

Effects of *Costus speciosus* ethanolic extract on male rats: The action mechanism and the ability to impregnate

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Abstract: To examine the action mechanism that mediates the anti-fertility effect of *Costus speciosus* extract, research was conducted on male Sprague-Dawley rats. *Costus* extract was given to male rats for 14 days at various doses, namely 275, 550 and 1,100 mg kg⁻¹ day⁻¹ in 0.5% sodium CMC. The results showed that *Costus* extract with doses ranging from 275 to 1,100 mg kg⁻¹ day⁻¹ was able to inhibit pregnancy among female rats by 10-70%. No obstacles in terms of sexual behavior were identified among male rats. The anti-fertility effect of *Costus* extract kicked in without involving a decreased level of male reproductive hormones.

Keywords: *Costus* extract, reproductive hormones, obstacles to pregnancy, sexual behavior.

INTRODUCTION

Costus speciosus is a plant used as traditional contraceptive drugs for men on an island in Sulawesi. *C. speciosus* leaves are processed traditionally by boiling or pounding and, afterwards, male adults will take this medicine as a contraceptive (Rahayu *et al.*, 2006). Previous research in our research group showed that ethanol extract (50%) of aerial parts of *C. speciosus* given to male rats inhibited the quality and quantity of sperm cells (Sari *et al.*, 2016). Administration of ethanol extract from *C. speciosus* for 90 days showed no symptomatic disorders in the behavior of male test animals and no changes were identified in vital organs as well as hematological and biochemical parameters of the blood and urine parameters. Decreased blood glucose and cholesterol levels were found in the research examining subchronic toxicity (Sari and Nurrochmad, 2016). This is not surprising since another research once reported the anti-cholesterol effects of the extract of *C. speciosus* water on rats induced with propylthiouracil (PTU). The water extract of *C. speciosus* at a dose of 275 mg kg⁻¹ day⁻¹ carried out an activity equivalent to simvastatin given at the dose of a therapy (Susanti *et al.*, 2017). The use of *C. speciosus* as an antidiabetic drug had been studied by researchers from India, based on its use in Ayurvedha to reduce the blood glucose level empirically in a number of regions in India (Bavarva and Narasimhacharya, 2008; Pawar and Pawar, 2012).

Some herbs have an anti-fertility activity through various mechanisms that have been studied for several years ago (Saravanan *et al.*, 2012). Cottonseed extract containing gossypol has been found to be able to inhibit spermatogenesis through hormonal inhibition i.e., by inhibiting testosterone, luteinizing hormones (LH), and

follicle-stimulating hormones (FSH) if administered with doses ranging from 20 to 30mg kg⁻¹ day⁻¹ for 4 to 6 weeks (Qian and Wang, 1984). Herbs, such as *Barleria peritonitis*, *Bupleurum sulphureum* and *Cichorium intybus*, are also known for their ability to inhibit sperm motility and the number of sperms through hormonal mechanisms (Singh and Gupta, 2016; Tatli-çankaya *et al.*, 2014). *Aegle marmelos* ethanolic extract also reduces the fertility of male rats through inhibition of testosterone and inhibits the integrity of the acrosomal membrane using sialic acids as the inhibitor (Chauhan *et al.*, 2007). *Menthae piperita* and *Mentha spicata* in the form of herbal tea (20g L⁻¹) were able to significantly increase the level of LH and FSH and to lower the level of testosterone (Akdogan *et al.*, 2004). In addition to the hormonal-related mechanism, some herbs also demonstrate an anti-fertility activity in male test animals by disrupting spermatogenesis processes. The water extract from *Azadirachta indica* administered for 28 days at a dose of 200mg kg⁻¹ caused damage to the seminiferous tubules and germ cells. Similarly, papaya seed extract is capable of causing damage to Leydig cells and seminiferous tubule vacuolization (Mishra and Singh, 2005; Udoh and Kehinde, 1999). On the other hand, the mechanisms of *C. speciosus* extract (CSE) in inhibiting spermatogenesis or male reproductive hormones have not been reported. Therefore, this research is aimed at examining the mechanisms that supposedly mediate the anti-fertility activity of CSE. The content of active compounds found in the ethanol extracted from *C. speciosus* includes steroids, phenols, saponins and sugars that might be able to inhibit fertility in male test animals (Devi and Urooj, 2010).

