

The effects of clove flower extract (*Syzygium aromaticum*) on the diameter and thickness of epithelial seminiferous tubules testis unilateral cryptorchidism model in white rat (*Rattus norvegicus*)

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Abstract

Objective: This study aims to determine the effect of clove flower extract (*Syzygium aromaticum*) on the diameter and thickness of the epithelial seminiferous tubules of white rats (*Rattus norvegicus*) testes induced by cryptorchidism.

Method: This study used 24 male white rats of the Sprague Dawley strain which were divided into six groups consisting of two groups with different intervals. The 18-day interval group consisted of the K-1, K+1 and P+1 treatment groups, while the 36-day interval group consisted of the K-2, K+2 and P+2 treatment groups. Data on the diameter and thickness of the seminiferous tubule epithelium were analyzed using the ANOVA test and continued with Duncan's test on data that showed significant differences ($P < 0.05$).

Conclusion: Based on the results of the study, it was shown that giving clove flower extract (*Syzygium aromaticum*) at a dose of 70 mg/kg for 18 and 36 days had no positive effect on reducing the diameter and thickness of the epithelium of the seminiferous tubules of the testes of white rats (*Rattus norvegicus*) induced by cryptorchidism surgery.

Keywords: Reproductive Health; Clove Flower Extract (*Syzygium Aromaticum*); Diameter of Seminiferous Tubules; Cryptorchidism; Thickness of Seminiferous Tubule Epithelium

1. Introduction

Cryptorchidism is one of the most common congenital abnormalities found in animals, particularly dogs and cats. The prevalence of cryptorchidism in dogs is reported to be 0.8%-10%. Cryptorchidism is characterized by the absence of one gonad (unilateral cryptorchidism) or both (bilateral cryptorchidism) in the scrotum [1]. Physiologically, the testicles in the scrotum have a lower temperature of around 2-4 °C compared to body temperature, which is 32-34 °C [2]. Abdominal hyperthermia can cause an increase in testicular temperature, resulting in oxidative stress. Oxidative stress is caused by increased production of Reactive Oxygen Species (ROS), decreased antioxidant enzyme activity, increased lipid peroxidation, and decreased H₂O₂ levels [3]. Ascension temperature in the environment of the retained testicles in the abdomen causes germ cell loss so that can result in reduced diameter and thickness tubule seminiferous which results in a decrease fertility [4].

ROS can be neutralized by the antioxidant activity of phenolic compounds. Cloves contain several active compounds such as eugenol, eugenol acetate, saponins, flavonoids, tannins, carfilene, sesquiterpenols, and naphthalene. The active

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compound eugenol in clove powder is very dominant, containing around 74-76% or 9381.7 to 14650 mg/100 grams and 0.15-0.24% eugenol acetate. Several studies have shown that eugenol in cloves can be used as an antioxidant [5,6]. Eugenol is able to stop damage oxidative consequence radical five times freer effective compared to the stated vitamin E as antioxidant strong [7].

The above considerations are the basis for the need to conduct this research to determine the effect of the extract. flower clove (*Syzygium aromaticum*) against diameter and thickness Epithelium of the seminiferous tubules of the testis of the unilateral cryptorchidism model of the rat white (*Rattus norvegicus*).

2. Materials and Methods

Materials used in this study is mouse white Male (*Rattus norvegicus*) Sprague Dawley strain 15-17 days old as many as 24 individuals, povidone iodine, xylazine, ketamine, silk thread as well polyglactin 6.0 thread used for operation induction cryptorchidism. Maintenance mouse white use material in the form of feed, drink and chaff as a cage base. Treatment therapy given use clove flower extract (*Syzygium aromaticum*) and aquades. In making preparation histopathology use 70% alcohol and formalin buffer. Fixation network using liquid paraffin, xylene, 70%, 80%, and 90% alcohol. The tools used in this study is 26G needle, 1cc syringe, gloves, mask, tampon, tools surgery sterile (blade, scalpel, tweezers, scissors) surgery, mosquito, needle holder) which will used in operation induction cryptorchidism.

Making extract methanol flower clove method soxhletation that is scales analytics, tools socket, glass measuring, stirrer and rotary evaporator. Maintenance animal tries done using 12 cages rat, scales weight, place feed and drink. Other equipment includes probe, microscope trinocular (Nikon Eclipse E200), label paper, tools write, digital camera.

