

The aphrodisiac effect and toxicity of combination *Piper retrofractum* L, *Centella asiatica*, and *Curcuma domestica* infusion

Nuning Rahmawati,¹ Moch. Saiful Bachri²

¹ Medicinal Plant and Traditional Medicine Research and Development Center, Ministry of Health of Indonesia

² Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta

Abstrak

Latar belakang: Cabe jawa (*Piper retrofractum* L) merupakan salah satu tanaman yang diketahui memiliki efek stimulan pada tubuh. Dari penelitian pendahuluan diketahui bahwa dalam bentuk infusa, LD_{50} nya rendah dan termasuk bahan yang tidak toksik. Infusa pada tikus putih dengan dosis 2,1 mg/10 gram berat badan mempunyai efek androgenik dan anabolik. Alkaloid utama dalam buah cabe jawa yang diduga merupakan senyawa aktif berkhasiat afrodisiaka adalah piperin. Pegagan (*Centella asiatica*) dan temulawak (*Curcuma domestica*) berfungsi sebagai bahan tambahan pendukung. Penelitian ini bertujuan untuk mengetahui toksisitas dan efek afrodisiaka infus ramuan buah cabe jawa, pegagan, dan temulawak pada tikus jantan.

Metode: Parameter efek afrodisiaka dilihat dari frekuensi introduction, climbing, dan coitus tikus jantan dibandingkan kontrol serta kadar hormon testosteron sebelum dan sesudah perlakuan. Toksisitas subkronik ditentukan dari kadar SGOT, SGPT, ureum, dan kreatinin sebelum dan sesudah pemberian infusa ramuan selama 3 bulan.

Hasil: Terdapat perbedaan yang signifikan parameter frekuensi climbing dan coitus antara kelompok kontrol dan kelompok yang diberi infusa ramuan ($P=0,032$). Sementara pemberian infusa ramuan *Piper retrofractum* L., *Centella asiatica*, dan *Curcuma domestica* tidak menyebabkan perbedaan yang signifikan kadar hormon testosteron tikus jantan sebelum dan sesudah perlakuan. Pemberian infusa ramuan dosis tinggi (5000 mg/200 g BB) menyebabkan perubahan yang signifikan kadar SGOT, SGPT dan ureum tikus kelompok perlakuan.

Kesimpulan: Infusa ramuan cabe jawa, pegagan, dan temulawak memiliki efek afrodisiaka pada libido tikus jantan galur SD dan pemberian ramuan dosis 5000mg/200g BB tikus setiap hari selama 3 bulan berturut-turut menyebabkan peningkatan yang signifikan kadar SGOT, SGPT, dan ureum tikus. (*Health Science Indones 2012;1:19-22*)

Kata kunci: afrodisiaka, toksisitas, *Piper retrofractum* L

Abstract

Introduction: *Piper retrofractum* L is a plant that acts as a stimulant on the body. A preliminary study showed that administration of infusion of 2.1 mg/10 g body weight had androgenic and anabolic effects in white mice. Piperine is the main alkaloid suspected to have an aphrodisiac effect. *Centella asiatica* and *Curcuma domestica* are the excipients. The objective of this research was to determine the toxicity and the aphrodisiac effect of a combination infusion of *Piper retrofractum* L, *Centella asiatica* and *Curcuma domestica* on Sprague-Dawley strain male rats.

Methods: Parameters for aphrodisiac effect were the frequency of introduction, climbing, and coitus of male rats. The concentration of pre and post-treatment of male rat testosterone hormone was determined using rat testosterone ELISA kit. Sub-chronic toxicity was determined from SGOT, SGPT, urea, and kreatinin concentrations of pre and post treatment of rats orally administered the combination infusion everyday for 3 months.

Results: There were significant differences in coitus and climbing frequencies between the male rat group administered combination infusion of *Piper retrofractum* L., *Centella asiatica*, and *Curcuma domestica* and the group not given the infusion ($P=0.032$). There was no significant difference between testosterone levels of the group administered the infusion and kontrol ($P=0.248$). Administering high dose (5000 mg/200 g BW) of infusion caused a significant difference in levels of SGOT and SGPT between pre and post-treatment.

Conclusion: The infusion of 1000 mg/200 g body weight had safe aphrodisiac effect on male Sprague-Dawley rats libido. (*Health Science Indones 2012;1:19-22*)

Key words: Aphrodisiac, toxicity, *Piper retrofractum* L.

