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Morella serrata (Lam.) Killick stabilizes biomembrane and rejuvenates sexual competence in male Wistar rats

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ABSTRACT

Ethnopharmacological relevance: *Morella serrata* L. is an indigenous medicinal plant to South and southern Africa with folkloric applications as aphrodisiac, laxative, antimicrobial, anti-inflammatory, analgesic, anti-hypertensive, antitussive and antidiabetic agent.
Aim of the study: This study evaluated the membrane stabilization and aphrodisiac potentials of its aqueous root extract in male Wistar rats.
Materials and methods: While the membrane stabilization of the extract was investigated against bovine erythrocytes (BE), the male rats for the aphrodisiac study were randomized into five groups with animals in group 1 given sterile placebo and served as control. The rats in group 2 were treated with 7.14 mg/kg body weight of PowMaxM, while animals in groups 3, 4 and 5 were administered with the extract (100, 200 and 400 mg/kg, respectively). All treatments (1 mL) were done once daily for 4 weeks via oral gavaging and their mating behavioural, testicular, spermatogenetic and antioxidant parameters were evaluated.
Results: With the exception of the mount, intromission and post ejaculatory latencies that were dose-dependently reduced by the extract, other mating parameters were significantly improved when compared with the control. Similar patterns of significant improvement were also observed on the testes-body weight ratio, quality and viability of sperm cells as well as testicular concentrations of proteins, cholesterol, glycogen, testosterone, follicle stimulating hormone, leuitenizing hormone and glutathione (reduced) subsequent to treatment with the extract. Although, administration of *M. serrata* had no significant ($p > 0.05$) effect on the testicular activity of gamma glutamyl transferase, those of lactate dehydrogenase, phosphatases (alkaline and acid), superoxide dismutase and catalase were significantly ($p < 0.05$) induced in the treated animals. The extract also conferred respective significant ($p < 0.05$) membrane stabilization potential of 66.02% and 60.87% on the BE against hypotonic solution and heat-induced hemolysis relative to 62.14% and 40.19% for ibuprofen. The effect elicited by the extract at the tested doses could partly be attributed to its antioxidant and adaptogenic constituents.
Conclusion: The data presented in this study have enriched biochemical information on the root extract of *M. serrata* as a viable source of phytonutrients that could be potentially useful for the development of aphrodisiac drugs, and thus lending scientific credence to its much touted sex enhancing attributes by the Basothos of the eastern Free State Province of South Africa.

1. Introduction

Morella serrata (Lam.) Killick (Myricaceae) is a shrub, usually 2 m tall and with characteristic brown resinous glands. It is characterized by narrow, toothed, simple alternate decussate leaves (80 mm long) with irregularly serrated veins. The flowers are unisexual and develop to drupe fruits with papillaceous endocarp (Schmidt et al., 2002). *M. serrata* (MS) is indigenous to southern and South Africa (Zukulu et al., 2012). A recent report by Lerato et al. (2015) presents MS in the top ten ranking (of the total eighty) botanicals frequently used ethnomedicinally in the Maseru district of Lesotho. In addition, the Xhosas and particularly Basothos of the eastern Free State Province of South Africa use its root decoction to treat flu, dysmenorrhea, headaches and as aphrodisiac (Moffet, 2010; Moteetee and van Wyk, 2011; Ashafa, 2013; Spell Caster Kenya, 2014). Because of its aphrodisiac effect, the plant is fondly and commonly called 'monna motsho (black man)' among the Basothos (Moffet, 2010). Its root decoction is ginger-like and has also found relevance as hypoglycaemic and laxative agent (Schmidt et al.,

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2002; Saheed and Omotayo, 2016). A study on its root extract also demonstrated that MS is non-toxic and practically safe for consumption (Sabiu and Ashafa, 2016a).

Erectile dysfunction (ED) and premature ejaculation are the commonest complaints of male patients presenting with sexual dysfunction (Lindau et al., 2007). ED is a global health challenge with incidence consistent with age, general dissatisfaction and other sexual ailments (Schouten et al., 2005; Amidu et al., 2010). Although, orthodox management therapies (psychotherapy, vacuum devices, surgery, penile implants and drugs) for ED are undoubtable (Ogah, 1999), affordability, sensitivity, social stigma (in the African context) and inherent side effects have undermined their applications (Rowland and Tai, 2003). Interestingly, a number of drugs from herbal sources (void of accessibility and affordability hitches, and with minimal side effect) have been shown to improve sexual competence and there is a keen global interest on the development of such (Ufelle et al., 2011; Sabiu et al., 2016a). It is also not surprising that phytotherapy has continued to be a sought-after alternative for individuals seeking to be sexually rejuvenated (Baljinder et al., 2010; Yakubu and Akanji, 2011; Sabiu et al., 2016a). Although, literatures have implicated MS to enhance sexual performance (Moffet, 2010; Ashafa, 2013; Spell Caster Kenya, 2014; Bruno et al., 2015; Sabiu and Ashafa, 2016a), evidence of its acclaimed capability to enhance sexual invigoration lacks scientific validation. Thus, this study investigated its root extract for aphrodisiac properties with a view to providing biochemical information on its touted effects. The membrane stabilization potential and GCMS analysis of the extract were also evaluated.