Surgical induction of cryptorchidism using the Tekayev method et al [8] modified. The procedure started with anesthesia, administered intraperitoneally at a dose of 0.2 mL per 100 g body weight, using a mixture of 3.75 mL Ketamine, 5.75 mL distilled water, and 0.5 mL Xylazine. After anesthesia, a small incision was made in the right inguinal region near the gubernaculum. The external inguinal ring was then used to separate the right gubernaculum from surrounding tissues and push it back into the abdominal cavity. To prevent the testicle from descending, the external inguinal canal was sutured closed. In cases of unilateral cryptorchidism, only one testicle was surgically induced, while the other testicle remained untreated to serve as a control for observing post-surgical changes. Following surgery, the rats were returned to their cages.

For the negative control group, a sham operation was performed by making an incision in the right inguinal area and isolating the right gubernaculum. The gubernaculum was manipulated by pushing it into the abdominal cavity to simulate cryptorchidism induction, then repositioned back to its original place. The incision was then closed with sutures. The experimental animals were euthanized using cervical dislocation on the 18th and 36th days after surgery. Both right and left testes were surgically removed, then fixed in 10% formalin buffer for subsequent histopathological examination.

Study This is study experimental with do test giving extract methanol flower clove (*Syzygium aromaticum*) in mice white (*Rattus norvegicus*) male cryptorchidism model. Design Research used that is Completely Randomized Design Factorial with grouping three treatment. Group treatment shared become six a group consisting of from K+1, K+2, K-1, K-2, P+1 and P+2. Group treatment consists of from 24 tails mouse white (*Rattus norvegicus*) among them as following: K-1: consists of from 4 tails animal try to do sham operation and given distilled water using a probe for 18 days. K-2: consists of from 4 tails animal try to do sham operation and given distilled water using a probe for 36 days. K+1: consists of from 4 tails animal try to do induction surgery cryptorchidism of the right testis and given distilled water using a probe for 18 days. K+2: consists of from 4 tails animal try to do induction surgery cryptorchidism of the right testis and given distilled water using a probe for 36 days P+1: consists of from 4 tails animal induced trial surgery cryptorchidism of the right testis and given therapy clove flower extract (*Syzygium aromaticum*) with a probe for 18 days. P+2: consists of from 4 tails animal induced trial surgery cryptorchidism of the right testis and given therapy clove flower extract (*Syzygium aromaticum*) with a probe for 36 days.

3. Results and Discussion

Research result from giving clove flower extract (*Syzygium aromaticum*) against diameter and thickness Epithelium of the seminiferous tubules of the testis of the unilateral cryptorchidism model of the rat white (*Rattus norvegicus*). Testes observed in the study This is the dexter testis that is performed induction cryptorchidism (located in the abdomen). Observation carried out on diameter and thickness epithelium tubule seminiferous as well as measured in a way

microscopic with help tool microscope trinocular use NIS-Elements by Nikon *software* with 400x magnification. Measurement conducted in 5 fields view, every roomy view observed 1 tubule seminiferous. The data obtained normality test was performed with the Shapiro-Wilk test and homogeneity test. Based on the normality test of the tubular diameter seminiferous shows normally distributed data ($p>0.05$). The results of the homogeneity test of the tubular diameter seminiferous shows the data has been homogeneous. Tubular diameter data seminiferous furthermore ANOVA test was carried out, then done analysis advanced with Duncan's test because own difference significant ($P\leq 0.05$).

Table 1 Average Tubule Diameter Seminiferous Rat Testis (*Rattus norvegicus*) Various Group Treatment

18-day interval treatment	X $\pm$ SD	36-day interval treatment	X $\pm$ SD
	Diameter of the seminiferous tubules (MM)		Diameter of the seminiferous tubules (MM)
K-1	221.85 b $\pm$ 9.95	K-2	219.72 a $\pm$ 9.95
K+1	200.46 ab $\pm$ 14.61	K+2	194.95 a $\pm$ 25.92
P+1	168.49 a $\pm$ 39.04	P+2	186.14 a $\pm$ 34.23

Description: Average value treatments followed by the same letter (ab) are stated No show difference real. The mean value followed by a different letter (A, B) indicates the real difference

Table 2 Average Epithelial Thickness Tubule Seminiferous Rat Testis (*Rattus norvegicus*) Various Group Treatment

