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WORD COUNT	3900	TIME SUBMITTED	24-JAN-2019 09:41AM
		PAPER ID	43804929

Medicinal Plants
Vol. 10 (1), March 2018, 52-57

doi :

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Research Article

Potential Efficacy of extracts of *Pimpinela alpina*, *Tribulus terestris* and *Eurycoma longifolia* in increasing sexual activity in male wistar rats

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Received: August 09, 2017; Accepted: February 27, 2018

ABSTRACT

Sexual dysfunction includes erectile dysfunction or impotence, ejaculation dysfunction and hypogonadism. It is a serious public health problem among young as well as old men worldwide, with a prevalence of more than 20%. In Indonesia, many indigenous plants have sex-stimulating effects include *Tribulus terestris* (tribulus), *Eurycoma longifolia* (pasak bumi) and *Pimpinela alpina* (purwoceng). In the present study, we evaluated the combination of *T. terestris*, *E. longifolia* and *Pimpinela alpina* (purwoceng) on the sexual behavior of male rats. Male Wistar rats were randomized to five groups (6 rats/group). Group 1: negative control. Group 2: positive control (sildenafil citrate 5 mg/kg b.w). Groups 3, 4 and 5: treatment with extract combination at 250 mg/kg b.w., 500 mg/kg b.w., 1000 mg/kg b.w. Mating behavior and mating performance were evaluated. Testosterone, FSH and LH were assayed by ELISA. The results showed that the extract combination (1000 mg/kg b.w.) significantly increased the frequency of mounting and intromission in comparison to the sildenafil group. The extract combination (1000 mg/kg b.w) increased serum hormone levels in comparison to sildenafil. It can be concluded that the extract combination at a dose of 1000 mg/kg b.w. increased sexual activity and hormonal concentrations in comparison to sildenafil treatment.

Keywords: *Pimpinela Eurycoma Tribulus*, hormonal, sexual activity

INTRODUCTION

Sexual dysfunction includes erectile dysfunction or impotence, ejaculation dysfunction, hypogonadism, etc. It is a serious public health problem among young as well as old men worldwide, with a prevalence of more than 20% (Ho *et al*., 2011; Laumann *et al*., 2005; Porst *et al*., 2007). This dysfunction leads to risk for aging and other etiological factors, including degenerative disease, increase in injuries and stress associated with industrialized lifestyles. Sexual relationship is among the most important social and biological relationship of human life by boosting the mood as well as interpersonal functioning. It does not only affect life expectancy, but also have a significant negative impact on an individual's wellbeing and quality of life (Fugl-Meyer and Fugl-Meyer, 2000). People have searched for ways to achieve sexual desire or sexual techniques from ancient times (Jain *et al*., 2010). Successful treatment of sexual dysfunction may improves not only sexual relationships, but also the overall superiority of life (Shin *et al*., 2010). It can be treated by both medical and surgical modalities. To achieve better sexual desire has led to the development and use of different substances known as aphrodisiacs. An enhances

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sex drive or sexual pleasure by crossing the blood brain barrier and mimicking or stimulating some area of sexual arousal in the central nervous system. These substances also act physiologically to increase blood flow to the penile area or increase the duration of sexual activity by numbing the genital area or even mimic the burning sensation of sexual intercourse.

Management of sexual dysfunction includes counseling of patient by an experienced psychiatrist or psychologist to restore confidence and improve patient's ability to obtain adequate erection, the use of vacuum erection devices, the use of surgical penile implants, hormonal treatments mainly with testosterone, or the use of specific drugs which increases firmness, maintenance of erection, frequency of orgasm, and level of desire (Taher *et al.*, 2001). Notably, this treatment is high in cost. The side effects include complications (e.g., infections) following surgical procedures, mechanical device failure, social stigma, and others such as headache, flushing, dizziness, visual disturbances, nasal congestion and priampism. Thus, despite advances in modern and herbal medicines, there remains an urgent need to develop safe and cost-effective drugs. For several hundred years, people around the world have used locally grown plants as supplements to energize, vitalize, and eventually to improve sexual functions. However, plant-derived and herbal remedies continue to be a popular alternative to treat sexual disorder and have also proven effective in improving sexual desire and sexual behavior in male animals (Rowland and Tai, 2003; Suresh *et al.*, 2000).

Many indigenous plants have been claimed to have a sex-stimulating effect in Indonesia. These included *Tribulus terestris* (tribulus), *Eurycoma longifolia* (pasak bumi) and *Pimpinela alpina* (purwoceng). Most studies published on this regard have generally targeted one plant at a time even though in the traditional medicine, most of the plants are used in formulations of groups of two or four plants (Ratnasooriya and Dharmasiri, 2000). The aim of present study was to determine the effects of combination extract (*Tribulus terestris* (tribulus), *Eurycoma longifolia* (pasak bumi)and *Pimpinela alpine* (purwoceng)) on sexual behavior and hormonal level of male rats.

