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Original Research Article

Anti-aging Effect of Plant Leaf Extract of Kersen (*Muntingia calabura* L.) on the Seminiferous Tubule of Mice Induced with D-galactose

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Abstract: One of aging-related changes in the testes in male is impairment of spermatogenic process that make spermatogonia, spermatocytes, and spermatids cells decreased which ultimately reduces fertility. There are many phytochemical compounds that are known to have anti-aging properties. One plant that is known to contain anti-aging compounds is the kersen or Jamaica cherry (*Muntingia calabura* L.). This study aims to reveal whether the phytochemicals in kersen leaf extract can restore the number of spermatogonia, primary spermatocytes, and spermatids, as well as the diameter and thickness of the seminiferous tubule epithelium in d-galactose-induced mice. There were 5 groups tested in this study. Group-1 was normal mice that were not induced and given the extract. Group-2 was a negative control where mice were induced with d-galactose but not treated. Groups 3, 4, and 5 were given 35, 70, and 105 mg/kg of cherry leaf extract, respectively. The results showed that kersen leaf extract was proven to be able to restore the number of spermatogonia cells, primary spermatocytes, and spermatids as well as restore the diameter and thickness of the seminiferous tubule epithelium. Thus, it can be concluded that kersen leaf extract has anti-aging properties in mice induced by d-galactose.

Keywords: Kersen, *Muntingia calabura*, d-galactose, anti-aging, testicular aging.

INTRODUCTION

Aging is a natural process characterized by a decline in the structure and function of body organs. In humans, aging causes changes in the face, body skin, muscle strength, internal organs, and hormone production. In the face, aging causes changes in the skeletal proportions of the midface and changes in the position and volume of soft tissues [1]. In the skin, aging causes changes in skin thickness, collagen fiber orientation, mechanical properties of the skin, and transport properties into or out of the skin [2]. In the endocrine system, aging causes a decrease in the production of the hormones testosterone, dehydroepiandrosterone (DHEA), and growth hormone. The decrease in hormone production then causes a decrease in organ function which has an impact on declining health [3].

One of the effects of aging that has received much attention is the decline in reproductive and sexual function. In men, the aging-related decline of sexual and reproductive function generally associated with a decline in testicular structure and function. Aging-related changes in the testes are characterized by decreased testicular volume, weight, and density, decreased testicular perfusion, occlusion and thickening of blood vessels, and increased weight and thickness of the tunica albuginea [4]. The decline in testicular structure and function can cause a decline in the spermatogenic process, sperm abnormalities, sperm dysfunction, and disruption of Sertoli cells and Leydig cells which ultimately reduces fertility in men [5].

Currently, there are many dietary phytochemicals that are thought to have anti-aging properties Si & Du (2014) in their review work found some phytochemicals that have anti-aging properties such as: phenolic compounds, terpenes, betalains, organosulfides, indoles and other organic acid. The phenolic compound includes flavonoids (polyphenols),

phenolic acids, hydroxycinnamic acids, lignans, tyrosol esters, stilbenoids, alkylresorcinols. The terpenes consisting of carotenoids, monoterpene, saponins, triterpenoid, and lipids. The anti-aging betalains are betacyanins, betaxanthins. Indoles compound includes iIndole-3-carbinol, sulforaphane, and allicin. Other organic acids that are thought to have anti-aging effect are oxalic acid and anacardic acid [6].

A much more complete list of natural compounds with anti-aging properties was written by Okkoro *et al*., (2021) in their review work. That, the anti-aging phytochemicals are: baicalein, epicatechin (ec), catechin, fisetin, kaempferol, quercetin, epigallocatechin gallate (egcg), chlorogenic acid, curcumin, rosmarinic acid, tambulin, sesamin, piceatannol, polydatin, oxyresveratrol, resveratrol, pentagalloyl glucose, tannic acid, naringin, chicoric acid, taxifolin, icariside ii, icariin, quercetin-3-o-glucoside, caffeic acid, gallic acid, juglone, salicylamine, salicyclic acid, 6-gingerol, tryosol, lappaol c, arctiin, matairesinol, arctigenin, calycosin, pyrroloquinoline quinine, chlorophyll. theophylline, caffeine, spermidine, tomatidine, reserpine, verminoside, ferulsinaic acid, fucoxanthin, glaucarubinone, ursolic acid, specioside, α-tocopherol, oleanolic acid, beta-caryophyllene, carnosol, carnosic acid [7].

