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Evaluation of the aphrodisiac potential of a chemically characterized aqueous extract of *Tamarindus indica* pulp

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ABSTRACT

Ethnopharmacological relevance: *Tamarindus indica* is an ingredient in the traditional aphrodisiac formulations in Africa and India. It is also a widely used food ingredient in other tropical countries.
Aim of the study: The present study was aimed to evaluate the aphrodisiac potential and reproductive safety profile of aqueous extract of *Tamarindus indica* in male Wistar rats.
Materials and methods: The aqueous extract was prepared by maceration of pulp followed by reduction of volume in rotavapor under heat followed by freeze drying. The prepared extract was characterized for contents of total phenol, flavonoid, and saponin. It was also subjected to phytoconstituent analysis using GCMS. Further, the extract was evaluated for acute toxicity study. The aphrodisiac and reproductive toxicity potential were evaluated in animals after grouping them in four with six animals each namely, normal control, standard (Sildenafil citrate, 4 mg/kg *p.o.*) and extract of *Tamarindus indica* treated groups at two dose levels, 125 and 250 mg/kg *p.o.* The study was conducted for 54 days with daily once dosing of extract and standard. Equal number of females was grouped without treatment for evaluation of parameters of sexual desire (mount frequency and intromission frequency) and parameters of sexual arousal (mount latency and intromission latency). These parameters were evaluated on day 14, 28, 42 and 54. Animals were sacrificed on day 54, testes were removed and studied for histopathological changes.
Results: The extract showed 6.6 mg gallic acid equivalent/g of total phenol, 2.3 mg catechin equivalent/g of flavonoid and 11.6% saponin. Forty chemical constituents were identified by GCMS analysis. In acute toxicity study, the extract was found to be safe till 2000 mg/kg *p.o.* Efficacy study showed significant ($p < 0.05$) improvement in parameters of sexual desire (mount frequency and intromission frequency) and parameters of sexual arousal on all observed days except mount frequency for 125 mg/kg on 42nd day and intromission frequency for both doses of tamarind compared to normal control. Improvements in these parameters were comparable to the standard drug. Histopathology study and sperm count suggested an increase in sperm production without any sign of toxicity in testis. Sperm motility significantly ($p < 0.05$) increased in the treatment groups that received extract at 250 mg/kg compared to normal control.
Conclusion: Aqueous extract of *Tamarindus indica* possessed aphrodisiac activity together with spermatogenic potential.

1. Introduction

Reproductive disorders and reproductive health hazards have become prominent public health concern. Sexual dysfunction is one among them. It is a serious medical and social problem which affects 10–52% males and 25–63% of females (Kotta et al., 2013). The sharp decline in reproductive health of the average male has been a growing concern worldwide. Sperm quality is reported to have declined world-

Abbreviations: Tamarind or TI, *Tamarindus indica*; MF, mount frequency; IF, Intromission frequency; ML, mount latency; IL, intromission latency; R. Time, Retention time; *p.o.*, per oral; GCMS, Gas chromatography Mass spectroscopy; ANOVA, Analysis of Variance; SEM, Standard Error of Mean

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wide in last 50 years by various research (Carlsen et al., 1992; Sk et al., 2008). A number of factors viz., nutrition, environment, socioeconomic status, sedentary lifestyle, and stress are known to associate with reproductive system disorders (Sharma et al., 2013). Many natural substances are being used as aphrodisiacs all over the world, like yohimbine, ground rhinoceros horn, mandrake plant etc. (Kotta et al., 2013). The research in sexual dysfunction study took paradigm shift after the introduction of the remedy for impotence (mainly erectile dysfunction) i.e., Viagra (sildenafil) in the 1990s. However, sildenafil was in the eye of a storm, owing to its side effects. Hence, the search for natural supplements is emphasized

Diet is one of the important factors playing a dominant role in maintaining the quality of sexual life. An awareness of the diet constituents and their implications on the sexual and reproductive activities of the male may help overcome the problems associated with sexual and reproductive life to some extent. In the present study, we evaluated the effect of the pulp of *Tamarindus indica* L., which is one of the most widely used ingredients of south Indian dish "Sambhar", a dish of day-to-day use. *Tamarindus indica* L. commonly known as tamarind is a tropical tree which belongs to the Fabaceae family, Caesalpiniaceae subfamily. Tamarind contains vitamins A, B and C and organic acids like citric acid, tartaric acid, and malic acid. The polyphenolics pericarp of Tamarind contains apigenin, catechin, epicatechin, naringenin, procyanidin B2, procyanidin dimer, procyanidin trimer, taxifolin, and eriodictyol, of total phenols. It exhibits high antioxidant capacity due to the presence of high phenolic content (Bhadoriya et al., 2011; De Caluwé et al., 2010). The pulp of *Tamarindus indica* is reported for its anti-inflammatory, anti-rheumatism, antipyretic, laxative, carminative properties and traditionally being used to alleviate sunstroke, datura poisoning, and alcoholic intoxication (De Caluwé et al., 2010). Tamarind is also reported to be traditionally being used in Africa and other tropical countries for aphrodisiac activity (Havinga et al., 2010; Kuru, 2014). Though various parts of tamarind tree are being used for the aphrodisiac purpose in various places, fruit is mainly used for this purpose by the people of Côte d'Ivoire (Havinga et al., 2010; Kerharo and Bouquet, 1950). Tamarind is found to be part of the Ayurvedic formulation to enhance the sperm count, where the kernel of tamarind mixed with fried Bishops weed (ajwain) is used to improve sperm count and to treat premature ejaculation (Dhiman et al., 2014). In 2007, Mallick et al., reported that tamarind present in a herbal formulation proved to be beneficial in streptozotocin-induced testicular dysfunction in diabetic rats (Mallick et al., 2007). Further, the finding of Singh et al., 2012, supported the reproductive safety of tamarind against fluoride-induced toxicity on the sperms in rats (Singh et al., 2012). The studies conducted on the safety issues of tamarind it was found that tamarind did not have mutagenicity in Ames, Bone Marrow Micronucleus and Sperm Aberration studies in mice (Zhang et al., 1995). In this report, an anti-clastogenic (anti-DNA damage effect) action attributing to tamarind was also reported.