2. Materials and methods

2.1. Chemicals, drugs, reagents and assay kits

Estradiol benzoate and progesterone were procured from Sigma Chemical (St. Louis, USA) and Shalina Laboratories (Mumbai, India), respectively. PowMaxM was a product of Beijing Kowloon Pharmaceuticals Co., Limited, Beijing, China. Experimental kits for protein, cholesterol, glycogen, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), acid phosphatase (ACP), and lactate dehydrogenase (LDH) were products of Randox Laboratories Limited (Co Antrim, United Kingdom) while those of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were obtained from Monobind Inc. (California, USA). The water used was glass-distilled and all other chemicals and reagents were of analytical grade.

2.2. Plant collection, authentication and processing

Subsequent to authentication at the Department of Plant Sciences, University of the Free State, South Africa, fresh roots of MS were obtained from its whole plants in its plantation at Phuthaditjhaba, Free State Province, South Africa. A voucher sample (AshMed/01/2014/QwaHb) was thereafter kept at the Herbarium of the institution for future reference. The roots were thoroughly rinsed under running tap and dried (40 °C) to constant weight in an Echotherm oven before pulverizing to smooth powder. Approximately 400 g of the pulverized sample was exhaustively extracted in distilled water (4 L) with continuous agitation on orbital Labcon Platform shaker (Laboratory Consumables, PTY, Durban, South Africa). The solution obtained was filtered and subsequently concentrated to a yield of 46.5 g (11.63%) extract (MSE) that was kept air-tight and refrigerated prior to use.

2.3. Experimental animals

The study was carried out subsequent to approval (NR-02-2013) by the Ethical Committee of University of the Free State, South Africa, and in accordance with the guidelines of National Research Council Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and principles of Good Laboratory Procedure (World Health Organization, 1998). Wistar rats (n=50 males, 50 females) with weight range 214.00–253.00 g were collected from the animal facility of the University of the Free State, Bloemfontein, South Africa. They were housed in clean cages kept in a well-ventilated house with optimum condition (temperature 23 ± 1 °C, photoperiod; 12 h natural light and 12 h dark; humidity; 45–50%). The animals were acclimatized to the animal house condition for 7 days during which they had *ad libitum* access to feed (Pioneer Food (Pty) Ltd, Huguenot, South Africa) and water.

2.4. Experimental protocol

The male rats were evenly distributed into five groups of 10 animals each. Animals in group 1 were given sterile placebo (distilled water) and designated as control. While rats in group 2 were placed on 7.14 mg/kg body weight (b.w.) of PowMaxM, animals in groups 3, 4 and 5 were treated with 100, 200 and 400 mg/kg b.w. dose of MSE (in distilled water), respectively. All administrations (1 mL) were done once daily for 4 weeks via oral intubation.

In preparation for mating, the female rats were brought to oestrus by successive subcutaneous administration of estradiol benzoate (10 μg/100 g) and progesterone (0.5 mg/100 g) at 48 h and 6 h respectively prior to pairing (Amin et al., 1996).

2.5. Assessment of male sexual performance

This study was executed between 20:00 and 23:00 h under dim light as previously described (Yakubu and Akanji, 2011). Briefly, following 24 h of last treatment on the male rats, the artificially primed female rats were paired (1:1) with the males. After 10 min of proceptive and precopulatory period, the rats were observed for 25 min from another room for display of copulatory characteristics. Sexual behaviours including mount frequency (MF), intromission frequency (IF), ejaculatory frequency (EF), mount latency (ML), intromission latency (IL), ejaculatory latency (EL), post-ejaculatory latency (PEL) and copulatory efficiency were recorded and computed.

2.6. Sperm motility, viability and counts

After 9 h of sexual performance study, the male rats were humanely sacrificed under halothane anaesthetization and the method of Salman and Ayoade (2008) was adopted in this assessment. Briefly, the caudal epididymis of the rats was dissected. An incision (about 1 mm) was made and drops of sperm fluid were squeezed onto the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. By computing the motile spermatozoa per unit area, the epididymal sperm motility was thereafter assessed. The sperm viability was also determined using Eosin/Nigrosin stain. For the sperm count, the epididymis was homogenized in normal saline (5 mL) prior to counting in the counting chamber of haemocytometer.

2.7. Testicular homogenate preparation

Following anaesthetization, the testes were also immediately but diligently excised from the rats, cleaned and homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were centrifuged at 10,000*g* (4 °C, 10 min) to obtain post-mitochondrial fractions and the resulting supernatant was stored (−20 °C) to ensure maximum release of the testicular fractions.

2.8. Determination of biochemical parameters

The testicular activities of lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), acid phosphatise (ACP), alkaline phos-