One of the plants whose extract contains several anti-aging compounds is the Jamaica cherry that in Indonesia is called kersen (*Mutingia calabura* L.). This plant, which grows very easily in Indonesia, is known to contain catechin, kaempferol, quercetin, gallic acid, and α-tocopherol in its leaves [8]. Other studies report that Jamaica cherry leaves are rich in flavonoids such as: 3,5-dihydroxy-7,40-dimethoxyflavone, 3,5-dihydroxy-7,8-dimethoxyflavone, 5-hydroxy-3,7,8-tri-methoxy flavones, 5,40-dihydroxy-3,7,8-dimethoxyflavone, 5-hydroxy-3,7,8,40-tetramethoxyflavone, 20,40-dihydroxy chalcone (isoliquiritigenin (cabreuvin), (2S)-50-hydroxy7,8,30,40-tetramethoxyflavan, 20,40-dihydroxydihydrochal-cone, and 3,4,5-trihydroxybenzoic acid [9].

To see whether the anti-aging chemical compounds contained in cherry leaf extract really have anti-aging properties, we tested the water extract of this plant's leaves on the seminiferous tubule function of mice induced by d-galactose. D-galactose was used because this compound has been proven to accelerate the aging process in test animals [10].

MATERIALS AND METHODS

Plant Sample and Extraction

Leaf samples of kersen were taken from Natar sub-district, South Lampung district, Lampung province. The fresh leaves are washed with running tap water. After being air-dried, the leaves are put in an oven at a temperature of 38-40ºC. Next, the leaves are ground with a blender until they become powder. Extraction is done by the maceration method using aquades solvent which is repeated three times until all the bioactives are dissolved.

Test Animals and Experimental Design

Male mice (*Mus musculus*) (25 individuals) aged 3 months, weighing around 25-30 grams, were used in this study. The mice were divided into 5 groups of 5 mice each. The first group was normal mice that were not induced by d-galactose and were not treated with the extract. The second group is the negative control group, namely mice induced with alloxan but not given the extract. Groups 3, 4, and 5 are groups of mice infused with alloxan and given 35 mg/kgBW, 70 mg/kgBW, and 105 mg/kg BW of kersen leaf extract, respectively. Treatment of all groups lasted for 35 days and during that time all mice were kept at room temperature and given standard food and drink *ad libitum*.

D-galactose Induction

All test mice, except the normal group, were induced with d-galactose at a dose of 150 mg/kg BW. Induction is performed intraperitoneally every three days for 35 days.

Testicular Dissection and Experimental Parameter

After 35 days of treatment, the experimental mice were sacrificed to have their testes taken. The testes of the mice that had been dissected were then taken and fixed using 10% formalin buffer. The testes were cut transversely to obtain histological preparations of the seminiferous tubules. The histological preparation of the seminiferous tubules was then observed under a microscope at 400x magnification. The experimental parameters used in this study were the number of spermatogonium, the number of primary spermatocytes, the number of spermatids, and the diameter and thickness of the tubular epithelium.

Statistical Analysis

The research data were analyzed using ANOVA and then tested using Duncan's post hoc test at a significance level of 5%.

RESULTS AND DISCUSSION

Spermatocytogenesis Parameters

The number of spermatogonia cells, primary spermatocytes, and spermatids produced by spermatocytogenesis in the seminiferous tubules of mice given five different treatments is presented in Table 1.

Table 1: Effect of plant leaf extract of kersen on the spermatocytogenesis parameters of seminiferous tubules in mice induced by d-galactose

Treatment	Spermatogonia ($\bar{x}$ ± SD)	Primary spermatocytes ($\bar{x}$ ± SD)	Spermatids ($\bar{x}$ ± SD)
Normal control	77.40 ± 10.96^{d}	141.32 ± 15.07^{c}	180.56 ± 19.75^{d}
Negative control	52.28 ± 3.68^{a}	97.84 ± 6.52^{a}	124.80 ± 10.07^{a}
Extract 35 mg/kg BW	58.92 ± 2.20^{ab}	115.80 ± 12.09^{b}	139.44 ± 11.58^{ab}
Extract 70 mg/kg BW	63.04 ± 7.50^{bc}	127.56 ± 14.03^{bc}	152.68 ± 17.43^{bc}
Extract 105 mg/kg BW	69.24 ± 5.17^{cd}	134.34 ± 10.74^{c}	165.24 ± 16.41^{cd}

