Aphrodisiac Properties of Methanolic Extract of *Smilax myosotiflora* Tubers in Male Rats

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Abstract

Smilax myosotiflora has been claimed in Malay traditional medicine to improve sexual functions in men. As this claim is not scientifically tested and proven, the present study aimed to investigate the effects of methanolic extract of *S. myosotiflora* tubers upon the expression on fertility and sexual behavior parameters in Sprague Dawley male rats. Forty eight sexually experienced male rats were divided into four groups (A-D). Rats in group A were administered with 0.4 ml of distilled water (vehicle) while groups B, C and D received 200, 400 and 800 mg/kg of the extract in 0.4 ml of the vehicle. After 30 days of treatment, fertility test was evaluated by pairing the male rats with 2 untreated females, 3 hours daily for a maximum of 2 weeks. Subsequently, sexual behavior was monitored and recorded by caging male rat with receptive female in estrus phase for 30 minutes. Results showed that *S. myosotiflora* extract significantly ($p<0.05$) improved fertility index. All doses of *S. myosotiflora* tested significantly ($p<0.05$) reduced mount and intromission latency and improved copulatory rate. The rats on 400 mg/kg showed a marked improvement ($p<0.05$) in the number of intromissions. Further, male rats that were treated with 800 mg/kg of the extract were found to significantly ($p<0.05$) reduced ejaculation latency and inter-intromission interval, and pronounced significant ($p<0.05$) increase in ejaculation frequency. In conclusion, oral administration of *S. myosotiflora* extract enhanced fertility and stimulated sexual behavior parameters in male rats.

KEYWORDS: *Smilax myosotiflora*; Sexual functions; Fertility test; Sexual behavior.

Introduction

In regards to the gradual increase in male sexual dysfunction cases, a number of treatment options have been developed. Unfortunately, these options are very high in cost, not easily accessible and associated with some serious side effects such as penis pain, urethral bleeding and infection (Schwarz *et al.*, 2006). Due to the increasing number of men seeking help for treatment of sexual dysfunction, more pharmacological research that provide cheaper and natural treatment options are really necessary. In consistent with this view, phytochemicals that have aphrodisiac potential in animals have gained a great reputation and have become known worldwide as an instant treatment (Adimoelja, 2000). Despite the widespread of phytomedicines, the

effects of a large portion of herbal plants used traditionally to treat sexual deficiency and related disorders have not been proven scientifically.

Smilax myosotiflora is a herbaceous climber from the family *Smilacaceae* and locally known as 'ubi jaga'. This plant is widely distributed in peninsular Malaysia, southern Thailand and Jawa (Zhari *et al.*, 1999). It is among the popular ingredient in several Malay traditional medicines and aboriginal herb preparations. Phytoconstituents of the methanolic extract of *S. myosotiflora* tubers included alkaloids, saponins, flavonoids, tannins, and coumarins (Dasuki *et al.*, 2012). According to its functions, the decoction of *S. myosotiflora* tubers has been consumed traditionally to strengthen male energy and intensify sexual performance, whilst the leaves and fruits which are used internally for treating syphilis and rheumatism (Ang *et al.*, 2004).

Previous studies have reported that the phenolic content of *S. myosotiflora* was responsible for the antioxidant activity (Dasuki *et al.*, 2012). Further, the extract has been shown to inhibit the growth of *Haemonchus contortus* (Wahab *et al.*, 2010). According to Asiah *et al*. (2007), *S. myosotiflora* has 4.3kDa bioactive peptide peak as same as *Eurycoma longifolia*. This protein has been proven to increase the expression of testosterone in rat leydig cells (Sambandan *et al.*, 2004). The present study therefore undertaken to evaluate the effects of *S. myosotiflora* tubers methanolic extract on fertility test and sexual behavior parameters of sexually experienced male rats. This is with a view to validate the acclaimed use of the plant tubers as sexual enhancer in the folk medicine of Malaysia.

Materials and Methods

Plant material

S. myosotiflora plants were collected from area of Sungai Sok, Kelantan, Malaysia. The plants were authenticated (voucher no. 11397) by Dr Rahmad Zakaria, botanist of Herbarium Unit, Universiti Sains Malaysia.

Preparation of extract

The tuber parts were dried at 50°C in the oven for 2-3 days until a constant weight was obtained. The dried tubers were grinded into fine powder (500g) and then extracted with methanol using soxhlet apparatus. The extract was concentrated through vacuum using rotary evaporator and left to dry at room temperature (Dhawan *et al.*, 2003). The resultant yield was reconstituted in distilled water to give the required doses of 200, 400 and 800 mg/kg body weight applied in this study.