MATERIALS AND METHODS

Plant material

Aerial parts of *C. speciosus* were harvested from an area in Sleman in March. Plants were determined by Djoko

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Santosa (Voucher No.814/IPS/2015) from the Department of Pharmaceutical Biology, the Faculty of Pharmacy, UGM.

Extraction

Aerial parts of *C. speciosus* were cut into thin slices, dried in the oven at 50-60°C, then ground into flour. Afterwards, the flour was extracted using 50% ethanol with the method of maceration at PT. Phytochemindo Reksa (Sari *et al.*, 2016). The resulting thick extract was then dried using starch. The dried extract was standardized according to the guidelines established by the Indonesian Herbal Pharmacope. The standardized extract did not contain arsenic, cadmium, mercury, and plumbum or bacterial fungus contaminants (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Candida albicans*). The extract contained diosgenin and phenolic compounds by 0.03% and 2.20%, respectively.

Dose and duration

A total of 40 male Sprague-Dawley rats, whose weight ranged from 180 to 200g, were used in this research. These test animals were housed in standard rat cages, maintained under standard conditions (12-h light/dark cycle; 25±3°C temperature; and humidity at 70-80), and given a standard laboratory chow and water *ad libitum*. Drugs or vehicles were administered to all animals by oral intubation.

Male rats were divided into 4 groups. The ethanol extract of *Costus speciosus* (CSE) was suspended into 0.5% Carboxymethylcellulose sodium (CMC sodium), and it was given every morning by 0.5mL to male rats for 14 days.

Group A consisted of rats belonging to the control group receiving 0.5mL of 0.5% CMC sodium Group B was administered CSE at the dose of 275 mg kg⁻¹ day⁻¹

Group C was administered CSE at the dose of 550 mg kg⁻¹ day⁻¹

Group D was administered CSE at the dose of 1,100 mg kg⁻¹ day⁻¹

Mating research

The male test animals were mated with female test animals which were in the proestrus cycle (1: 1). Sexual behavior of the male rats was observed on Days 1, 6, and 11 of CSE administration. Observation was conducted for 1 hour at night from 19:00 to 20:00. Observation of the sexual behavior was performed during the mating activity using parameters such as the mounting frequency (MF) and the intromission frequency (IF). Sexual behavior was recorded using a video camera. Female rats which had been mated with male rats were examined to identify sperm plaques the next morning. If these sperm plaques are found among female rats, it means that conception has

occurred (counted as Day 0 of pregnancy). On Day 19 (two days before giving birth normally), female rats were sacrificed and underwent a cesarean in order to count the number of feti.

Autopsy schedule

Male rats, after undergoing administration of CSE for 14 days, were sacrificed using mild anesthesia. The right and left testes were stored in 2.5mL of phosphate-buffered saline (PBS 0.01 M, pH 7.4) as the transport medium.

The test animals were sacrificed with light anesthesia and a surgery was performed afterwards. The right and left testes were stored in the transport medium of 2.5mL of PBS. These testes were placed in a Petri dish to remove fat and have them decapsulated. These testes were then carefully chopped until fine parts were obtained. After that, the medium of Dulbecco's Modified Eagle's Medium (DMEM) by 7mL and collagenase by 0.25 mg mL⁻¹ were added to these chopped testes to form a suspension. This suspension was stirred using a water bath shaker with a rotation speed of 6 and at a temperature of 37°C for 40 minutes. The suspension was filtered to separate the seminiferous tubules from Leydig cells. Fractions resulting from filtration were centrifuged at 2000 rpm for 10 minutes to separate the intratesticular fluid from Leydig cells and other cells. The supernatant resulting from centrifugation was then taken as a sample to measure the level of FSH, LH, and intratesticular testosterone. The levels of the hormones FSH, LH, and testosterone were measured using ELISA ELISA (DRG® FSH ELISA (EIA-1288), DRG® LH ELISA (EIA-1289), DRG® Testosterone ELISA (EIA-1559)). The intratesticular fluid sample was diluted 4 times using DMEM as the medium and followed by the ELISA testing procedures.

