Jurnal. ARRB M Kanedi 2017

By Hendri Busman

WORD COUNT 3428
TIME SUBMITTED 19-JUN-2019 09:09AM
PAPER ID 47998820

Annual Research & Review in Biology

X(X): XX-XX, 20YY; Article no.ARRB.36975
ISSN: 2347-565X, NLM ID: 101632869

Root Extract of Purwoceng (*Pimpinella pruatjan*) Enhances Aggressiveness, but Not Libido, in Male Mice

Mohammad Kanedi[1*], Sutyarso[1], Hendri Busman[1], Nuning Nurcahyani[1] and Wiwit Nurkhasanah[1]

[1]Department of Biology, Faculty of Mathematics and Sciences, University of Lampung, Bandar Lampung, Indonesia.

Authors' contributions

This work was carried out in collaboration between all authors. Author MK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors Sutyarso and HB managed the analyses of the study. Author NN and WN managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/36975
Editor(s):
(2)
Reviewers:
(1)
(2)
(3)
(4)
Complete Peer review History:

Original Research Article

Received 25th September 2017
Accepted 20YY
Published 20YY

ABSTRACT

Aims: Purwoceng herbs, *Pimpinella pruatjan* Molk., are widely marketed in Indonesia; it is believed that the plant was used by ancient Javanese kings as an aphrodisiac. Unfortunately, the claim that purwoceng is an effective aphrodisiac lacks support from biological and pharmacological studies. This study aimed to test whether purwoceng extract affects aggressiveness and sexual behaviour in mice.
Study Design: The study employed a completely randomised design using five concentration levels repeated three times.
Place and Duration of Study: The study was conducted at the Department of Biology, Faculty of Mathematics and Sciences, University of Lampung, Bandar Lampung, Indonesia, between

**Corresponding author: E-mail: wegayendi@yahoo.com;*

December 2016 and March 2017.
Methodology: Male mice (*n* = 20), aged 4 months and weighing 25–30 g, were divided into four groups. Group 1 comprised animals treated individually with 0.5% CMC (carboxymethyl cellulose; as controls). Groups 2, 3 and 4 were treated with 0.8 mg, 1.6 mg and 3.2 mg, respectively, of purwoceng extract suspended in 0.5% CMC. All treatments were administered orally with a total suspension volume of 0.5 ml per feed, once daily for 35 days.
Results: The results showed that the mice treated with purwoceng extract had a shortened latency of attacks and increased frequency of attacks. However, none of the sexual behaviour parameters of the mice, including courtship latency, mounting latency, mounting frequency and latency of copulation, were affected by the purwoceng extract.
Conclusion: It is suggested that ethanolic root extract of purwoceng has the potential to enhance aggressiveness, but not libido, in male mice.

Keywords: Purwoceng; pimpinella pruatjan; pimpinella alpine; libido; aggressive behavior.

1. INTRODUCTION

Purwoceng is the Indonesian name for *Pimpinella pruatjan* Molk., an herbaceous plant belonging to the family Apiaceae/Umbelliferae (carrot family). At the local level, especially in Java, Indonesia, purwoceng herbs are widely marketed due to the folk belief that the plant was used by ancient Javanese kings as an aphrodisiac medicine; however, biological and pharmacological studies of these plants are limited. Beyond reports from local researchers, few scientific reports on the phytochemical content or biological, biochemical and pharmacological activities of these plants have been published in reputable international journals accessible via the internet.

Among local researchers, the taxonomic determination of these plants is still confusing; some use the term *Pimpinella pruatjan*, while others use *Pimpinella alpina*. According to the Plant List, *Pimpinella pruatjan* Molk. is synonymous with *Pimpinella panatjan* Mirb. ex Rosenthal, while *Pimpinella alpina* Koord. is synonymous with *Pimpinella javana* DC. and *Anisometros alpina* Hassk. [1]. Furthermore, it has been suggested on the Useful Tropical Plants Database that *Pimpinella pruatjan* Molk. is synonymous with *Carum panatjan* Baill., *Pimpinella panatjan* Mirb. ex Rosenthal., *Heterachaena alpina* Zoll. and *Anisometros alpina* Hassk. [2]. In Java, *P. pruatjan* is found in restricted areas of the Dieng Plateau, Central Java, on Mount Pangrango in West Java and in the mountainous area in East Java [3].