Experimental animals

Inbred adult Sprague Dawley rat of males (250-270g) and females (200-220g) were supplied by Animal Research and Service Centre (ARASC), Universiti Sains Malaysia, Kelantan, Malaysia. The rats were caged at a temperature of 22 ± 1 °C with a reversed light dark cycle (light from 2000 h to 0800 h) and relative humidity of 50±5%. The rats were fed with excess standard animal feed and water *ad libitum* always available. All male rats were trained for sexual experience by exposing each of

male rats to a female rat in behavioral estrus for overnight. The male rat was considered as sexually experience if the present of sperm was detected in vaginal smear in the subsequent morning.

Experimental design

Forty eight male rats were randomly divided into four groups (A-D) of 12 each. Group A were orally administered once daily with 0.4 ml of distilled water (vehicle) while groups B, C and D received 200, 400 and 800 mg/kg of the extract in 0.4 ml of vehicle for 30 days prior to mating and throughout mating period (OECD, 2001). All experimental procedures on rats were conducted in accordance to USM Guide For The Care and Use of Laboratory Animals and approved by Animal Ethics Committee [PPSG/07(A)/044/(2009)(50)].

Fertility test

After 30 days of treatment, each male rat was separated into a single cage and was allocated with two untreated female rats (1:2 ratio), 3 hours daily for a duration of 14 days. During this period, at least two estrous cycles of female rats should have elapsed while exposed to the males (Kuriyama and Chahoud, 2004). Vaginal smears from the female rats were collected daily after the 3 hours test time. The day of sperm detection in the vaginal smear was considered as day 0 of pregnancy. The female rats were sacrificed at day 21 post-conception. The following fertility parameters were then calculated according to the standard procedures (Gill-Sharma *et al.*, 2001):

(a) Mating index: Number of males that make any of his female partner sperm positive within 14 days / total number of males involved in mating × 100.
(b) Fertility index: Number of days elapsed until the male rats had first fertilized its female partner.
(c) Libido index: Number of sperm positive females / total number of females involved in mating × 100.
(d) Pregnancy index: Number of pregnant females / number of sperm positive females × 100.
(e) Live Fetus index: Number of live offspring / total number of offspring during autopsy at day 21 of pregnancy × 100.
(f) Litter size index: Number of pups per pregnant females can vary within group from 0-17. Non pregnant females were assigned as 0, if any, in the control group were also included.

Sexual behavior

Each of male rats was allowed to rest for at least 3 days before they were assessed for their sexual behaviors. Afterwards, the male rat was placed in a transparent cage, in a silent room under dark/light illumination (red light, 75W). After 5 minutes of adaptation period, a receptive female in behavioral estrus was introduced gently to the cage. The estrous cycle of the female rat was determined according to standard procedures (Yener *et al.*, 2007). The mating behaviors were recorded for 30 minutes using a video compact recorder. The following parameters were then computed and calculated according to standard methods (Dasuki *et al.*, 2011):

(a) Mount latency (ML): Time (in second) from the introduction of the female to the first mount.
(b) Intromission latency (IL): Time (in second) from introduction of the female to the first intromission.
(c) Ejaculation latency (EL): Time (in minute) from the first intromission to the first ejaculation.
(d) Mount frequency (MF): Total number of mounts observed in 30 minutes.
(e) Intromission frequency (IF): Total number of intromissions observed in 30 minutes.
(f) Ejaculation frequency (EF): Total number of ejaculations observed in 30 minutes.
(g) Post-ejaculatory interval (PEI): Time (in minute) from the first ejaculation until the next intromission.
(h) Intromission ratio (IR): Total intromissions divide by total mounts plus intromissions
(i) Inter-intromission interval (III): Ejaculation latency divides by total intromissions.
(j) Copulatory rate (CR): Total mounts plus intromissions divide by the time from the first mount until ejaculation (not the ejaculation latency).

Statistical analysis

Data with normal distribution were analyzed using one-way ANOVA, followed by Bonferroni post-hoc test in case of overall significant effects. Data which not normally distributed were analyzed using Kruskal Wallis test and followed by Mann Whitney U test for comparison between groups. Proportion was analyzed using Chi square test or Fisher's exact test. For all of the statistical comparisons, the level of significant difference was defined as $p<0.05$. The statistical analyses were performed using SPSS software (version 20.0).

Results

Effects on fertility test

Indices of fertility test were illustrated in Table 1. The proportion of females that were impregnated by male rats (mating index) did not present any considerable effect. The methanolic extract of *S. myosotiflora* after 30 days of treatment at all doses was able to improve significantly ($p<0.05$) fertility index by reducing the days elapsed for the males to fertilize their female partners. Although the ratio of pregnant per sperm-positive females (pregnancy index) appeared to increase especially in the groups that received 400 and 800 mg/kg of extract, it did not reach statistically significant. The libido, live fetuses and litter size indices of male rats treated with various doses of the plant extract remained statistically unaffected.

