

EFFECTS OF 50% ETHANOLIC EXTRACT OF *ANDROGRAPHIS PANICULATA* ON SEXUAL BEHAVIOR AND SERUM HORMONAL ASSAY IN MALE SPRAGUE DAWLEY RATSDasuki MS^{1*}, Wan MH¹, Hasnan J², SitiAmrah S² and D' Souza UJ³¹School of Health Sciences, ²School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.³Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, Malaysia.*Corresponding author: drdasuki@usm.my Phone number: +6097677581 Fax number: +6097677515

ABSTRACT: *Andrographis paniculata* has been used for centuries as a medicinal herb for the treatment of various ailments. The present study was aimed to investigate the possible effect of *A. paniculata* extract on male sexual behavior parameters and sex hormone levels. Fifty Sprague Dawley male rats received 50% ethanolic extract of *A. paniculata* by gavaging for 10 weeks at the doses of 0.5, 1, 10, 100, 1000 mg/kg, while another 10 male rats were given distilled water. Sexual behavior study was carried out on day 45th of treatment by pairing with a naturally receptive female (1:1). At the end of treatment period, the male rats were sacrificed by ethyl ether inhalation. The bloods were centrifuged to obtain serum for determination of hormone levels. Results showed that sexual behavior parameters in treatment groups were found to be comparable with that of the control indicating no effect on sexual motivation and performance. Treatment of *A. paniculata* was significantly increased serum testosterone level, while FSH and LH concentrations remained statistically unchanged at any of the dose levels. Based on these findings, ingestion of *A. paniculata* did not induce any adverse effect on sexual behavior and improvement of testosterone observed might be contributed for therapeutic use of this plant as sex enhancer.

Keywords: *Andrographis paniculata*, 50% ethanolic extract, sexual behavior, sex hormone

INTRODUCTION

Andrographis paniculata is a perennial herbal shrub, which belongs to the family of *Acanthaceae*. This plant is widely distributed in tropical and subtropical Asia, south-east Asia and India (Datta *et al.*, 2012). It is a predominant constituent of at least 26 Ayurvedic formulations in Indian Pharmacopoeia (Sonia, 2014). Phytochemicals investigation of *A. paniculata* reveals the presence of diterpenoids, flavonoids and polyphenols, where andrographolide is detected as a major component (Koteswara Rao *et al.*, 2004). According to its functions, the extract of *A. paniculata* has been described to have multiple pharmacological properties such as antithrombotic (Thisoda *et al.*, 2006), anti-platelet aggregation (Amroyan *et al.*, 1999), antibacterial activity (Mishra *et al.*, 2009), anti-inflammatory (Xia *et al.*, 2004), antidiarrhoeal effect (Gupta *et al.*, 1990), antiviral (Wiert *et al.*, 2005), antipyretic (Panicha *et al.*, 2007), immunostimulant (Xu *et al.*, 2007) and anticancer activities by inhibition of cell cycle progression (Shi *et al.*, 2008). It also act as a hypoglycaemic agent (Zhang and Tan, 2000), and used for treatment of upper respiratory tract infection (Coon and Ernst, 2004). Several *in-vivo* studies showed that *A. paniculata* has antifertility effects which caused cessation of spermatogenesis in male rats (Akbarsha *et al.*, 1990) and female rats become infertile and may abort (Zoha *et al.*, 1989). Inversely, study by Burgos *et al.* (1997) found that treatment of *A. paniculata* for 60 days did not produce any testicular toxicity effect in male rats. In a study with human subjects, no significant negative effect of *A. paniculata* (fixed combination Kan Jang) on male semen quality and fertility could be observed, and rather, the claim by the principally of volunteers was feeling of enhanced sexual potency during masturbation (Mkrtchyan *et al.*, 2005). Therefore, in the present study an attempt has been made to investigate the effect of 50% ethanolic extract of *A. paniculata* on sexual behaviors and serum hormonal assay in male rats.

MATERIALS AND METHODS**Preparation of plant extract**

The extraction of *A. paniculata* was performed using a mixture of 50% ethanol and 50% water by NOVA laboratories, Selangor, Malaysia. Standardized extract of *A. paniculata* was supplied in liquid form, dark green color.

The extract was subjected to freeze drying (Heto LL3000) at -50°C overnight. Later, the dried pure *A. paniculata* extract was scraped using a spatula and was pulverized into powder with an electric blender (Khind, Malaysia). The extract was then reconstituted in distilled water to give the required doses of 0.5, 1, 10, 100, 1000 mg/kg body weight applied in this study.

Animals

A total of 60 adult male rats and 120 adult female rats of Sprague Dawley were obtained from Animal Research and Service Centre, Universiti Sains Malaysia (USM), KubangKerian, Kelantan, Malaysia. The rats weighing between 180-200 grams (aged 8-10 weeks) were housed in PVC cages (HebeiTuohua Metal Products Co., Ltd., China) and maintained under controlled environmental condition at temperature of $22\pm 25^{\circ}\text{C}$ with a 12 h light / 12 h dark cycle. All animals had free access to tap water and were fed with a standard pellet diet (Gold Coin Feed Mills, Malaysia). All rats were allowed to acclimatize for one week prior to the treatment. 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 experienced if the present of sperm was detected in vaginal smear in the subsequent morning (Dasukiet al., 2011).

Treatments

Sexually experienced male rats were randomly divided into six groups (I-VI) of 10 each. Group I received 0.4 ml of distilled water and served as control. Groups II, III, IV, V and VI received 0.5, 1, 10, 100 and 1000 mg/kg of *A. paniculata* extract in 0.4 ml of vehicle respectively. The different doses of the extract were administered orally to the rats via a gastric tube (Instech Solomon, USA) 2 hours after the onset of darkness once daily for 10 weeks (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 USM/PPSP/050(1).