*Values in the same column followed by the same superscript is not different statistically at $\alpha = 0.05$

The data in Table 1 clearly shows that d-galactose induction in male mice significantly reduced the number of spermatogonia, primary spermatocytes, and spermatids. However, the administration of Jamaica cherry leaf extract was able to restore the number of these cells according to the increase in dose. Even at the highest dose (105 mg/kg BW) the number of spermatogonia, primary spermatocytes, and spermatids was statistically the same as the number in the group of normal mice.

Diameter and Thickness of Seminiferous Tubules

The effect of administering the extract to mice induced with d-galactose (negative control) compared to normal mice (normal control) and induced mice given kersen leaf extract is presented in Table 2. A visual depiction of the diameter and thickness of the seminiferous tubule epithelium is shown in Figure 1.

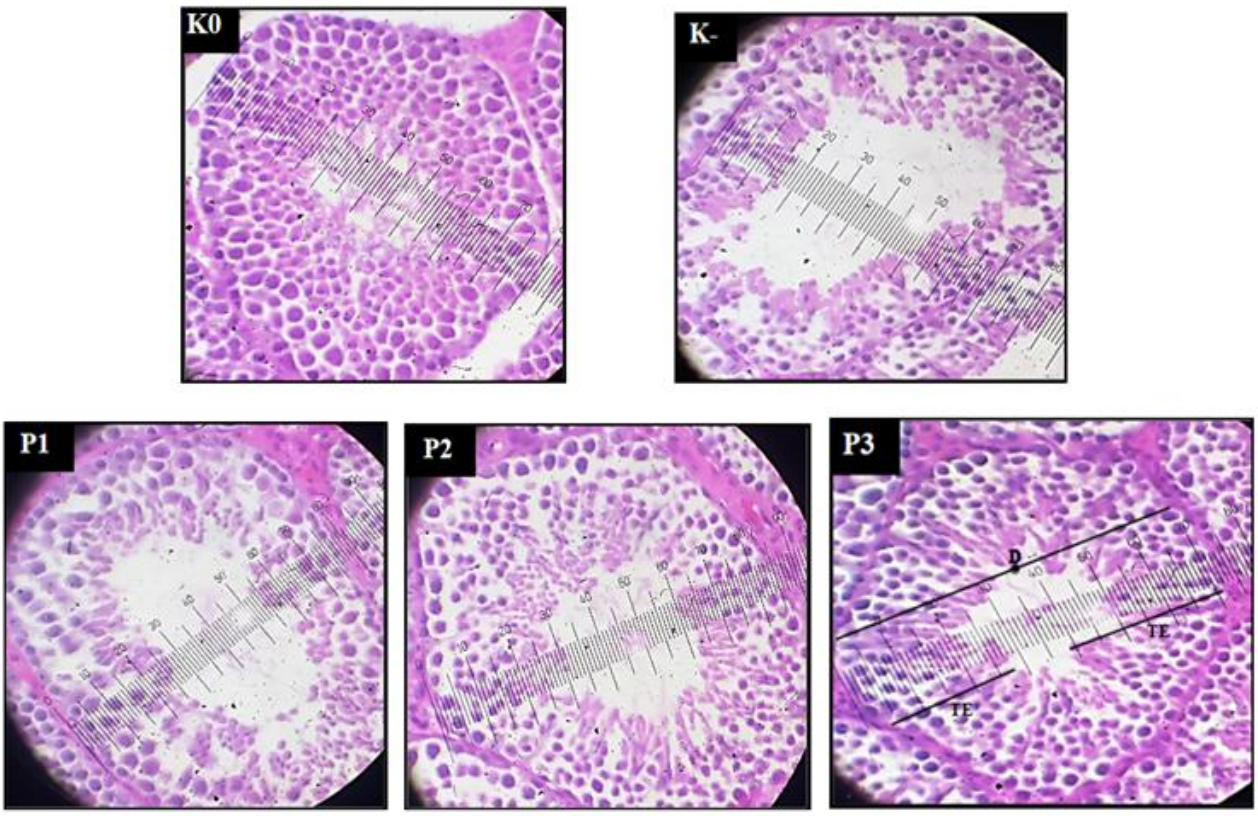

Figure 1: Photographs depicting diameter and thickness of seminiferous tubule of mice due to treatment given to the d-galactose-induced mice for 35 days. K0: normal control; K-: negative control; P1, P2 and P3 are mice received leaf extract of kersen at the doses of 35, 70, and 105 mg/kg BW respectively

Table 2: Effect of plant leaf extract of kersen on the diameter and thickness of seminiferous tubules in mice induced by d-galactose

Treatment	Diamater of seminiferous tubules in µm ($\bar{x}$ ± SD)	Thickness of seminiferous tubule epithelium in µm ($\bar{x}$ ± SD)
Normal control	87.40±4.87^{c}	37.92±3.15^{c}
Negative control	68.40±7.76^{a}	19.32±0.92^{a}
Extract 35 mg/kg BW	71.40±8.50^{a}	24.36±3.07^{b}
Extract 70 mg/kg BW	75.60±5.98^{ab}	28.32±5.01^{b}
Extract 105 mg/kg BW	82.20±6.37^{bc}	35.20±4.83^{c}

*Values in the same column followed by the same superscript is not different statistically at α = 0.05

Similar to spermatocytogenesis parameters, d-galactose induction significantly decreased the diameter and thickness of the seminiferous tubule epithelium of mice. Kersen leaf extract was also seen to restore the diameter and thickness of the seminiferous tubule epithelium. At the highest dose, kersen leaf extract was able to restore the diameter and thickness of the tubule epithelium equal to the normal group.