Regardless of the taxonomic confusion concerning purwoceng, the study results of local researchers on the medicinal benefits of these plants remain worthy of consideration. For instance, a phytochemical study showed that the root extract of this plant contained alkaloid, glycoside, coumarin, triterpenoid–steroid and saponin, whereas flavonoids and tannins were detected in leaf extracts [4]. Another study on the meristem culture of the plant using thin-layer chromatography found that *Pimpinella pruatjan* Molk. contained stigmasterol [5].

The folklore that claims the purwoceng plant has aphrodisiac effects seems to be supported by several studies. Ethanolic plant extract of *Pimpinella alpina* was found to enhance sexual behaviour parameters in Swiss Webster male mice [6]. This effect is likely due to some bioactive components of purwoceng plant extracts that play a role in increasing testosterone and LH (luteinising hormone) levels [7].

Because purwoceng extract is claimed to increase sexual behaviour and is known to increase the levels of testosterone and LH hormone, logically, this plant extract should affect other virility behaviours, such as aggression. This study was designed to examine and verify whether the root ethanolic extracts of purwoceng affect the testosterone-related virility behaviour, especially the parameters of aggressiveness and sexual drive.

2. MATERIALS AND METHODS

2.1 Plant Samples and Extraction

The part of the purwoceng plant used in this study was the root. Dry plant root powder was purchased from an herbalist in the city of Surakarta, Central Java, Indonesia. The extraction was carried out by soaking the powder (100 mg) in 70% ethanol (500 ml) and

macerating the mixture for 24 h. Supernatant was collected every 24 h for 3 days and then evaporated under low pressure until a brownish, viscous extract formed. To ready the extract for administration to the test animals, the yielded ethanolic extracts were suspended in distilled water containing 0.5% CMC (carboxymethyl cellulose). Ethanol solvent was used to mimic what was done by Caroline [6], while CMC was used because the ethanolic extract could not be dissolved in water. The stock solution was then subjected to a serial dilution in accordance with the concentration levels set for the experiment.

2.2 Animals and Experimental Design

The male and female Swiss albino mice used as test animals in this study were obtained from Lampung Veterinary Centre, Indonesia. The male mice (n = 20), aged 4 months and weighing 25–30 g, were divided into four groups. Group 1 comprised animals treated individually with 0.5% CMC (as control). Groups 2, 3 and 4 were treated with 0.8 mg, 1.6 mg and 3.2 mg, respectively, of purwoceng extract suspended in 0.5% CMC for 35 days. These doses were based on Nasihun's (2009) work, in which a dose of 25–50 mg was applied in Sprague–Dawley rats [7]. All treatments were administered orally with a total suspension volume of 0.5 ml per feed. Both during acclimatisation and throughout the study, the test animals were kept indoors at room temperature, with food and water available *ad libitum*.

2.3 Aggressive Behaviour Tests

To assess the aggressiveness of the treated animals, male–male fight tests were performed. The tests were carried out in an open round plastic tray with a diameter of 40 cm and height of 25 cm. The tray was divided into two halves, which were separated by a removable cardboard partition. To begin the test, a treated male (test male) and an adult neutral male (opponent) were allowed to adapt to their half-tray environment with the partition closed for at least 5 min (**Fig. 1**). Starting from when the cardboard partition was removed, the fight activities of the mice were then observed for 10 min. Throughout the test, videotaping was performed to observe the following parameters: latency of threats, latency of attacks and attack frequency. Latency of threats was defined as the time from when the partition was removed until a first sign of threat was displayed. Latency of attacks was the time from when the partition was removed until a first attack was seen. The attack frequency was the number of attacks by the test animals.

2.4 Sexual Behaviour Tests

To assess the libido potential of the male subjects, male mice that had been treated with different concentrations of purwoceng extract were mated with oestrous virgin females. By using a tray designed similarly to the fight test tray, both males and females subjected to the tests were allowed to adapt to the tray environment with the partition closed for about 5 min (**Fig. 1**). When the carboard partition was removed, the mating activities of the mice were observed for 30 min. Throughout the experiment, videotaping was performed to observe the following parameters: courtship latency, mounting latency and mounting frequency. Courtship latency was the time from when the partition board was opened until the male displayed the first courtship action. Mounting latency was the time from when the cardboard was removed until a first mounting action was shown by the test males. Mounting frequency was the total number of attempts made by the male to ride on the female's back.

