Aphrodisiac properties of \textit{Tynanthus micranthus} Corr. & Mello ex. Schum in male mice

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In traditional medicine, a variety of plants have been used as sexual stimulants. Within the genus \textit{Tynanthus} (Bignoniaceae) some species are known by their aphrodisiac effects, including \textit{Tynanthus micranthus} Corr. & Mello ex. Schum. The purpose of this study was to evaluate the sexual behavior of male mice treated with a crude extract of the aerial parts (leaves and stems) of \textit{T. micranthus}, for this, the extracts were prepared and orally administered (5 and 10 mg/kg) to different groups of male Swiss mice. Mating behavior was filmed and compared with control (distilled water) and test drug (sildenafil citrate), 1 and 3 h after treatment. The animals treated with the extract presented a significant reduction in the latency time for mounts and an increased frequency of mounts, especially after three hours of treatment, in a dose-related manner, as compared to the control group. These results prove the influence of \textit{T. micranthus} on sexual behavior of mice and allow the inclusion of this species in the list of aphrodisiac plants of the genus.

Key words: Aphrodisiac properties, sexual behavior in male mice, erectile dysfunction, natural products.

INTRODUCTION

Although impotence or erectile dysfunction (ED) is not a life-threatening condition, it directly affects the quality of life of men and their partners. It can be described as the inability to achieve or maintain a sufficient penile rigidity, and therefore allow the satisfactory sexual intercourse (Mackay, 2004). In Brazil, a study using assessment tools with men aged 18 to 80 years showed that 45.1% of subjects had ED (31.2% mild, 12.2% moderate and 1.7% severe). Compared with men aged 18 to 39 years, those aged 60 to 69 years had a 2.2% greater risk of ED, while this risk tripled for those aged 70 or more (Abdo et al., 2006).

Due to advances in knowledge on the pathophysiology and treatment of virility-related problems, there are currently some options of valid treatment that will depend on the type and severity of the symptoms, possible
contraindications or intolerance to the proposed treatment, and the patient's socio-economic status. Treatment for erectile dysfunction is individualized according to the cause presented by the patient, which may be psychological in nature or the result of an organic dysfunction (Hatzimouratidis et al., 2010).

Nowadays, inhibitors of phosphodiesterase-5 are the most widely used oral therapy for impotence (Mackay, 2004), followed by other treatments such as intracavernous injection of vasoactive drugs (Hatzimouratidis et al., 2010), psychological monitoring (Santamaría, 2010) and, in some cases, implantation of a penile prosthesis (Al-Enezi et al., 2011).

As an alternative treatment, some plants called aphrodisiacs have been used since ancient times, indicated for the treatment of sexual problems by ancient Greek and Arab physicians such as Hippocrates (460 b.C.), Dioscorides (70 a.C.), Raazi (926 a.C.) and Ibn-Sina (1038 a.C.) (Tajuddin et al., 2003). Tynanthus micranthus Corr. & Mello, also known as “Cipó-cravo”, belongs to the Bignoniaceae family, which has a large number of species with important biological and pharmacological activities previously proven (Castillo and Rossini, 2010).

Until now, there are no study reporting the ethnobotanical data of T. micranthus, however in folk medicine this plant is used for aphrodisiac purposes. These effects have been described for two species of the Tynanthus genus: Tynanthus panurensis (Plaza et al., 2005) and Tynanthus fasciculatus (Vilegas et al., 1993; Melo et al., 2010; Mendes, 2011). The study published by Custodio et al. (2010) showed eugenol as the main component of the essential oil extracted from T. micranthus; hence the strong smell of cloves that exudes when the plant is rubbed. Further phytochemical studies on its chemical components, performed by Cansian et al. (2012), indicated the presence of chemical groups such as flavonoids (dihydroflavonol and leucoanthocyanidins), steroids, triterpenes and heteroside anthraquinone. This same study describes the isolation and identification of the phytosteroid beta-sitosterol and flavone apigenin in the species. For these reasons, we carried out this study, aiming to demonstrate the effects of the crude ethanolic extract of T. micranthus on male mice sexual behavior, compared to distilled water (negative control) and sildenafil citrate (positive control).