MATERIALS AND METHODS

The selected plants were collected from The Center of Study Traditional Plants, Ministry of Health, Republic of Indonesia in Tawamangu, Karanganyar, Central Java, Indonesia. The plants were identified and authenticated by The Indonesia Science Institute (LIPI). The collected plant material was made free from foreign organic matter.

Pimpinela alpina, Tribulus terestris and *Eurycoma longifolia* were selected. The selected parts were allowed to get air dried and triturated to make powder form individually. The prepared powders were mixed in the ratio 1:1:1. The mixed powder was extracted with Aquadest 10:1 (10 L Aquadest for 1 kg powder). The extract was evaporated by rotary evaporator.

Wistar rats (3-4 months) weighing approximately 200–300 g of either sex housed in standard conditions of temperature (24 ± 2°C), relative humidity (55 ± 5%), and light (12 hours light/dark cycles) were used. They were fed with standard pellet diet and water *ad libitum* (Hu *et al.*, 2009). The research was been done at a laboratory of preclinical study and a laboratory of biomolecular investigation at the Faculty of Medicine Sriwijaya University, Palembang, Indonesia, with approval of The Ethics Committee of Bioethics and Humaniora Unit, Faculty of Medicine Sriwijaya University, Palembang, Indonesia, (No. 217/kptfkrsmh/2016).

The rats were fasted for 18 hours with water *ad libitum*. The extract was administered in six different doses: 250, 500, 1000, 2000, 4000 and 8000 mg/kg body weight, per oral. The animals were observed for clinical signs and symptoms of toxicity every 30 minutes up to 6 hours on the first day and thereafter, everyday up to 7 days (Chauhan *et al.*, 2007). Acute toxicity studies showed no mortality or changes in behavior observed at dose up to 8000 mg/kg b.w. Dose selected for aphrodisiac activity was 250 mg/kg b.w., 500 mg/kg b.w., and 1000 mg/kg b.w. Animals were randomly divided into five groups with six animals per group. Group I represented the control animals treated with normal water only; Groups II, III and IV were treated with oral suspension extract at doses of 250, 500 and 1000 mg/kg BW, respectively; and group V served as a positive control treated with sildenafil citrate of 5 mg/kg BW. All the treatments were continued for 2 weeks.

Mating behavior studies were carried out in a separate room under dim red illumination according to the standard procedure. Healthy male Wistar rats showing brisk sexual activity and female animals showing regular estrus cycle were selected for the study. The male rats were placed in a rectangular Plexiglass chamber, 10 minutes before the introduction of a primed female and get acclimatized to the chamber conditions. The primed female was then

introduced into the chamber with one female to one male ratio and the mating behaviors observed for first week and second week after commencement of the extract treatment. The following mating behavior parameters were recorded: a. Mount frequency (MF): the number of mounts without intromissions from the time of introduction of the female until ejaculation; b. Intromission frequency (IF): the number of intromissions from the time of introduction of the female until ejaculation; c. Mount latency (ML): the time interval between the introduction of the female and the first mount by the male; d. Intromission latency (IL): the interval from the time of introduction of the female to the first intromission by the male (characterized by pelvic trusting and springing dismount); e. Ejaculation latency: the time interval between the first intromission and ejaculation (characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity). The experiment was terminated when the male rat began to mount the female followed by intromission after a brief period of inactivity (Subramoniam *et al.*, 2007; Carro-Juarez *et al.*, 2004).

After 2 week treatment, the male rat of each group was placed in a separate cage with estrus female animals for 1 day (male : female = 1:5). The next day morning, the vaginal smear of each female rat was examined under a microscope for the presence of sperm. The number of sperm-positive females was recorded in each experimental group and in comparison to values observed in the control group (Carro-Juarez *et al.*, 2004).

The blood was collected from retro orbital venous plexus of all animals after termination of experiment. Blood samples were spun at 2500 rpm for 10 minutes in a table top centrifuge. The serum samples were separated to measure the concentration of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. FSH, LH and testosterone were measured by ELISA kit (Sunlong Biotech), according to the protocol provided with each kit.

At the end of the study, the animals were killed by an overdose of anesthesia agent (ketamine). The right-hand side of the epididymis was removed and used for sperm analysis and left-hand side was used for morphological study, main and accessory reproductive organs were dissected and weighed.

