

## THE COMBINATION INFUSION OF *Talinum paniculatum* (Jacq.) Gaertn., *Centella asiatica* (L.) Urb. AND *Curcuma xanthorrhiza* Roxb. EFFECTS ON TESTOSTERONE LEVELS AND INTRODUCTION, CLIMBING AND COITUS FREQUENCIES IN MALE SPRAGUE-DAWLEY RATS

***Efek Infusa Kombinasi Talinum paniculatum (Jacq.) Gaertn., Centella asiatica(L.) Urb. dan Curcuma xanthorrhiza Roxb. terhadap Kadar Testosteron dan Frekuensi Introduction, Climbing dan Coitus Tikus SD***

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### ABSTRACT

*Talinum paniculatum* (som Java) reported to have many pharmacological activities such as increasing spermatozoa quality, cell regenerations, fertility, and antibacterial effect both empirically and scientifically. This study aimed to prove the combination infusion effects of som Java, gotu kola, and temulawak on introduction, climbing, and coitus levels as well as testosterone levels in male Sprague Dawley (SD) rats. SD rats were divided into 2 groups, control group received aquadest, treatment group received formula a mixture of som java *Talinum paniculatum* root, gotu kola (*Centella asiatica*) herb, and Javanese turmeric (*Curcuma xanthorrhiza*) rhizome with the dose of 491,2 mg/200 g BW for 7 consecutive days. Artificial estrus of female SD rats were induced by administering estradiol valerat 2 mg/200 g BW 48 hours before observation. The determined parameters were levels of testosterone as well as introduction, climbing and coitus frequencies. Administration of estradiol valerat led to artificial estrus induction of female SD rats. The administration of the combination infusion dose 491.2 mg/200 g BW significantly increased testosterone level as well as introduction and climbing frequencies of male SD rats as of 27.77%; 86.39% and 69.17% respectively.

**Keywords:** *Talinum paniculatum*, testosterone, aphrodisiac, climbing, coitus

### ABSTRAK

Som jawa (*Talinum paniculatum*) secara empiris dan ilmiah terbukti telah banyak dimanfaatkan oleh masyarakat untuk mengatasi berbagai masalah kesehatan diantaranya untuk meningkatkan kualitas dan kuantitas spermatozoa, regenerasi sel, fertilitas, dan sebagai antibakteri. Penelitian ini dilakukan untuk membuktikan efek peningkatan kadar hormon testosteron serta frekuensi *introduction*, *climbing*, dan *coitus* tikus jantan SD dari pengaruh infusa kombinasi som jawa, pegagan, dan temulawak. Hewan uji tikus galur SD dibagi menjadi 2 kelompok, yaitu kelompok kontrol diberikan akuades, sedangkan kelompok perlakuan diberikan infusa ramuan akar som jawa, herba pegagan, dan rimpang temulawak dosis 491,2 mg/200 g bb selama 7 hari berturut-turut. Induksi estrus artifisial tikus betina dengan pemberian estradiol valerat 2 mg/200 g bb dilakukan 48 jam sebelum pengamatan. Parameter yang diukur meliputi kadar testosteron, frekuensi *introduction*, *climbing*, dan *coitus*. Dibandingkan dengan kelompok kontrol, pemberian infusa ramuan tidak berefek pada frekuensi *coitus*, namun mampu meningkatkan kadar testosteron serta frekuensi *introduction* dan *climbing* tikus jantan sebesar 27,77; 86,39 dan 69,17%.

**Kata kunci:** *Talinum paniculatum*, testosteron, introduction, climbing, coitus

## INTRODUCTION

Aphrodisiac is an agent that contributes to an arouses sexual desire (Singh *et al.*, 2012). Nowadays, the prevalence of sexual dysfunctions was quite high and tended to increase with age, especially in men (Nicolosi *et al.*, 2004). Sexual dysfunction can be influenced by various factors such as psychological disorders, neurological disorders, chronic disorders, systemic diseases, and life styles (Singh *et al.*, 2013). Tan *et al.* (2011) reported the correlation between erectile dysfunctions and metabolic syndrome (Tan *et al.*, 2011). Diabetes mellitus as one of metabolic disorder also often associated with sexual dysfunction, fertility, and impotence (Kamaruzaman & Noor, 2017). There are many kinds of sexual dysfunction. The most frequent sexual dysfunctions for women are desire and arousal dysfunctions, while premature ejaculation and erectile dysfunction are the most common sexual dysfunctions for men (Mccabe *et al.*, 2016). In human life, sexual relationships take a high priority in social and biological relationship (Singh *et al.*, 2013). It has been stated that aphrodisiac combined with healthy life style will attain an improved sexual life (Erhabor & Idu, 2017).