Male Sexual behaviors

The experiment was carried out on the 45th day after the commencement of *A. paniculata* administration. The sexual behavior of male rat was conducted in a silent room under the presence of dark/light illumination (red light, 75W). After 5 minutes of adaptation period in the copulation cage (transparent cage), 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 (Sony Handycam). The following parameters were then computed and calculated according to standard methods (Wan et al., 2013).

- 1) Mount latency: Time (in second) from the introduction of the female to the first mount.
- 2) Intromission latency: Time (in second) from introduction of the female to the first intromission.
- 3) Ejaculation latency: Time (in minute) from the first intromission to the first ejaculation.
- 4) Post-ejaculatory interval: Time (in minute) from the first ejaculation until the next intromission.
- 5) Intromission frequency: Total number of intromissions observed in 30 minutes.
- 6) Intromission ratio: Total intromissions divide by total mounts plus intromissions
- 7) Inter-intromission interval: Ejaculation latency divides by total intromissions.
- 8) Copulatory rate: Total mounts plus intromissions divide by the time from the first mount until ejaculation (not the ejaculation latency).

Hormonal assay

At the end of treatment period, the male rats were sacrificed by ethyl ether (Merck, Germany) inhalation. Blood samples were collected from the inferior vena cava and were allowed to clot for 10 min at room temperature. Subsequently the blood samples were centrifuged at $224 \times g$ for 10 min with Uniscop Laboratory Centrifuge (model SM800B, Surgifriend Medicals, Essex, England) to obtain serum for hormonal assay. Testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) radioimmunoassay test kits are products of DRG Diagnostics, GmbH, Germany. Serum samples were assayed by using the procedure described by DRG Diagnostics. This was based on the principle of radioimmunoassay of competitive binding between the sample serum and the standards for a constant amount of the antisera (Tietz, 1995).

Statistical analysis

Testosterone concentrations and sexual behavior parameters were subjected to non-parametric Kruskal Wallis test in order to calculate Median and inter-quartile range. Significant differences between groups were determined by Mann Whitney U test. FSH and LH concentrations were subjected to ANOVA test in order to calculate Mean and standard error mean. Significant differences between groups were determined by Bonferroni post-hoc test. The level of significant difference was defined as $p < 0.05$. The statistical analyses were performed using SPSS software (version 20.0).

In addition, treatment of *A. paniculata* standardized extract (10% andrographolide) up to 1000 mg/kg showed no signs of dose-dependent toxicity. The body weight gain and feed consumption were also not affected at any of the dose levels (Allan et al., 2009). On the contrary, study by Akbarsha and Murugaian (2000) showed that ingestion of *A. paniculata* for 48 days caused sperm counts to decrease, the spermatozoa were not motile and several of them possessed abnormalities.

In the present study, *A. paniculata* was administered to male rats to determine its effects on rat sexual behavior and serum hormonal levels. In rat model of sexual behavior, mount latency, intromission latency and post-ejaculatory interval are considered as measures of sexual arousability and motivation, whereas intromission frequency, intromission ratio and copulatory rate as measures of potency and performance (Yakubu et al., 2008). Although *A. paniculata* has been known as a health-promoting herb (Datta et al., 2012), the results showed that no doses of the plant extract produced any significant changes in sexual motivation and performance. The brain area of medial preoptic is an important area that responsible for the regulation of male sexual behavior, including genital reflexes, sexual motivation and copulatory performance (Giuliano and Allard, 2001). Changes in the level of neurotransmitter in medial preoptic area such as dopamine have been demonstrated to facilitate mating and to enhance sexual responsiveness (Hull et al., 2004). Thus, the unaltered of sexual behavior in male rats indicates that treatment of *A. paniculata* did not interfere with the concentration of dopamine and its availability. However, this plant may affect the rats in other conditions, as reported in experiments with other plant species in non-copulating male rats (Ang and Ngai, 2001), the rats with impotent symptoms (Zanoli et al., 2009) or different fractions of substance in differently aged animals (Ang et al., 2003).

Sexual behavior and erection are largely dependent on androgen which may act through central and peripheral mechanism (Mills et al., 1996). Chronic daily treatment of *A. paniculata* showed a prominent increase in serum testosterone level, while concentrations of FSH and LH remained unaffected. Although some reports showed that the extract of *A. paniculata* at doses 1g/kg in male rats for 50 days (Burgos et al., 1997; Allan et al., 2009), could not produce any significantly effect on testosterone level, it should be noted that, in this study high testosterone level was observed at week 10 (70 days), but not at week 7 (49 days). Surprisingly, the elevation of testosterone level in this study did not affect the overall male sexual behavior parameters. Studies have shown that increase in testosterone helps to improve sexual function and libido (James and Nyby, 2002), in addition to the intensity of orgasm and ejaculation (Traish, 2009). Besides, etiology of erectile dysfunction has been correlated with low serum testosterone levels (Lejeune et al., 2013). Enhanced levels of testosterone after treatment of plant extract could be foreseen as a possibility for usage of the herb in treatment of erectile dysfunction.

CONCLUSION

The results of this study established that various doses of *A. paniculata* extract did not induce any changes to male sexual behavior parameters. While for the effect on sex hormones, increase in the testosterone observed with *A. paniculata* supplementation is beneficial in this regard. However, further studies on higher mammals are needed to evaluate the possible therapeutic use of *A. paniculata* in sexual dysfunction.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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