18-day interval treatment	X $\pm$ SD	36-day interval treatment	X $\pm$ SD
	Thickness of epithelial seminiferous tubules (MM)		Thickness of epithelial seminiferous tubules (MM)
K-1	48.32 b $\pm$ 10.02	K-2	46.88 a $\pm$ 9.40
K+1	27.05 a $\pm$ 9.61	K+2	26.54 a $\pm$ 24.15
P+1	30.78 a $\pm$ 11.19	P+2	38.19 a $\pm$ 14.00

Description: The mean value followed by a different letter (a, b) indicates the real difference

The testicles are sensitive organs to radical free that can result in damage from spermatozoa membranes and can also cause disorders in the spermatogenesis process. Membrane cells spermatogenic own unsaturated fatty acids fed up double. Therefore that, they prone to with ROS capable oxidize proteins, lipids and acids deoxyribonucleic acid so that can cause damage cell [8]. Group K+1 treatment after induced surgery cryptorchidism with giving distilled water for 18 days show decline mean diameter of tubule seminiferous. Group K+2 treatment after induced surgery cryptorchidism with giving distilled water for 36 days show decline mean diameter of tubule seminiferous under group K+1. Condition This in accordance with study Previously Moon et al [9], that change histological related with cryptorchidism result in decrease in tubular diameter significant seminiferous group. K+1 treatment after induced surgery cryptorchidism with giving distilled water for 18 days show decline average thick epithelium tubule seminiferous. Group K+2 treatment after induced surgery cryptorchidism with giving distilled water for 36 days show decline average thick epithelium tubule seminiferous under group K+1. Findings this is also appropriate with study Abd-El-Hafez et al [10], that existence difference significant in thickness germinal epithelium and tubule diameter seminiferous in the testes that experience cryptorchidism with the testicles located in scrotum.