Many natural substances have been empirically utilized for aphrodisiac. *T. paniculatum* Gaertn is one of natural aphrodisiac widely used in Asia as reproductive tonic (Thanamool *et al.*, 2013). In Indonesia, *T. paniculatum* is well known as Som Java or Java Ginseng. Java Ginseng is commonly used for many medicinal purposes. The daily oral administration of ethanol extract of Java Ginseng roots in concentration of 1.4 mg/20 g bw for 9, 18, and 27 consecutive experimental days could shorter mounting latency as well as increase mounting frequency against Balb male mice induced by ethinylestradiol as of 0.56 ug/20 g bw/day (Winarni, 2007). Previous research demonstrated that the root of *T. paniculatum* is the richest source of steroidal saponins and potential to be used in many medicinal purposes. The multiple pharmacological effects of *T. paniculatum* have been reported, include for vitality and maintain blood circulation, testosterone levels as well as motility and number of sperm increasing whereas saponin was determined as main chemical compound responsible to those effects (Solim & Manuhara, 2016).

Previous research revealed that the combination form of *C. xanthorrhiza* Roxb with *Piper retrofractum* L showed aphrodisiac activity indicated by the increase of climbing frequency as well as coitus frequency against male rats (Rahmawati & Bachri, 2012) . The safety data of those formula compositions has proven in previous studies. Ethanol extract of *C. xanthorrhiza* Roxb doses of 300, 2000 and 5000 mg/kg bw had no side effects as well as toxic effects and did not cause the death of Swiss female mice (Devaraj *et al.*, 2013). Standardized extract of *C. asiatica* Urban leaves had been tested in Sprague-Dawley rats for acute oral toxicity, sub-chronic toxicity, and mutagenic potential. The study found that the extract did not produce mortality or significant changes in the clinical signs (Deshpande *et al.*, 2015). Another acute toxicity studies found that *C. asiatica* whole plant was safe to Swiss mice with a dose of 3, 5 and 7 g/kg body weight orally (Pingale, 2008).

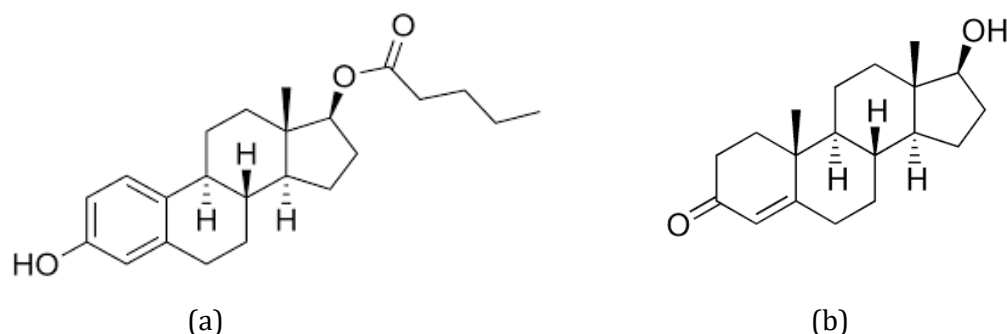


Figure 1. Chemical structure of estradiol valerate (a) and testosterone hormone (b)

The combination formula of *T. paniculatum*, *C. asiatica* and *C. xanthorrhiza* were expected to provide a better synergistic effect rather than in single formula administration. Many researches related to *T. paniculatum* pharmacological activity in single composition has been implemented, however the efficacy data in the combination form is not available yet. Therefore, this research needs to be conducted to prove the effect of combination infusion of *T. paniculatum* root, *C. asiatica* herb and *C. xanthorrhiza* rhizome against male Sprague Dawley (SD) rats' libido.

**METHODS**

**Preparation of Infusion of Simplicias**

Simplicias of *T. paniculatum*, *C. asiatica* and *C. xanthorrhiza* were provided by Postharvest Laboratory of Medicinal Plant and Traditional Medicine Research and Development Center, Ministry of Health. Determination of main component dose of *T. paniculatum* root was based on previous preclinical trials. The additional components doses, *C. xanthorrhiza* rhizome and *C. asiatica* herb were based on empirical use, subsequently converted to rat dose (multiplied by an extrapolation factor of 0.0182).