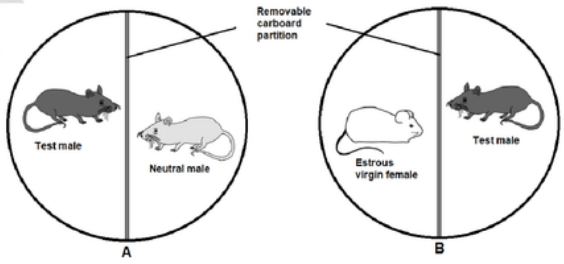

Fig. 1. Diagram depicting the animals' positioning in the open tray set for aggressive behaviour (A) and sexual behaviour tests (B)

2.4 Data Analysis

The data, presented as the mean ± SEM (standard error of the mean), were analysed using one-way ANOVA (analysis of variance). When a significant difference was detected by ANOVA, the treated groups were then compared with each other and the control group using the LSD (Least Signicant Diference) test.

3. RESULTS AND DISCUSSION

The effects of purwoceng root extract on aggressive behaviour of male mice are presented in Table 1. Based on the ANOVA results, there was no significant difference in the latency of attacks between groups (F = 0.867, P = 0.480). However, there were significant differences in the latency of attacks (F = 7.875, P = 0.002) and frequency of attacks (F = 7.693, P = 0.002). The LSD tests showed that purwoceng root extract significantly shortened the latency of attacks and increased the frequency of attacks (α = 0.05). It is inferred that root extract of *Pimpinella pruatjan* affects the aggressiveness of male mice.

In contrast to the aggressiveness test, the test results from treated males mating with oestrous virgin females did not show any difference in any observed sexual behaviour variables (Table 2). The ANOVA results for the mean values of courtship latency (F = 1.241, P = 0.33), mounting latency (F = 0.518, P = 0.68), mounting frequency (F = 1.237, P = 0.33) and latency of copulation (F = 0.075, P = 0.97) clearly showed that there was no significant effect of *Pimpinella pruatjan* root extract on the sexual behaviour parameters of mice.

The results of this study did not demonstrate that purwoceng plant extract positively affects sexual behaviour in male mice, as reported by Caroline [6]. However, in her experiments, Caroline only used two parameters of mating behaviour – introducing (attempts to engage in courtship) and mounting. Between the two, introducing, but not mounting, was significantly affected by the purwoceng extracts.

Assuming that Taufiqurrahman's report properly describes the real condition that male rats given plant extract of purwoceng had elevated testosterone [8], the results of this study may be associated with that phenomenon. As shown in Table 1, male mice treated with purwoceng root extract were significantly more aggressive than those in the control group.

It has long been known that testosterone not only affects aggressiveness in male animals, but also does so in females [9]. In rats, testosterone has been associated with both intra- and interspecific aggression, including adult mouse killing in females that were neonatally androgenised. In monkeys, testosterone is related to aggressiveness and dominance, accounting for aggressive displays during the mating season [10]. In humans, increased circulating testosterone is associated with significant increases in anger–hostility, but only minor changes in mood [11].

Table 1. Aggressive Behaviour Parameters of Male Mice Treated with Purwoceng (*Pimpinella pruatjan* Molk.) Root Extracts

Treatment	Threat Latency (sec)	Attack Latency (sec)	Attack Frequency
0.5% CMC	70.0 ± 51.290[a]	224.4 ± 31.649[a]	2.6 ± 1.691[a]
0.8 mg extract in 0.5% CMC	9.4 ± 2.561[a]	17.6 ± 8.588[b]	28.4 ± 2.713[b]
1.6 mg extract in 0.5% CMC	7.6 ± 5.627[a]	63.8 ± 59.079[b]	18.0 ± 5.992[b]
3.2 mg extract in 0.5% CMC	68.2 ± 54.619[a]	227.8 ± 45.852[a]	18.0 ± 4.183[b]

Data are presented as the mean ± SEM. Values in the same column followed by the same superscript did not differ at α = 0.05. CMC: Carboxymethylcellulose