MATERIALS AND METHODS

Plant

The plant material was collected in Quinta do Sol, Paraná, Brazil, and was identified by botanist Dr. Gerd Hatschbach in the Municipal Botanical Museum of Curitiba, Paraná, where a voucher sample of the species was deposited under number 231.071. The leaves and stems extracts were prepared separately in a hot closed Soxhlet apparatus using absolute ethanol as solvent extractor for eight hours. The crude extracts were filtered and concentrated on a rotary evaporator, yielding 100 and 120 ml, with a solid content of 525 and 628 mg/ml, to stems and leaves, respectively. The concentrated extract was kept in water-bath at 40°C until complete solvent removal.

Animals

Male and female mice of the Swiss strain, aged approximately 60 days and weighing between 25 and 30 g, were used for the experiments. The animals were kept under controlled parameters of temperature (20 ± 2°C) and light, using artificial lighting to set a light/dark photoperiod of 12 h each, considering the light period from 10:00 PM to 10:00 AM (reversed cycle). Water and commercial diet were provided ad libitum. All procedures were authorized beforehand by the Ethics Committee on Animal Use of the Department of Biological Sciences of the Universidade Federal do Paraná, under process number 23075.108893/2011-23, and were performed in accordance to the ethical principles established by the Experimental Animal Brazilian Council, and the requirements of the “Guide for the Care and Use of Experimental Animals (Canadian Council on Animal Care)”.

Sexual behavior assessment

Sexual receptivity of females was induced by synthetic female sex hormones. Approximately 52 h before mating, 10 UI of equine chorionic gonadotropin (ECG Novormon®) were administered intraperitoneally, followed by 10 UI of human chorionic gonadotropin (HCG-Vetecor®) 48 h afterwards (Neto et al., 2011). Males were divided into 4 groups of 7 to 9 animals. The first group received distilled water and was considered as negative control. The second and third groups, labeled CP300 and CP600, received 5 and 10 mg/kg of the crude extract (prepared with stems and leaves extracts equally weighed) of T. micranthus, respectively. The fourth group was treated with sildenafil citrate at a concentration of 0.71 mg/kg (Singh and Gupta, 2011), being the positive control. All treatments were given orally. Sexual behavior was evaluated according to Tajuddin et al. (2003) with minor modifications. The behavior evaluation was carried out in the dark period, since mice have sexual nocturnal habits and their metabolism is more active in this period. One hour after treatment, each male was introduced in a transparent polypropylene box, remaining isolated for the setting period of ten minutes. Then, a female was introduced into the box, contact with the male was filmed for 20 min, thereafter they were separated. After 3 h treatment, the male was placed back in contact with the same female and was filmed for a further 20 min. Then, the following parameters were analyzed:

A) Mount latency (s): Time elapsed between the introduction of the female into the box and the first mounting or attempt to mount;
B) Decision of yes or not to mount;
C) Number of mounts or attempts to mount;
D) Presence of sperm in the vaginal smear.

For the animals that did not seek the female during the analyzed period, the mount latency was recorded as 1200 s, which corresponds to the total filming time.

Statistical analysis

The statistical comparison among controls and treated groups was obtained using the Kruskal-Wallis test followed by the Dunns post-hoc test for mount latency and t-test for the number of mounts, with a confidence level of 95% (p < 0.05).
RESULTS

At the end of the first hour of treatment, groups CP300, CP600 and positive control showed a reduction of the mount latency period of 49 ± 16%, 58 ± 19% and 29 ± 16%, respectively, which was not statistically different from the negative control group (Figure 1A). After three hours of treatment, however, the CP300 group reduced its mount latency period in 68 ± 15% when compared to the negative control (Figure 1B). Similar results were observed in the CP600 group, which reduced its mount latency period in 78 ± 15%, again comparing to the negative control. The positive control also reduced the mount latency period in 61 ± 11% (Figure 1B).