The study findings are expressed as mean ± SD. The results were analyzed using Student's t-test and one-way ANOVA followed by post-hoc testing. Data were considered statistically significant at minimum level of $p< 0,05$.

RESULTS

All doses of extract combination were able to significantly decrease mount and intromission latencies, when compared to Aquadest control and standard drug (sildenafil). It also significantly decreased ejaculatory latency. Ultimately it resulted in an increased percentage of mounting frequency and intromission frequency in comparison to Aquadest control and standard drug (sildenafil). The extract combination at 1000 mg/kg BW significantly increased the frequency of mounting and intromission, in comparison to the sildenafil group (Table 1).

Daily administration of extract combination for 2 weeks to male rats resulted in a dose-dependent increase in mating performance as compared to the control group, (Figure 1).

The extract combination had significant effect on testosterone, LH and FSH concentration in serum in comparison to the control group (Figure 2). The level of testosterone, LH, and FSH increased gradually with dose dependency in all the experimental groups. The extract combination 1000 mg/kg BW showed an increase of serum hormone levels in comparison to sildenafil.

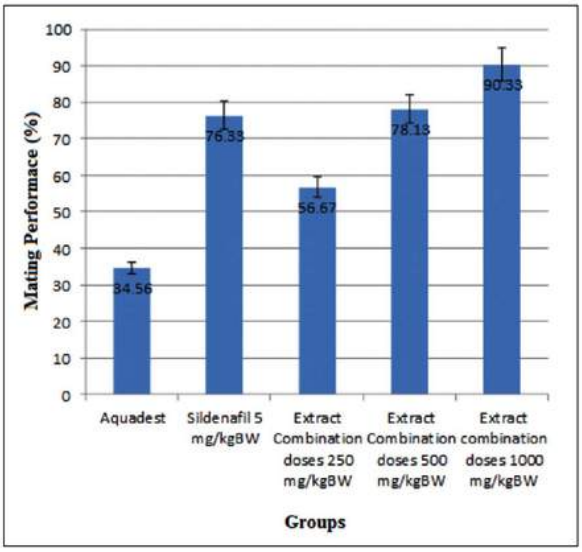

Figure 1: Effect of extract combination on mating performance rats

Table 1: Effect Extract Combination on Mating Behaviour

Mating Behaviour	Aquadest		Extract 250 mg/kgBW		Extract 500 mg/kgBW		Extract 1000 mg/kgBW		Sildenafil 5 mg/kgBW	
	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
MF	71.28±0.78	68.23±8.27	70.78±1.65*	99.23±1.21*	70.11±1.14*	117.54±1.45*	71.98±1.43 NS	128.54±2.11*	72.27±1.45*	121.12±3.65*
IF	72.31±1.11	74.56±2.23	72.03±1.33NS	119.22±2.01*	72.45±3.23 NS	121.43±2.11*	73.34±2.54*	137.99±3.12*	72.76±2.54NS	132.67±2.87*
ML	7.54±0.23	7.78±0.11	7.77±0.22 NS	4.21±0.18*	7.56±0.44 NS	3.89±0.27*	7.89±0.22 NS	2.99±0.14*	7.23±0.13 NS	3.78±0.21*
IL	8.23±0.22	8.16±0.19	8.25±0.23 NS	5.13±0.32*	8.34±0.78 NS	4.91±0.33*	8.32±0.32 NS	3.65±0.14*	8.11±0.14 NS	4.78±0.65*
EL	236.11±1.11	240.13±1.01	238.11±0.23*	756.11±1.21*	240.23±2.21*	811.13±1.23*	236.13±1.56NS	998.12±0.23*	223.13±1.43*	897.12±2.34*

All values were expressed as Mean±SD (n=6); significant difference from control,*p<0,05; NS= not significant, MF=mounting frequency, IF=intromission frequency, ML=mounting latency, IL=intromission latency, EL=ejaculation latency

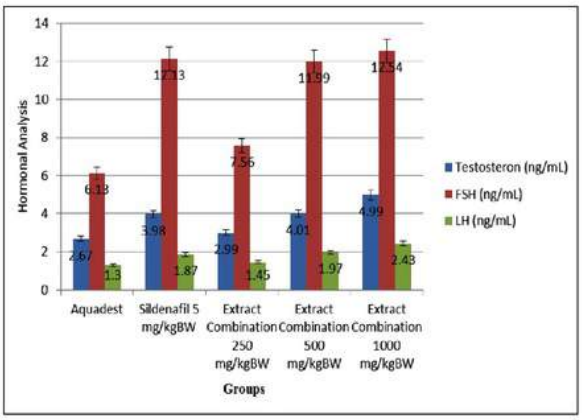

Figure 2: Effect of extract combination on serum testosterone, FSH and LH in male rats

DISCUSSION

This study examined the effect of the extract combination on male sexual competence in rats, with sildenafil citrate as positive reference drug. To the best of our knowledge, this is the first study to report the extract combination enhanced the sexual behaviors of male rats in comparison to the control. The present study provides special evidence that the extract combination is a potent stimulator of sexual behavior, particularly on sexual arousal in male rats.