The word aphrodisiac is derived from Aphrodite, the Greek goddess of love and refers to any substance or thing that may stimulate or intensify sexual desire.¹ Prevalence of sexual dysfunction in men is higher than in women. In a recent study conducted in the Boston area, 52% of men between the ages of 40 and 70 reported some degree of erectile dysfunction.² Therefore, the study of aphrodisiacs is important because they may provide a means to treat the psychological components of sexual dysfunction as opposed to the current treatments, surgical implants and injection therapy, which only treat the mechanical component.³

Piper retrofractum L. commonly known as Javanese chili, has been used in folk medicine for many years, especially as an aphrodisiac. This plant is a stimulant on the nerve cell and may improve health. Some preliminary studies of Javanese chili fruit showed that infusion at a dose of 2.1 mg/10 g body weight had an androgenic and anabolic effects in white mice.⁴ Based on research conducted by Isnawati *et al.* (2002) an extract of *Piper retrofractum* L. did not cause gene mutations in five strains of bacteria.⁵ Piperine is the main alkaloid compound of Javanese chili suspected to have aphrodisiac effect.^{6,7}

Several studies have been done on only *Piper retrofractum* L. but none has studied *Piper retrofractum* L. in combination with other plants.

The purpose of this investigation was to determine the toxicity and aphrodisiac properties of a combination infusion *Piper retrofractum* L., *Centella asiatica* and *Curcuma domestica* on male rats.

METHODS

This is an experimental pharmacological study using healthy Sprague Dawley (SD) white rats (*Rattus norvegicus*), male and female, 2.5 to 3-month-old. Rats were obtained from the Experimental Animal Care Unit of Universitas Gadjah Mada, Indonesia. For the aphrodisiac study, there were control and treatment groups (each group consisted of 1 male rat and three female rats with three times replications for each). While for sub-chronic toxicity study, there were 1 control group and 3 treatment groups [low dose (200 mg/200 g BW), medium dose (1000 mg/200 g BW) and high dose (5000 mg/200 g BW)]. Each group consisted of 6 male and 6 female rats.

Determination of main component dose of *Piper retrofractum* L. (42 mg/200 g BW) was based on previous preclinical trials. The additional components, *Curcuma domestica* (182 mg/200 g BW) and *Centella asiatica* (109.2 mg/200 g BW), were based on empirical use, subsequently converted to rat dose (multiplied by an extrapolation factor of 0.0182).

Before the study began, experimental rats were acclimatized for 7 days, housed in the experimental pharmacology laboratory of Medicinal Plant and Traditional Medicine Research and Development Center. They were given by drink *ad libitum* and fed with food pellets.

In the aphrodisiac study, the treatment male rat group was orally administered the combination infusion daily for 7 consecutive days. On the fifth day (48 hours before observation), female rats were subcutaneously injected with estradiol valerate 50 mg/rat to induce artificial estrus. Then, on the seventh day male rats placed in a cage with three females were observed for male rats sexual behaviors, which included the frequency of introduction, climbing, and coitus at 06.00-07.30 p.m. The frequency of introduction was counted when male rats started to approach the female rats and kiss around the tail or body parts of female rats. Frequency of climbing was counted when the male rat ride the female rats, while frequency of coitus was counted the male rats mount the female rats. Orbital venous blood samples were obtained through the male rats' eyes and performed at days 0 and 7. Blood was centrifuged and then the serum used for determination of testosterone levels with rat testosterone ELISA kit.

To study sub-chronic toxicity, three treatment groups were orally administered the combination infusion daily with doses stated above for a period of 3 months. Orbital venous blood samples were then collected through the male rats' eyes and performed at days 0 and 90. Blood was centrifuged and the serum used for determination of SGOT, SGPT, urea, and kreatinin levels, assessed with SGOT, SGPT, urea, and kreatinin Diasys reagents by spectrophotometry. Statistical analysis using both paired and unpaired t-test by SPSS version 16.⁸

This toxicity and aphrodisiac study received ethical clearance from Ethics Committee of National Institute of Health Research and Development, Ministry of Health of Indonesia.

RESULTS

As many as 72 experimental rats were included in this assessment, consisted of 42 female rats and 30 male rats.

Table 1. Frequency of introduction, climbing and coitus of in male rats with combination infusion

| Groups | Control | Treatment | P |
|--------------|---------|-----------|-------|
| Introduction | 2.0 | 7.0 | 0.100 |
| Climbing | 3.7 | 17.0 | 0.032 |
| Coitus | 0.0 | 3.7 | 0.032 |

In the aphrodisiac study (Table 1), shows that there were significant increase in coitus and climbing frequencies between male rat group administered combination infusion of *Piper retrofractum* L., *Centella asiatica* and *Curcuma domestica* and the control group (P=0.032).