Ethical approval

This research has been approved by the ethical committee of Animal Ethics, Integrated Laboratory, Universitas Gadjah Mada (UGM) 00058/04/LPPT/XI/2016.

STATISTICAL ANALYSIS

The number of feti, MF, IF, testes' weight, the cholesterol level and the hormone level were statistically analyzed using ANOVA followed by the post-hoc test (95% confidence level).

RESULTS

CSE's anti-fertility effect was evidenced by the percentage of fertility of pregnant female rats after they mated with male rats treated with CSE in various doses. CSE with doses ranging from 275 to 1,100 mg kg⁻¹ day⁻¹ was able to inhibit fertility by 10 to 70%, in which the number of feti produced was not significantly different (table 1).

Table 1: The effect of CSE treatment on the fertility test of male rats

Group	Number of mated males	Number of mated females	Number of pregnant females	Number of viable fetuses	Fertility test (%)	Inhibition of fertility (%)
CMC sodium 0.5%	10	10	10/10	8.80±2.99	100	0
EC 275 mg kg ⁻¹ day ⁻¹ in CMC sodium 0.5%	10	10	9/10	8.67±1.59	90	10
EC 550 mg kg ⁻¹ day ⁻¹ in CMC sodium 0.5%	10	10	8/10	8.31±2.37	80	20
EC 1,100 mg kg ⁻¹ day ⁻¹ in CMC sodium 0.5%	10	10	3/10	5.67±1.70	30	70

Number of viable fetuses' values are means ±S.E.M. from 3-10 rats per group

Table 2: Inhibition of the intratesticular testosterone level

Group	Testes weight (g)	Cholesterol level (mg/dL)	Testosterone level (ng/mL)	LH level (ng/mL)	FSH level (ng/mL)
CMC sodium 0.5%	1.97±0.35	10.31±2.10	8.81±0.98	1.01±0.05	1.10±0.02
CSE 275 mg kg ⁻¹ day ⁻¹ in CMC sodium 0.5%	1.94±0.54	9.65±2.68	9.44±0.07	1.05±0.03	1.10±0.04
CSE 550 mg kg ⁻¹ day ⁻¹ in CMC sodium 0.5%	1.98±0.37	9.84±2.06	9.34±0.39	1.09±0.05	1.01±0.08
CSE 1,100 mg kg ⁻¹ day ⁻¹ in CMC sodium 0.5%	1.96±0.45	10.40±2.97	9.65±0.98	1.10±0.06	1.09±0.07

Values are means ± S.E.M. from 10 rats per group. All the groups did not differ significantly (P> 0.05).

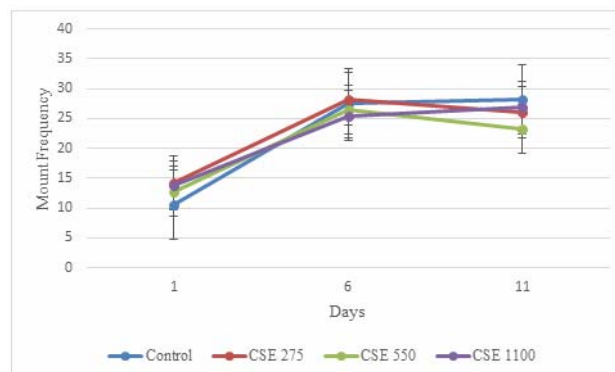
In fig. 1, it can be seen that the mounting frequency occurring on Days 1, 6 and 11 after CSE administration did not differ significantly between the treatment groups with CSE administration, with doses ranging from 275 to 1,100mg kg⁻¹ day⁻¹ and the control group (P>0.05). The same thing was found for the intromission frequency.

To determine the mechanism that mediates the anti-fertility effect of CSE, the levels of cholesterol and reproductive hormones such as testosterone, FSH and LH were examined. Table 2 shows that CSE administration with doses ranging from 275 to 1,100 mg kg⁻¹ day⁻¹ did not affect testes' weight, intratesticular cholesterol levels, and all the reproductive hormones in male rats (testosterone, FSH and LH).