Table 1. Dosage of each formula composition

Composition	Dosage (mg/200 g bw)	Total dosage (mg)
Som Java	200	491,2
Gotu kola	109,2	
Javanese turmeric	182	

Based on table 1 and previous preclinical research, it is obtained a comparison of Som Java, gotu kola and Javanese turmeric as of 10:5:9 with the total dose of 491.2 mg/200 g bb. The ratio of the herbs weight and distilled water was 10% w/v with the calculation as of:

Table 2. Herbs weight for 50 ml infusion stock for SD rats

Som Java	Gotu kola	Javanese turmeric
200 mg x $\frac{50 \text{ ml}}{200 \text{ mg}}$ = 5000 mg	109,2 mg x $\frac{50 \text{ ml}}{200 \text{ mg}}$ = 2730 mg	182 mg x $\frac{50 \text{ ml}}{200 \text{ mg}}$ = 4550 mg
2 ml	2 ml	2 ml

Each ingredient was weighed according to calculations and homogenised with aquadest up to 50 ml, the mixture then be added aquadest with volume as of two times the herbs weight. An infusion was made by heating the mixture for 15 minutes started after the temperature reached 90°C, the infusion was filtered into a glass beaker and given 2 ml/200 g bw orally to a rat.

### **Experimental Animals**

As of twenty four Sprague Dawley 2-3 month-old, male and female rats were used in this experimental study. Rats were obtained from the Experimental Animal Care Unit of Universitas Gadjah Mada, Indonesia. Rats were divided into control and treatment groups, each group consisted of 4 rats (1 male and 3 female) with 3 replication of each group (in total 6 groups). The number of experimental rats was determined based on Ferderer Formula,  $(t-1)(n-1) \geq 15$ , in which "t" is groups number and "n" is the minimal number of animal used in each group (in this study, n was 4 for six groups of it has fulfilled Ferderer Formula).

### **Animal Treatments**

Before the study began, experimental rats were acclimatized for 7 days, then housed in the experimental pharmacology laboratory of Medicinal Plant and Traditional Medicine Research and Development Center (MPTMRDC). The rats were given ad libitum drink and fed with standard pellets. Artificial estrus of female rats were induced by per oral administration of estradiol valerate 2 mg/200 g bw 48 hours before conducting libido parameters observation. The combination infusion of *T. paniculatum* root, *C. asiatica* herb, and *C. xanthorrhiza* rhizome were given to male rats for 7 consecutive days. Rats body weight were recorded on day-0 and day-7. Determination of testosterone levels was conducted before and after treatment using testosterone ELISA kit whereas blood was taken via retro orbital plexus venous of rats. On the 7<sup>th</sup> day at 18.30 pm for the duration of 180 minutes, the experimental observation was conducting on some parameters of aphrodisiac activities. The first parameter was the onset of introduction phase, climbing as well as coitus phase. The 2<sup>nd</sup> parameter were the frequencies of introduction, climbing and coitus of the control and treatment groups in separately individual cages in which one male rat was caged with three female rats in estrus phase within lightless condition. Male rats started introduction phase when they began to get closer around female rats, kissing the female rat's body, while climbing phase defined as when male rats climbed up above the female rat's body and coitus phase when sexual intercourse happened, the genitalia of the male rat entered the female rat's genitalia. The body weight of experimental rats was also monitored in this current study before and after treatment.

### **Data Analysis**

All data collected from control and treatment experimental groups were analyzed using paired and unpaired t-test of SPSS.

### **Ethical Approval**

All research involving experimental animal as subject need ethical approval. This study had an ethical approval on 2011 from Ethics Committee of National Institute of Health Research and Development.

## **RESULTS AND DISCUSSION**

The mixture of *T. paniculatum*, *C. asiatica* and *C. xanthorrhiza* shown to have aphrodisiac effect on Sprague-Dawley (SD) white rats (*Rattus norvegicus*) as observed animal in this experimental pharmacological study. Induction of artificial estrus of female rats was performed by administering estradiol valerate 2 mg/200 g bw 48 hours before observation. Estradiol valerate known as natural estrogen going to replace the use of ethinyl estradiol in order to minimize the risk for thromboembolism (Ahrendt *et al.*, 2010). Within a few minutes, estradiol valerate could be quickly absorbed, hydrolyzed and converted to estradiol, whereby exerting the same estrogenic effects. One milligram of estradiol valerate is equivalent to 0.76 mg of ethinyl estradiol. Two mg of estradiol valerate can have the same biological effect of 4 to 20 µg ethinyl estradiol. A research reported the use of estradiol valerate might contribute in reducing the risk

for thrombosis. In different kind of concentrations, estradiol valerate showed inhibitory effect on the follicle-stimulating hormone and the stimulation of the endometrium cell as well as maturation of vaginal epithelial cells and protein synthesis in the liver. The research results showed the administration of estradiol valerate 2 mg/200 g bw 48 hours before observation induced artificial estrus on most of female rats (Ahrendt et al, 2010). Other research reported that estrogen plays an important role in the female animal estrus phase in term of physiological, behavioral, and ovulation manifestations as well as luteinizing hormone release and increase blood circulation in the genital organs (Hafez & Hafez, 2000).