Effects on sexual behavior

Table 2 demonstrated the sexual behavior parameters of male rats. All doses of *S. myosotiflora* were able to significantly decrease ($p<0.05$) ML and IL. The rats that were treated with 400 mg/kg of extract showed a marked improvement ($p<0.05$) in the number of intromissions (IF). Further results revealed the male rats that received 800 mg/kg of the extract pronounced a significant reduction ($p<0.05$) in EL. This dose also significantly increased ($p<0.05$) the EF. A trend of reduction was observed in the III parameter, which reached statistical difference ($p<0.05$) at the dose of 800 mg/kg. The prosexual activities of the plant extract was further

manifested by significantly increased ($p<0.05$) CR in dose-dependent manner. Though not statistically significant, the other sexual parameters such as MF, PEI and IR were also observed to enhance.

Discussion

The search for aphrodisiac substances that can fix sexual deficiency dates back to millennia (Melnyk and Marcone, 2011). Folk medicine of Malaysia has alluded to the use of numerous of plant species as sexual enhancer including *S. myosotiflora*. The decoction of its tubers is popularly claimed as an alternative therapy for treating sexual dysfunction (Wan Hassan, 2006). In order to understand the scientific reasons behind this folk claim, the effects of methanol extract of *S. myosotiflora* on male reproductive functions were investigated in this study.

Fertility test is designed to assess the fecundity and sexual competency of male rat and also to provide measures of the functional consequences of reproductive damage (Manson and Kang, 1994). The findings of the present study indicated male rats that were administered with *S. myosotiflora* demonstrated significant effect in fertility index. It was suggested that the sexual capability of treated rats were improved as evidence of the day elapsed for male rats to make their females 'sperm positive' were reduced as compared to control group. Other parameters such as mating, libido, pregnancy, live fetus and litter size indices remained statistically unchanged. The enhancement of male fertility in this study could be attributed to antioxidant properties possessed by the plant extract (Dasuki *et al.*, 2012). Antioxidants have been demonstrated to enhance the process of spermatogenesis and synthesis of steroid hormones such as glucocorticoids, androgens and estrogens and thus leading to improvement of mating performance in male rats (Brinkhaus *et al.*, 2000).

Sexual behavior in male is a complicated phenomenon which under the control of endocrine, central and peripheral nervous systems. Sexual behavior in animal models is considered useful in predicting the potential similar effects of chemicals in human (Hull and Dominguez, 2007). The mating behavior of male rat when cohabited with estrus (receptive) female consists of repeated series of mounts and intromissions, ultimately leading to ejaculation. ML and IL are considered as a mirror of sexual motivation of the rats and it normally acts inversely proportional to sexual motivation or desire (Yakubu and Akanji, 2011). All doses of *S. myosotiflora* extract produced significant reduction in ML and IL. This indicated that the hesitation time for the male rats to move towards receptive females was reduced. Hence, it might suggest that *S. myosotiflora* intake groups were extremely aroused and increased in sexual eagerness.

Any alterations in MF or/and IF are considered as a reflection of libido, strength, potency, sexual performance and vigour in male rats (Kpomah *et al.*, 2012). Treatment of *S. myosotiflora* especially at dose 400 mg/kg showed a remarkable increase in the number of complete intromissions. Even no dose-dependent effect was seen, the other two doses of *S. myosotiflora* (200 and 800 mg/kg) also demonstrated an increment in IF, but those two groups did not reach

statistical different. Such elevation in the number of intromissions by male rats suggests that the penile tumescene and rigidity as well as accessory muscle that help in sustaining erection of the male sexual organs were fully functioning (Yakubu and Afolayan, 2009).

Ejaculation in male rats is achieved after certain number of intromissions, approximately 8 to 12. EL is a useful indicator of sexual pleasure and performance, whereas EF represents strength, vigour and stamina (Abdulwaheb *et al.*, 2007). In this experiment, the extract had caused the reduction of EL in dose-dependent manner. However, only dose at 800 mg/kg was able to influence EL significantly. Yet again, the highest dose of the extract was able to increase significantly the number of ejaculations (EF) in male rats. This is mostly due to shortened latencies in the first ejaculation, and to lesser extent to second ejaculation. It is clear evidence that sexual pleasure and performance were enhanced in extract-treated rats, particularly at the dose of 800 mg/kg.