DISCUSSION

The use of herbs with an anti-fertility effect is often disapproved of by men due to their concern about the resulting effect on sexual behavior (Liu *et al.*, 2010). Men dislike the anti-fertility effect allegedly inhibiting the level of testosterone because of the view that inhibition of reproduction, especially inhibition of testosterone, will inhibit sexual behavior in men. Testosterone does not directly affect sexual behavior. Testosterone triggers dopamine release in the medial preoptic area of the hypothalamus (MPOA), which is the area controlling sexual behavior (Hull *et al.*, 1997). In this research, an analysis was conducted of the effect of CSE administration in various doses on the sexual behavior of male rats, namely the mounting frequency and the intromission frequency. CSE administration with doses

ranging from 275 to 1,100mg kg⁻¹ day⁻¹ did not affect sexual behavior of male rats.

**Fig. 1:** Effect of CSE treatment on mount frequency of male rats.

This suggests that CSE administration with doses ranging from 275 to 1,100 mg kg⁻¹ day⁻¹ for 11 days did not affect the sexual behavior of male rats. These results remove men's doubts and assure them they do not need to worry about any negative effect on their sexual behavior arising from CSE consumption. This indicates that the CSE mechanism in inhibiting the number and quality of sperm in the previous research (Sari *et al.*, 2016) reinforced by the inhibition of pregnancy in female rats in this research (10-70%) might not occur via the hormonal mechanism as previously predicted, considering that CSE contains steroids, especially in the form of diosgenin. Inhibition of fertility occurring in CSE administration might occur via the non-hormonal mechanism, namely disturbances to germ cells, seminiferous tubules and Leydig cells; it is even highly likely that inhibition of hyaluronidase occurs

as found in some herbs empirically used to produce an anti-fertility effect in various traditional medicines (Daniyal and Akram, 2015). The root extract of *Martynia annua* containing pelargonidin-3,5-diglucoside, oleic acid, cyanidin-3-galactoside and apigenin is able to inhibit spermatogenesis in male rats through hormonal mechanisms, namely the reduction of LH and testosterone levels (Dhingra *et al.*, 2013; Mali *et al.*, 2002). Another research suggests that the leaf and fruit extracts of *M. annua* also contain anti-androgen compounds capable of growing furs in rats made to suffer from alopecia with the induction of testosterone. This extract contains glycosides, sterols, and phenolic compounds (Itankar *et al.*, 2013). *Menthae piperita labiatae* and *Mentha spicata labiatae* in the form of herbal tea (20g L⁻¹) can significantly increase LH and FSH levels and lower the testosterone level. *Methae piperita* and *spicata* contain terpenoid compounds, namely menthol and carvone, of above 50% (Akdogan *et al.*, 2004). The ability to inhibit pregnancy in the test animals varied in several herbs. In this research, CSE administration with doses ranging from 275 to 1,100 mg kg⁻¹ day⁻¹ during 14 days managed to inhibit pregnancy by 10 to 70%, while the stem extract of *Sarcostemma acidum* with doses ranging from 50 to 100 mg kg⁻¹ day⁻¹ given for 60 days managed to inhibit pregnancy among female test animals by 30 to 100% (Verma *et al.*, 2002).

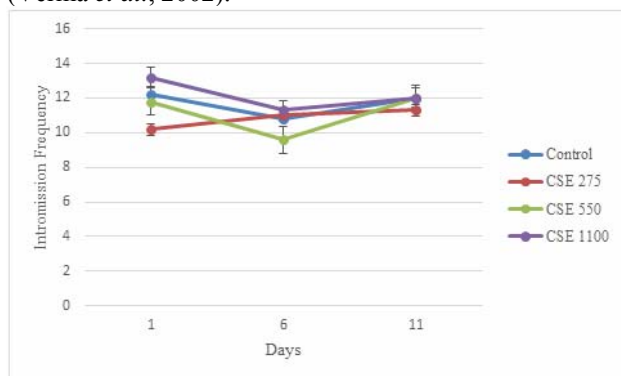


Fig. 2: Effect of CSE treatment on intromission frequency of male rats.

CONCLUSION

CSE administration with doses ranging from 275 to 1,100 mg kg⁻¹ day⁻¹ was able to reduce the level of pregnancy among rats by 10 to 70% without inhibiting the sexual behavior of male rats. It is likely that the anti-fertility effect of CSE is not generated using the hormonal mechanism.

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