Table 3. SGPT, SGOT, urea, and creatinine concentration of male rats with combination infusion

| | | Pre-treatment | | Post-treatment | |
|------------|---------------|---------------|-------|----------------|-------|
| | | Mean | P | Mean | P |
| SGOT | Control | 132.7 | 1.000 | 140.3 | 1.000 |
| | Low dose | 143.7 | 0.351 | 181.7 | 0.351 |
| | Moderate dose | 136.8 | 0.000 | 132.3 | 0.000 |
| | High dose | 124.2 | 0.038 | 212.3 | 0.038 |
| SGPT | Control | 59.5 | 1.000 | 54.2 | 1.000 |
| | Low dose | 62.3 | 0.216 | 69.5 | 0.216 |
| | Moderate dose | 72.2 | 0.010 | 56.0 | 0.010 |
| | High dose | 73.2 | 0.005 | 53.3 | 0.005 |
| Urea | Control | 57.8 | 1.000 | 40.8 | 1.000 |
| | Low dose | 51.5 | 0.016 | 37.0 | 0.016 |
| | Moderate dose | 43.7 | 0.680 | 41.7 | 0.680 |
| | High dose | 53.2 | 0.007 | 37.8 | 0.007 |
| Creatinine | Control | 0.48 | 1.000 | 0.48 | 1.000 |
| | Low dose | 0.48 | 0.041 | 0.58 | 0.041 |
| | Moderate dose | 0.53 | 0.750 | 0.58 | 0.750 |
| | High dose | 0.53 | 0.809 | 0.52 | 0.809 |

From the results of the sub-chronic toxicity study (Table 3), administration of *Piper retrofractum* L., *Centella asiatica* and *Curcuma domestica* infusion of high dose (5000 mg/200 g BW) caused a significant increase in levels of SGOT (P=0.038) and SGPT (P=0.005). However, moderate dose did not cause a significant increase in levels of urea (P=0.680) and creatinine (P=0.750), while the administration of low dose did not cause significant increase in levels of SGOT (P=0.351) and SGPT.

Table 2. Pre and post-treatment concentration of testosterone hormone in male rats with combination infusion

| Groups | Pre-treatment | | Post-treatment | |
|---------|---------------|-------|----------------|-------|
| | Mean | P | Mean | P |
| Sample | 0.751 | 0.248 | 1.385 | 0.248 |
| | 0.771 | | 0.832 | |
| | 0.938 | | 1.099 | |
| Control | 0.896 | 0.460 | 0.945 | 0.460 |
| | 0.516 | | 0.486 | |
| | 0.605 | | 0.661 | |

But, as shown in Table 2, there was no significant difference between the testosterone levels of male rats administered by combination infusion and control (P=0.248). It can be concluded that the administration of *Piper retrofractum* L., *Centella asiatica* and *Curcuma domestica* infusion did not cause a significant increase in testosterone levels of male rats.

DISCUSSION

This study was an experimental pharmacological study with Sprague-Dawley (SD) white rats (*Rattus norvegicus*) as experimental animal. The limitation of this study were the mood of experimental rats were influenced by environment factors where the observation of introduction, climbing and coitus of male rats conducted. The presence of observer also influenced rat mood.

The administration of combination infusion was found to have a positive aphrodisiac effect on increasing the rat sexual libido. This could be caused by a specific compound contained in *Piper retrofractum* L. fruit. In general, plants possessing aphrodisiac activity contain saponins, alkaloids, and other compounds. *Piper retrofractum* L. fruit contains piperine, an efficacious compound as a body reinforcement and blood circulation acceleration. *Centella asiatica* and *Curcuma domestica* were used as additional support material.

In theory, differences in the sexual behavior intensity between control and treatment group could be caused by differences in blood steroid levels (testosterone) of male rat blood. Steroid hormones in the form of testosterone in animals, produced by the gonads, play an important role in stimulating the sexual behavior occurrence. Increased androgenic hormones would affect the increased sexual libido. This study, where the infusion was a combination of *Piper retrofractum* L (42 mg/200 g), *Centella asiatica* (109.2 mg/200 g) and *Curcuma domestica* (182 mg/200 g), did not result in a significant change on testosterone hormone level of pre and post-treatment. But there was a higher coitus frequency on male rats. A study by Sa'roni *et al.* on androgenic effects of administering only *Piper retrofractum* L. infusion (2.1 mg/10 g BW) also did not find a significant increase of testosterone levels.⁴ Therefore both studies showed the same results.

Preliminary toxicity study of piperine, produced by chloroform extraction of *Piper retrofractum* L., showed that piperine was absolutely toxic against *Artemia salina* L (LC₅₀ = 7.498 ppm)⁹ while administering the combination infusion of *Piper retrofractum* L., *Centella asiatica* and *Curcuma domestica* of high dose of dose of 1000 mg/200 g body weight everyday for 3 months to Sprague-Dawley rats were considered safe.

In conclusion the infusion of *Piper retrofractum* L (42 mg/200 g), *Centella asiatica* (109.2 mg/200 g) and *Curcuma domestica* (182 mg/200 g) possessed an aphrodisiac effect on male rats libido. Oral administration of the infusion up to a dose of 1000 mg/200 g body weight to Sprague-Dawley rats were considered safe.

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