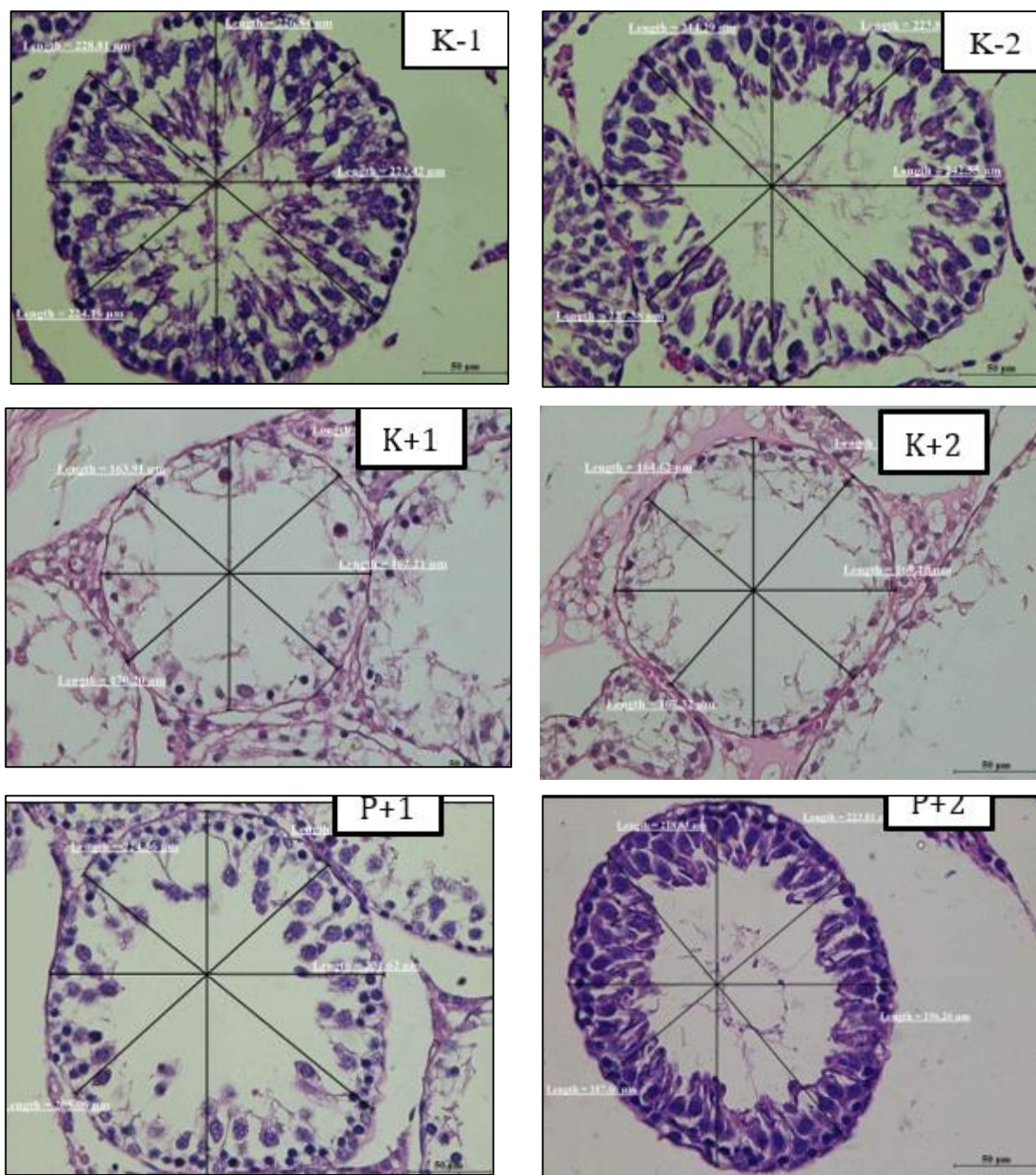


Figure 1 Overview of seminiferous tubules microscopic image with staining haematoxylin eosin 400x magnification (trinocular microscopy; Nikon Eclipse 200). The black line shows the measurement of the diameter of the seminiferous tubules. Note: The K+1 and K+2 treatment groups (cryptorchidism and aquadest for 18 and 36 days) showed a decrease in the diameter of the seminiferous tubules compared to the K-1 and K-2 treatment groups (sham and distilled water operation for 18 and 36 days). The P+1 and P+2 treatment groups (cryptorchidism and clove flower extract 70 mg/kg BW for 18 and 36 days) showed an increase in diameter of the seminiferous tubules

In general, macroscopic induced testis cryptorchidism experience atrophy compared to the testicles which are in the sac scrotum. This is in accordance with Lee et al [11] states that after 30 days post induction cryptorchidism, operated testicle far smaller than scrotum and sperm no found in the cauda epididymis testis cryptorchidism compared to the testicles located in scrotum.