Table 2. Sexual behaviour parameters of male mice treated with Purwoceng (*Pimpinella pruatjan* Molk.) root extracts

Treatment	Courtship Latency (sec)	Mount Latency (sec)	Mount Frequency	Copulation Latency (sec)
0.5% CMC	5.4 ± 1.965	198.2 ± 157.896	9.4±2.159	233.0±153.796
0.8 mg extract in 0.5% CMC	2.2 ± 0.583	177.6 ± 71.201	17.6±3.982	297.8±99.375
1.6 mg extract in 0.5% CMC	4.6 ± 1.435	188.8 ± 99.071	14.8±2.888	308.8±99.375
3.2 mg extract in 0.5% CMC	5.0 ± 0.632	45.2 ± 13.654	14.6±2.993	264.4±87.903

Data are presented as the mean ± SEM. None of the mean values in the same column showed significant difference by ANOVA at α = 0.05.

There are other explanations for the effects of purwoceng that could be related to testosterone-associated biological phenomena. As reported by Mariani and colleagues (2015), from plantlet extracts of the meristem culture of purwoceng (*Pimpinella pruatjan* Molk.), thin-layer chromatography successfully detected stigmasterol [12]. When administered to mice for 20 days, stigmasterol is known to reduce serum triiodothyronine (T3), thyroxin (T4), glucose concentration and the activity of hepatic glucose-6-phosphate, with a significant increase in insulin [13]. In laboratory animals, administration of T4 was found to increase SHBG (sex hormone binding globulin) concentration, peripheral aromatisation of androstenedione and testosterone levels [14].

Beyond studies in the literature have revealed positive relationship between aggressive behaviour and testosterone levels, some studies have suggested that increased aggressiveness in animals is not always associated with increased testosterone. In prairie voles for instance, castration (making the subject incapable of producing testosterone) did not affect the frequency of aggression in males, and thus, it can be inferred that aggressive behaviour may be independent of gonadal steroid hormones in this rodent [15].

Regardless of whether the increased aggressiveness related directly or indirectly to testosterone levels, another important finding of this study, as shown in Table 2, is that purwoceng extract did not affect the sexual behaviour parameters in male mice. This may be related to the properties of the active chemicals contained in the purwoceng extract. However, due to a lack of phytochemical data on *Pimpinella pruatjan*, the explanation of the absence of sexual effects of purwoceng extracts on male mice should refer to the results of studies on the same genus.

Plant extract of anise, *Pimpinella anisum*, contains β-linalool, methyl chavicol, α-terpineol, *cis*-anethole, *trans*-anethole and p-anisaldehyde [16]. *Trans*-anethole has been found to show antifertility, anti-implantation, antiprogestational and antiandrogenic activities in rats [17]. A more comprehensive study on anise plant extract showed that anise oil administration caused several histopathological changes, including inhibition in Sertoli cell numbers; inhibited inhibin and glutathione s-transferase (GST) expression; decreased testosterone, T3 and T4 hormones; and inhibited sperm counts and sperm motility [18]. These seemingly contradictory results appear to logically support Adimoelja's comment that research reports on the phytochemical properties and medical benefits of *Pimpinella pruatjan* are still inconsistent [19].

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Despite the inconsistency of the effects of purwoceng extract on the sexual behaviour of the experimental animals, this study's findings clearly showed that purwoceng extracts increase the aggressiveness of male mice. This phenomenon is possibly related to the role of some active compounds, such as flavonoids and tannins, contained in purwoceng, which may have increased the testosterone levels. As reported by Peiris et al. (2015), plant extracts of *Cardiospermum halicacabum* containing tannins, flavonoids and terpenoids significantly increased testosterone levels, leading to increased fertility in male rats [20]. Taken together, the study results confirm that there are still many aspects that need to be revealed to ascertain whether purwoceng is beneficial to health in general, especially in terms of reproductive health and libido, or whether it should be avoided because of its side effects.

