were maintained at 25 ± 1 °C. Study protocol was reviewed and approved by institutional animal ethics committee before the start of the work.

2.4. Acute toxicity study

Acute toxicity study was performed on female mice in accordance with the Organization for Economic Cooperation and Development guidelines for the testing of chemicals, (OECD 425: Acute Oral Toxicity-Up-and-Down-Procedure), 2006.

Three month old female mice weighing 27 ± 3 g were dosed with 2 g/kg body weight of *Cinnamomum cassia* methanol extract. Mice were fasted for 4 h before dosing and food was allowed only after 2 h of treatment. The extract was triturated using mortar and pestle with distilled water containing 1% Tween 20. A vehicle control (1% Tween 20) group was maintained.

Animals were observed at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and thereafter, daily for a total period of 14 days for any adverse event (time of onset, length of recovery period).

2.5. Sexual behavior study

Male Wistar rats were divided into 4 groups of six rats each i.e. group I (young rat treated with 1% Tween 20 in water), group II (aged rat treated with 1% Tween 20 in water), group III (aged rat treated with 100 mg/kg body weight CCME) and group IV (aged rat treated with 20 mg/kg body weight sildenafil). CCME and sildenafil were suspended in water containing 1% Tween 20 and administered orally. Sexual behavior study was performed as described in published literature.[8–13] A week before start of study, male rats were sexually trained in presence of female rats for 3 days. Ovariectomized female rats[14] for the study were brought to estrous phase by administration of diethyl stilbestrol (1 mg/kg, p.o, administered 2 days before the study) and progesterone (5 mg/kg, s.c., administered 6 h prior to the study). Male rats were dosed orally for 28 days and sexual behavior was observed on 28th day for 30 min in the evening. Following sexual behavior parameters of male rats were observed in presence of female rat in a wooden box (45 × 50 × 35 cm) covered with glass lid and illuminated with red light.

Mount latency (ML): time from the introduction of female into the cage of the male up to the first mount.

Intromission latency (IL): time from the introduction of the female up to the first intromission by the male.

Mount frequency (MF): number of mounts before ejaculation.

Intromission frequency (IF): number of intromission before ejaculation.

Ejaculation latency (EL): time from the first intromission of a series up to the ejaculation.

Post-ejaculatory interval (PEI): time from the first ejaculation up to the next intromission by the male.

2.6. Sperm analysis

After completion of sexual behavior study, male animals were anesthetized with ether and sacrificed by cervical dislocation. Left epididymis and shaft of penile tissue from each rat was excised. Epididymis was used for sperm parameter study whereas shaft of penile tissue was kept in 7% formalin saline solution for histopathology.

Sperm count and analysis was performed by the methods as reported earlier.[13,15–18] Briefly, the epidydimises were transferred to beakers containing 30 mL of saline–triton–merthiolate solution (saline solution containing 0.05% triton-X and 0.01% merthiolate) and teased with surgical blade. Sperms were allowed to diffuse into the solution for 15 min and volume was made up to 50 mL with distilled water. Subsequently, 0.5 mL of 1% eosin Y solution was added to the sperm solution and mixed well. 10 μL of this sperm solution was placed on Neubauer chamber for sperm count study. Sperm heads were counted using light microscope (Olympus CX41, Olympus, USA; magnification: 200×) and mean sperm count in all 4 corner square were counted using the following formula.

$$\text{No. of sperms per caudal epididymis} = \frac{\text{Mean count} \times 50(\text{total volume})}{0.01 \times 0.01(\text{Volume of counting chamber})}$$

Live sperm ratio was calculated by the following formula.

$$\text{Live sperm percentage} = \frac{\text{Total number of live sperm}}{\text{Total number of sperms counted}} \times 100$$

Defective sperms (coiled sperms, tail less sperms, sperms with bent neck, midpiece and tails) were observed and percentage was calculated using the following formula.

$$\text{Defective sperm percentage} = \frac{\text{Total defective sperms counted}}{\text{Total number of sperms counted}} \times 100$$