Based on the data obtained in this study, it is proven that the extract of kersen leaves (*Muntingia calabura* L.) has anti-aging properties against aging in the testes of mice induced by d-galactose. The anti-aging properties are most likely due to the high antioxidant content in the extract of cherry leaves. As stated by previous researchers, the antioxidant activity test using the DPPH technique showed that cherry leaf extract has very high antioxidants with an IC_{50} value of 3. 030 µg/ml. Intracellular antioxidant activity tested using modified NBT method showed that leaf extract of kersen reduced intracellular reactive oxygen species (ROS) level [11].

The antioxidant properties of kersen leaf extract are thought to be due to the high content of total phenolics, sugars, organic acids, amino acids, phytosterols, phenolics and terpene glycosides [12]. The existence of phenolic compounds as antioxidants in cherry leaves was also revealed by previous researchers who testing anti-oxidant activity using DPPH and superoxide scavenging assays [13].

Other studies have successfully shown that the presence of phytochemicals that have anti-aging properties such as gallic acid and quercetin are those that have strong antioxidant properties. The antioxidant properties of these compounds make kersen leaf extracts have the potential as antidiabetic herbal ingredients [14].

In alloxan-induced hyperglycemia Wistar rats (*Rattus novergicus*), *Muntingia calubara* leaves extract known to have downregulate ROS and MDA levels [15]. Not only has antidiabetic and anti-hyperglycemic properties, kersen leaf extract also has anti-hypercholesterolemia properties. Research using mice as test animals that were fed with high cholesterol for 14 days, Benu *et al*., (2024) successfully proved that kersen leaf extract is effective in reducing hypercholesterolemia [16].

Finally, the anti-aging effect of kersen leaf extract has also been proven by Sulistyoningrum *et al*., (2019). In the D-galactose-induced skin aging mouse model, aqueous leaves extract of kersen at the dose of in 70 mg/kg had anti-aging properties in vivo where plasma MDA level decrease and fibroblast count of the skin increase [17].

CONCLUSION

Kersen leaf extract was proven to be able to restore the number of spermatogonia cells, primary spermatocytes, and spermatids as well as restore the diameter and thickness of the seminiferous tubule epithelium. So that it can be concluded that kersen (*Muntingia calabura* L.) leaf extract has anti-aging properties in mice induced by d-galactose.

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Conflict of Interest: Authors declare there is no conflict of interest.

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