4. CONCLUSION

Ethanolic root extract of purwoceng, *Pimpinella pruatjan* Molk., has the potential to increase aggressiveness, but not libido, in male mice.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

The author is very grateful to Ms. Meri Jayanti for her assistance in preparing samples of purwoceng plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. The Plant List: Version 1. Published on the Internet; http://www.theplantlist.org/ (2010)
2. Fern K., 2014. Useful Tropical Plants Database 2014. Availabel at:

http://tropical.theferns.info/viewtropical.php?id=Pimpinella+pruatjan

3. Rostiana O. Pruatjan (Pimpinella pruatjan Molk.): The rooted herbal medicine of Indonesia for aphrodisiac properties. Nat Prod Chem Res 2014, 2:5
4. Mariani T.S., Isna S. and Chia T.F.. Study on Meristem Culture of Purwoceng (Pimpinella pruatjan Molk.) and Its Stigmasterol Detected by Thin Layer Chromatography. International Journal of Pharmacological and Pharmaceutical Sciences Vol:2, No:12, 2015.
5. Caroline C. Pengaruh Ekstrak Etanol Herba Purwoceng (Pimpinella alpina) Terhadap Perilaku Seksual Mencit Swiss Webster Jantan (Effect of ethanolic herbs extract of purwoceng, Pimpinella alpina, on the sexual behavior of Swiss Webster male mice), 2011. Available at: http://repository.maranatha.edu/2435/1/0810070_Abstract C.PDF.
6. Nasihun T. 2009. Pengaruh Pemberian Ekstrak Purwoceng (Pimpinella alpina Molk) Terhadap Peningkatan Indikator Vitalitas Pria Studi Eksperimental Pada Tikus Jantan Sprague Dawley.(Effect of purwoceng, Pimpinella alpina Molk. extr on male vitality indicators based by an experimental study on Sprague Dawley rats). Sains Medik 009; 1 (1) : 53-62
7. Taufiqurrachman. The effect of buceng extracts on androgen production in Sprague Dawley male rats. Med J Indones 12;21:28-31
8. Flores D.L. and Crews D. Effect of Hormonal Manipulation on Sociosexual Behavior in Adult Female Leopard Geckos (Eublepharis macularius), a Species with Temperature-Dependent Sex Determination. Hormones and Behavior, 95; 29: 458–473.
9. Giammanco M., Tabacchi g., Giammanco S., Di Majo D. and La Guardia M. Testosterone and aggressiveness. Medical science monitor: international medical journal of experimental and clinical research, 2005; 11(4): RA136-45
10. O'Connor D.B., Archer J. and Wu F.C.W. Effects of Testosterone on Mood, Aggression, and Sexual Behavior in Young Men: A Double-Blind, Placebo-Controlled, Cross-Over Study . *The Journal of Clinical Endocrinology & Metabolism*, 2004; 89(6): 2837–2845
11. Mariani T.S., Isna S. and Chia T.F. Study on Meristem Culture of Purwoceng (Pimpinella pruatjan Molk.) and Its Stigmasterol Detected by Thin Layer Chromatography. International Journal of Pharmacological and Pharmaceutical Sciences Vol:2, No:12, 2015.
12. Panda S, Jafri M, Kar A and Meheta BK: Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. oterapia 2009; 80 (2): 123126.
13. Choksi N.Y., Jahnke G.D., St.Hilaire C. and Shelby M. Role of Thyroid Hormones in Human and Laboratory Animal Reproductive Health. Birth Defects esearch (Part B), 2003; 68:479–491
14. Demas G.E., Moffatt C.A., Drazen D.L. and Nelson R.J. Castration Does Not Inhibit Aggressive Behavior in Adult Male Prairie Voles (Microtus ochrogaster). Physiology & ehavior, 1999; 66(1):59–62.
15. European pharmacopoeia, 3rd ed. rasbourg, Council of Europe, 1996.
16. Dhar SK. Anti-fertility activity and hormonal profile of trans-anethole in rats. Indian Journal of Physiology and Pharmacology, 5, 39:63–67.
17. Ibrahim A.A.M. Correlation between fennel- or anise-oil administration and damage to the testis of adult rats. Egyptian Journal og Biolo 2008; 10: 62-76.
18. Adimoelja A. Phytochemicals: modern views and breakthrough in traditional herbal management of sexual dysfunctions. Papers in: 3rd AOAC Meeting, Chiba, Japan (2000). Available at: www.tribumen.lt/assets/Pdf/Klinikiniai.../Phytochemicals.doc

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