The prosexual effect of *S. myosotiflora* was further manifested by the significant increase in CR. Such enhancement in CR indicates sustained increase in interest, stamina, focus and agility in the sexual acts (Tajuddin *et al.*, 2005). Last but not least, administration of *S. myosotiflora* also pronounced a significant decrease in III, in dose-dependent manner. However, only male rats at the dose of 800 mg/kg did reach statistical different. The reduction of III values is an indication that significant and sustained of penile erection was activated (Abdulwaheb *et al.*, 2007).

Earlier report showed that *S. myosotiflora* contains stigmasterol, sitosterol and campesterol. Stigmasterol has been demonstrated to be acting as an intermediate in the biosynthesis of androgens, corticoids and estrogens (Hasnah and Shaida, 2000). Sexual behavior and penile erection are critically depended on androgens which may act through central and peripheral mechanisms (Mills *et al.*, 1996). Thus, the improvement of sexual behaviors observed in this study could be due to the alteration of androgens in male rats.

In conclusion, the present study indicated that methanolic extract of *S. myosotiflora* enhanced fertility and stimulated sexual behavior parameters in male rats. However, the mechanisms of *S. myosotiflora* extract exert its aphrodisiac effects may await further studies.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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Tables

Table 1: Effects of *S. myosotiflora* extract on fertility test.

Parameters	Control (n=12)	*S. myosotiflora* extract			p value
		200 mg/kg (n=12)	400 mg/kg (n=12)	800 mg/kg (n=12)	
[y] Mating index (%)	12/12 (100)	12/12 (100)	12/12 (100)	12/12 (100)	$p > 0.05$
[z] Fertility index (day)	6.50 ± 5	3.00 ± 2	3.00 ± 4	3.00 ± 3	$p < 0.05^*$
[y] Libido index (%)	22/24 (92)	24/24 (100)	22/24 (92)	24/24 (100)	$p > 0.05$
[y] Pregnancy index (%)	16/22 (73)	17/24 (71)	19/22 (86)	21/24 (88)	$p > 0.05$
[y] Live Fetus index (%)	176/176 (100)	150/150 (100)	177/177 (100)	220/221 (99)	$p > 0.05$
[z] Litter Size index	9.50 ± 12	7.00 ± 11	8.00 ± 4	11.00 ± 6	$p > 0.05$

[y] = Fisher's exact test. [z] = Kruskal-Wallis test. Values are median ± IQR.
n = number of rats. * Significant level: $p<0.05$.

Table 2: Effects of *S. myosotiflora* extract on sexual behavior parameters.

Parameters	Control (n=12)	*S. myosotiflora* extract			p value
		200 mg/kg (n=12)	400 mg/kg (n=12)	800 mg/kg (n=12)	
[y]ML (sec)	22.00 ± 44.00	8.00 ± 3.75	3.50 ± 5.50	3.00 ± 2.00	$p < 0.05^*$
[y]IL (sec)	73.00 ± 123.25	12.00 ± 7.50	9.00 ± 8.50	8.00 ± 8.75	$p < 0.05^*$
[z]EL (min)	15.89 ± 1.45	15.17 ± 1.50	14.42 ± 1.50	10.63 ± 1.05*	$p < 0.05^*$
[z]MF	7.67 ± 0.89	10.33 ± 1.08	10.25 ± 1.22	7.92 ± 1.10	$p > 0.05$
[z]IF	15.33 ± 2.05	23.83 ± 2.49	26.50 ± 2.72*	23.33 ± 1.99	$p < 0.05^*$
[y]EF	1.00 ± 1.00	2.00 ± 0.75	2.00 ± 0.00	2.00 ± 1.00*	$p < 0.05^*$
[z]PEI (min)	6.60 ± 0.31	6.66 ± 0.29	6.55 ± 0.29	6.56 ± 0.22	$p > 0.05$
[z]IR	0.65 ± 0.03	0.69 ± 0.03	0.72 ± 0.02	0.75 ± 0.03	$p > 0.05$
[y]III	1.05 ± 0.68	0.63 ± 0.58	0.56 ± 0.45	0.48 ± 0.37*	$p < 0.05^*$
[z]CR	1.63 ± 0.31	2.42 ± 0.26	3.09 ± 0.60	3.38 ± 0.55	$p < 0.05^*$

[y] = Kruskal-Wallis test. Values are median ± IQR. [z] = One-way ANOVA test. Values are mean ± SEM. ML= mount latency; IL= intromission latency; EL= ejaculation latency; MF= mount frequency; IF= intromission frequency; EF= ejaculation frequency; PEI= post-ejaculatory interval; IR= intromission ratio; III= inter-intromission interval; CR= copulatory rate. n = number of rats. * Significant level: $p<0.05$.