The mating behavior study revealed that the doses of extract combination significantly increased MF and IF, in comparison to the control group and the doses 1000 mg/kg BW of extract combination increased MF and IF, in comparison to the standard group. The doses of extract combination significantly decreased ML and IL, in comparison to the control group and the doses 1000 mg/kg BW of extract combination decreased ML and IL, in comparison to the standard group. MF and IF are considered to be indices of libido and potency, while ML and IL are also indicators of sexual arousal (Sekar *et al*., 2009; Gundidza *et al*., 2009). The significant increases in MF and IF and the decreases in ML and IL indicate that libido and potency were enhanced by extract combination. Furthermore, the prolongation of EL is an indicator of prolonged duration of coitus. PEI is considered to be an index of potency, libido, and the rate of recovery from exhaustion after the first series of mating. This indicates that the treatment of extract combination remarkably delayed EL, with no negative effect on the other parameters of sexual behavior, and with no

locomotor alterations throughout the observation period. The delayed EL and increased penile erection in treated male rats indicated the involvement of NO in the intervention (Surender and Gupta, 2011). These observations support the role of extract combination in improvising sexual function.

The continued administration of various doses of extract combination for 2 weeks increased testosterone, LH and FSH levels. An increase in testosterone level has been associated with increased sexual desire, penile tumescence and rigidity, as well as increased use of accessory muscles that contribute to sexual activity (Kaufman and Cannon-Smith, 2007; Carro-Juarez *et al*., 2009).

Research on various animal and human models indicates that there is a strong correlation between sexual behavior and brain neurotransmitters such as dopamine and 5-HT (Yeh *et al*., 2008). The motor control of ejaculation in animals is modulated by serotonin and its receptors (Carro-Juarez and Rodriguez-Manzo, 2003). Testosterone may also facilitate male sexual behavior by increasing dopamine release in the medial preoptic area and potentiating nitrergic neurotransmission in brain, which resulted in stimulation of hypothalamic–pituitary–gonadal axis (Chauhan *et al*., 2011; Zamble, *et al*., 2008). Also, increase in testicular weight indicates the number as well as motility of sperm (OECD, 2001). Increased serum testosterone levels after administration of extract combination could thus be considered as one of the contributing factors responsible for the overall increased sexual performance in the treated groups, especially for lengthening of EL and increased copulatory ability in rats.

Several bioactive agents may be responsible for increasing endogenous testosterone levels and enhancing male sexual behavior. The mechanism of these agents includes steroids by rising androgen production (Gauthaman *et al*., 2002) flavonoids by enhancing testosterone synthesis or by preventing its metabolic degradation (Subramoniam *et al*., 1997; Chauhan *et al*., 2010); alkaloids by dilating the blood vessels in the sexual organs (Tajuddin *et al*., 2005); and saponin by activating gonadal tissues and CNS via-NO dependent mechanism (Yakubu *et al*., 2008). Thus, the improvements in sexual function demonstrated in the current study might be due to the presence of such compounds in extract combination.

Organ–body weight ratio is an index of inflammation or cellular constriction. The increase in the testes–body weight ratio observed may be attributed to increase the secretory activity of the testes. Increased testicular weight and high protein concentration of the testes indicate enhancement of testicular growth as FSH. Testosterone, LH, and FSH are hormonal marker of androgenicity (Mbongue *et al*., 2005). Increased testicular weight and hormonal concentrations indicate that the extract combination has androgenicity potential. FSH is responsible for the initiation, maintenance and production of normal sperm in pubertal rats. The significant increase in serum FSH suggests enhanced sperm cell production in Sertoli cells.

CONCLUSION

The study concluded that the cumulative dose of extract combination could enhance overall sexual function and performance in male rats by increasing the levels of FSH, LH, testosterone and spermatozoa concentration. The results suggest that the extract combination may be a new promising aphrodisiac, which could be used to improve the sex lives of men.

COMPETING INTERESTS

The authors declare no competing interest.

AUTHORS CONTRIBUTION

First author Radiyat Umi Partan conducted research and wrote paper, second author Rachmat Hidayat designed study and third author Mgs Iran Saleh reviewed the paper.

ACKNOWLEDGEMENTS

The author thank to the Sriwijaya University for facilitating this research

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