The administration of combination infusion of *T. paniculatum*, *C. asiatica*, and *C. xanthorrhiza* at concentration of 491.2 mg/200 g bw for seven consecutive days led to increase the percentage of introduction and climbing phase frequency of treatment male rats group compared to the control group as of 86.39 and 69.17 % respectively. However there was a difference starting time between introduction phase of treatment and control groups as of 30.30% as shown in table 2. The secondary metabolites contained in *T. paniculatum* determined to be responsible for its estrogenic activity as reported in previous study (Thanamool et al., 2013). Testosterone level of male rats was another parameter for determining aphrodisiac activity of the formula. From previous studies, it is known that administration of medicinal plant to experimental animals generally begins to show the aphrodisiac effect on the 7<sup>th</sup> day, so that in this study the observations of the aphrodisiac parameters were made on the 7<sup>th</sup> day (Sugiarto & Setiantiningrum, 2010). Traditional medicines have incomparable advantages and disadvantages when used to develop new drugs, such as onset of any pharmacological effect, abundant clinical experiences, and their unique diversity of chemical structures and biological (Yuan et al., 2016).

Table 3. Frequencies of introduction, climbing and coitus of experimental male rats

Group	Replication	t intro (minutes)	t coitus (minutes)	Frequency		
				Introduction	Climbing	Coitus
Treatment	1	2	-	16	11	0
	2	4	-	12	16	0
	3	4	-	16	9	0
Average		3.3		14,7	12	0
Control	1	1	-	2	3	0
	2	2	-	2	4	0
	3	4	-	2	4	0
Average		2.3	-	2	3.7	0
Percentage (%)		30.30		86.39*	69.17*	0

\*counted by formula ((Freq of I or C treatment - control)/Freq treatment) x 100%

This study revealed that the administration of *T. paniculatum*, *C. asiatica* and *C. xanthorrhiza* effect on testosterone levels of male rats compared to control group. The provision of the combination infusion for seven consecutive days shown positive effect on testosterone hormone levels of male rats indicated by the presence of significantly increase of testosterone levels before and after treatment compared to control group with p value < 0.05 as seen in table 4.

Table 4. Testosterone levels of male rats before and after treatment

Group	Replication	Testosterone level	P (95%)
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		Before treatment	After treatment	
Treatment	1	1.046	1.415	0.012*
	2	1.066	1.326	
	3	1.158	1.437	
Control	1	0.896	0.945	0.460
	2	0.516	0.486	
	3	0.605	0.661	

\*based on paired t-test with 95% of confident level (p value < 0.05, significantly different)

Pair t-test statistical analysis showed a significant increase in testosterone levels in male-treated rats before and after treatment ( $p < 0.05$ ), while there were no differences in testosterone levels before and after treatment in male control rats ( $p > 0.05$ ). These results are in accordance with the previous research in which single adduction of *T. paniculatum* could increase testosterone level of male mice, shortening mounting latency and increase mounting frequency (Winarni, 2007). Asiaticoside contained in *C. asiatica* as well as xanthorrhizol in *C. xanthorrhiza* which contributed in rejuvenating body system expected to have synergistic effect in combination with *T. paniculatum* on male rat libido (Bhavna & Jyoti, 2011). Stigmasterol and  $\beta$  sitosterol contained in *T. paniculatum* considered to responsible on testosterone level increasing by supplying cholesterol for pregnenolon forming of testosterone synthesis (Winarni, 2007). Eseyin (2018) reported that stigmasterol's chemical structure positively related to testosterone synthesis and it was contributes to its aphrodisiac potentials. In male, testosterone levels do not have a correlation in sexual activity but in female, testosterone levels are positively related to sexual satisfaction and the frequency of sexual intercourse (Zitzmann & Nieschlag, 2001).

Table 4. Rats body weight before and after treatment

Groups	Rats body weight (g)		p (95%)
	Before treatment	After treatment	
Treatment	187	191	0.321
	190	210	
	162	162	
Control	133	153	0.069
	151	185	
	126	139	

The rat body weights were measured on the day-0 and day-7. The results of the study revealed that the administration of infusion combinations formula in experimental rats showed no significant changes ( $p > 0.05$ ) in body weight between the treatment and control groups as shown in table 4. This indicated that the combination of infusion for 7 consecutive days gave the same effect on body weight changes both on treatment and control groups. Bias factor for the use of laboratory animals became the limitation of this study in which introduction phase, climbing and coitus frequencies are specified as parameters that can be influenced by several factors, including stress and environmental factors.

## CONCLUSION

In conclusion, the administration of combination infusion of *T. paniculatum*, *C. asiatica* and *C. xanthorrhiza* at a dose of 491.2 mg/200 g bw for 7 days consecutively significantly increased testosterone level as well as introduction and climbing frequencies of male SD rats as of 27.77%; 86.39% and 69.17% respectively.

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