During the analysis, it was observed that some males did not seek the female, which conducted us to verify the decision parameter of whether or not to mount, which is expressed as the sum of "yes" and "no" for each group (Figure 2). In contrast to the negative control group, in which only 11% of the animals showed interest for the female, in the CP300 group 78% of animals presented a positive response for the mount decision, with the same behavioral pattern observed for the two time points analyzed. The CP600 group showed a total of 71 and 86% positive responses at the first and third hours, respectively. The positive control group showed a significant increase in positive responses at the third hour, being 44 and 89% of animals with a "yes" response for mount decision in the first and third hours, respectively.

Both treatment groups, as well as the positive control group showed an increase in the number of mounts or
attempts of mounts (Figure 3). The results show that both concentrations of *T. micranthus* crude extract were able to increase the number of mounts in the two time points analyzed, being more significant for three hours after treatment with the higher dose (CP600 group). The positive control showed a significant increase in the number of mounts only in the third hour after treatment. The presence of sperm in the vaginal smear was only observed in one female from the CP300 group for both time points analyzed, and in one female from the CP600 group in 3-h' time point. The absence of sperm in the majority of females can be explained by the reduced contact time between the couple, or by lack of penetration. This latter parameter was not assessed, since the objective was to observe the increased interest of the male by the female, thus demonstrating the aphrodisiac activity of the plant.

**DISCUSSION**

The crescent search for medicinal plants with aphrodisiac potential sparked the interest of researchers in gathering information from various plants that showed significant pharmacological activity. These review papers report results obtained from *in vivo* or *in vitro* assays, and try to suggest a possible mechanism of action relating to the main chemical components isolated from the plants. Some well-known aphrodisiacs cited are *T. terrestres*, *A. sativa*, *G. biloba*, *P. ginseng*, *S. aromaticum*, *T. afrodisiaca*, and other plants (Patel et al., 2011; Malviya et al., 2011; Singh et al., 2012; Singh et al., 2013).

The phytosteroid beta-sitosterol and the flavone apigenin were isolated from the aerial parts of *T. micranthus*, which also indicated the presence of chemical groups such as flavonoids (dihydroflavonol and leucoanthocyanidins), steroids, triterpenes, heterside anthraquinone, plus eugenol found in its essential oil (Cansian et al., 2012; Custódio et al., 2010). The improvement of sexual performance in animals treated with the extracts, as showed by us, can be explained by the rich phytochemical composition of the plant, suggesting several pathways of sexual activity stimulation. Phytochemicals, such as flavonoids, have been reported in the literature as directly affecting male sexual functions (Malviya et al., 2011), leading to smooth muscle relaxation (vasodilation) in the corpus cavernosum by activation of the nitric-oxide (NO) - cyclic guanosine monophosphate (cGMP) via, or by interacting with central pathways involved in libido or sexual desire (Estrada-Reyes et al., 2013).

Studies have shown aphrodisiac activity in some plants such as cloves (*S. aromaticum*) and nutmeg (*Myristica fragrans*) (Tajuddin et al., 2003). These plants, as well as *T. micranthus*, have eugenol-rich essential oils, which have been reported in studies as vasodilating and smooth muscle relaxant agents (Criddle et al., 2003; Tajuddin et al., 2005).

Another suggested mechanism of action involves the neurotransmitters responsible for smooth muscle relaxation in the corpus cavernosum. Drugs that induce changes in neurotransmitters levels or their activity in cells may alter sexual behavior, which is often reinforced by high testosterone levels. The classical theory of testosterone treatment is the stimulation of sexual desire and, in doing so, restores erectile functioning (Sureshkumar et al., 2000; Tajuddin et al., 2003; El-Tantawy et al., 2007).

According to our findings, animals treated with the crude extract showed a greater sexual activity after the third hour of treatment, in a dose-dependent manner, thus demonstrating the activity of *T. micranthus* on enhancing sexual behavior in mice. Further studies may complement these results in order to define the mechanism of action and the components that determine such activity.
Conclusion

This study showed that the crude ethanolic extract of *T. micranthus* enhanced the sexual behavior of male mice, thus supporting the use of this plant in folk medicine as an aphrodisiac.

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Conflict of Interest

Authors have not declared any conflict of interest.

REFERENCES