However, even today a detailed and systematic study to assess the aphrodisiac and pro-fertility potentials of tamarind was not carried out. Therefore, the present study was planned to assess the safety of the extracts of pulp and seeds of *Tamarindus indica* (Tamarind or TI) on the reproductive system by evaluating its aphrodisiac potentials and effects on quality and quantity of sperms in male Wistar rats.

2. Materials and methods

2.1. Chemicals

Sildenafil citrate (Penegra® tablets) were procured from pharmacy dispensing wing of Kasturba Medical College, Manipal. Sodium methyl cellulose was procured from Rankem, India. Folin- Ciocalteau reagent was procured from Sigma Aldrich, USA. Aluminium chloride was procured from Spectrochem Private Ltd, India. Diethyl ether was procured from Himedia Laboratories, India. Dulbecco's Modified Eagle's medium and Eosin-Y were procured from Himedia, India.

2.2. Collection of plant material and preparation of aqueous extract

Tamarindus indica (TI or tamarind) fruits were collected from Udupi in the month of March-April. The fruit was authenticated by Dr. Richard Lobo, Associate Professor, Department of Pharmacognosy and a voucher specimen (Specimen No. PP 617) was submitted to the herbarium of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka-576104, India. The aqueous extract was prepared by maceration method. The pulp was macerated with chloroform-water (10 ml of chloroform in 1000 ml of distilled water) for 7 days with intermittent stirring (Sodde et al., 2015). The contents were filtered using a muslin cloth. The volume of extracted liquid was reduced in rotavapor at 40 °C. The final drying was performed by lyophilisation. The percentage yield of extract was 16.7%.

2.3. Characterization of extract

2.3.1. Presence of phenol

The total phenolic content is determined by the method called Folin- Ciocalteau method using the Folin-Ciocalteau reagent (a mixture of phosphotungstic acid and phosphomolybdic acid and some amount of lithium salts). The standard plot was made with different concentrations of gallic acid *i.e.,* 10, 40, 80, 120, 200 µg/ml. The concentration of extract used in the study was at 200 µg/ml. Briefly, to 200 µL of extract/standard solution, 1.5 ml of Folin-Ciocalteau reagent was added and incubated in dark. After 5 min, 1.5 ml of 20% sodium carbonate was added and incubated for 90 min. The absorbance was taken at 725 nm and concentration of total phenol in the extract was calculated using the standard plot (Blainski et al., 2013)

2.3.2. Presence of flavonoid

The total flavonoid content was determined by aluminium chloride method. Aluminium chloride forms acid stable and acid labile complexes with keto and hydroxyl groups of flavones and other flavonoids. For the study, the standard plot was made with catechin at different concentrations 20,40,60,80 and 100 µg/ml. Test solution used in the study was used at a concentration of 1 mg/ml. Briefly, to 1 ml catechin/extract solution, 4 ml water, and 0.3 ml sodium nitrite was added and incubated for 5 min. To the solution, 0.3 ml of 10% aluminium chloride was added and incubated for 1 min. Then, 2 ml of 1 M sodium hydroxide added and volume was made up to 10 ml with water. The absorbance was taken at 510 nm (Chang et al., 2002).

2.3.3. Presence of Saponin

Extract (5 g) was mixed with 50 ml 20% ethanol and heated for 90 min at 55 °C with periodic agitation. The mixture was filtered and again heated for 90 min at 55 °C with 50 ml 20% ethanol. Both solutions were mixed and volume was reduced to about 40 ml at 90 °C. The solution was added to the separating funnel containing 40 ml diethyl ether and shaken vigorously. This step was repeated till aqueous layer became clear. The diethyl ether layer was discarded and 60 ml *n*-butanol was added. The resultant mixture was washed twice with 10 ml 5% sodium chloride and evaporated to dryness on a water bath. Further drying of the residue was done in the oven to a constant weight and represented as percentage total saponin (weight of residue/weight of sample × 100) (Edeoga et al., 2005).

2.3.4. Gas chromatography- Mass spectroscopy (GCMS) analysis of extract

The extract was analyzed GCMS by dissolving in methanol to obtain a final concentration of 1 mg/ml. GCMS was carried out using Shimadzu GCMS-QP2010S instrument. Column used was RTX-5, with