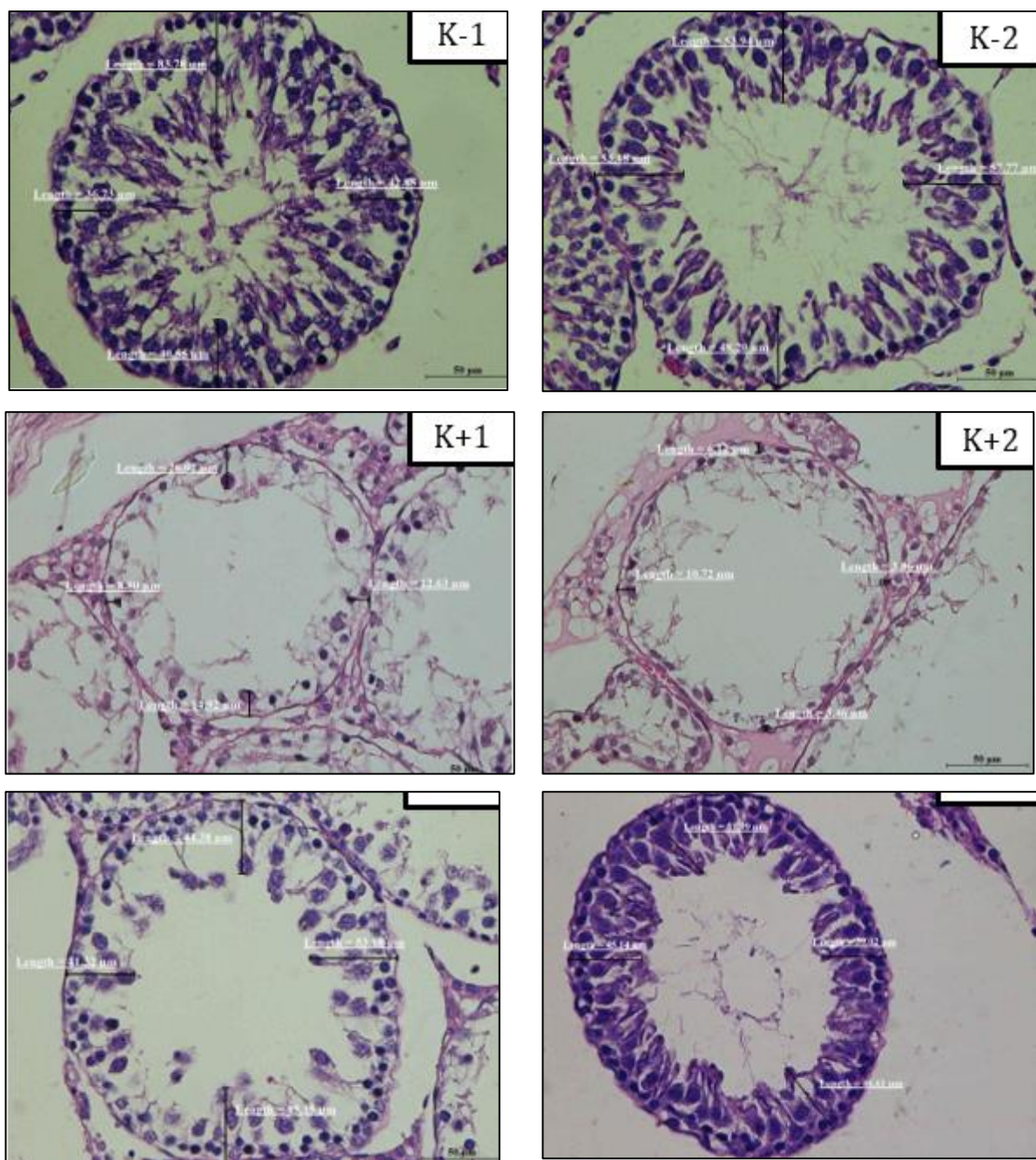


Figure 2 Overview of seminiferous tubules microscopic image with staining haematoxylin eosin 400x magnification (trinocular microscopy; Nikon Eclipse 200). The black line shows the measurement of the thickness of epithelial seminiferous tubules. Note: The K+1 and K+2 treatment groups (cryptorchidism and aquadest for 18 and 36 days) showed a decrease in the thickness of epithelial seminiferous tubules compared to the K-1 and K-2 treatment groups (sham and distilled water operation for 18 and 36 days). The P+1 and P+2 treatment groups (cryptorchidism and clove flower extract 70 mg/kg BW for 18 and 36 days) showed an increase in thickness of epithelial seminiferous tubules

The effect of eugenol as content clove flower extract that work as antioxidant own influence an increase that is not significant to the diameter of the tubule seminiferous. Group P+1 treatment after induced surgery cryptorchidism with giving dose clove flower extract 70mg/ KGBB for 18 days show decline mean diameter of tubule seminiferous, whereas group P+2 treatment after induced surgery cryptorchidism with giving dose clove flower extract 70mg/ KGBB with a longer interval of 36 days show existence improvement mean diameter of tubule seminiferous compared to group P+1 but own average under K+1 and K+2 groups. Research results the average diameter indicates decline can influenced by

factors effect antioxidants in extracts flower cloves responded in a way different in testicular tissue. One of the possible reasons for matter This that is difference pharmacology from material chemistry on the target [12].

Group P+1 treatment after induced surgery cryptorchidism with giving dose clove flower extract 70mg/ KGBB for 18 days show decline average thick epithelium tubule seminiferous, whereas group P+2 treatment after induced surgery cryptorchidism with giving dose clove flower extract 70mg/ KGBB with a longer interval of 36 days show existence improvement mean diameter of tubule seminiferous compared to P+1 group. Improvement in thickness epithelium tubule seminiferous which is not significant can influenced by factors dose. Other studies have shown characteristic biphasic from dose extract flower different cloves on the testicles. Higher doses low increase product testosterone, whereas higher dose tall cause decline production testosterone so that induce spermatogenesis disorders. Changes serum testosterone levels can be caused by changes activity enzymes in the testes that regulate testicular androgenesis. clove flower extract with dose tall own contraindications with a number of enzymes in the testes, while if given with dose low can increase Serum testosterone levels [13].

4. Conclusion

Based on the results of data analysis, it was shown that administration of clove flower extract at a dose of 70 mg/kg body weight for 18 and 36 days did not provide a significant improvement effect on the reduction in diameter and thickness of the seminiferous tubule epithelium.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Statement of ethical approval

This research received ethical clearance No. 1.KEH.011.01.2022, issued by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga.

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