

Conference Paper

Effect of Propolis on Spermatogenic Cells Number and Diameter of Seminiferous Tubules in Male Mice (*Mus musculus*)

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Abstract

The present research aimed to determine the effect of propolis in spermatogenic cells number and seminiferous tubules diameter. Group Po served as control group, P1 (1,6 mg/0,5ml/day), P2 (3,2 mg/0,5 ml/day), P3 (6,4 mg/0,5 ml/day), and P4 (12,8 mg/0,5 ml/day) were given propolis ethanolic extract treatment orally and killed after 14 days. Spermatogenic cells number (spermatogonial cells, primary spermatocytes, spermatids) and seminiferous tubules diameter were observed. The result showed a lower number of spermatogonial in P1 and P2 groups, primary spermatocytes reduction in P2 group, spermatids increased in P1, P3, and P4 group. Seminiferous tubules diameter decreased in P2 and P3 group. P2 group (3,2 mg/0,5 ml/day) showed the lowest result in all parameters ($p < 0.05$). However, oral administration of propolis at these dose for 14 days may decrease spermatogonial cells and primary spermatocytes and increase spermatids number in adult mice.

Keywords: Propolis; seminiferous tubule; spermatogenic cells; testis histology.

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1. Introduction

Livestock productivity improvements can be made through improved feed, livestock management, and implementation of the breeding program, but direct research on livestock breeding itself may cost large financing and take relatively long time. To obtain a model that can be applied empirically in livestock animals researcher often used animal experiments. Mice is one of the experimental animals that can be used

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as research material because it has superior properties and meets the requirements of the standard to represent few farm animals condition [1].

Fulfillment the need for mice in large quantities can be done in many ways one of them by accelerating the breeding through increased fertility of male mice. High fertility of males will accelerate the process of mating and fertilization so that faster gestation in females would be obtained [2].

Reactive Oxygen Species (ROS) are a class of free radicals that produced in various organs including the testis as a physiological event. ROS are highly reactive oxidizing agents that stimulate oxidation and DNA damage in cells [3]. Spermatozoa's plasma membrane contains a high concentration of unsaturated fatty acid which can easily damage by oxidative stress, which means ROS production is a potential danger for spermatozoa's activity [4].

Propolis has been used in folk medicine for centuries. It is known that propolis possesses antimicrobial, antioxidative, anti ulcer and anti tumor activities [5]. Antioxidants are compounds which suppress the production of ROS [3, 6]. Propolis can improve the condition of the sick body pathology, works as an antioxidant and antibiotics, as well as raise the body's immune system both humoral and cellular because it contains flavonoids approximately 15% [7].

Twelve different flavonoids, pinocembrin, acacetin, chrysin, rutin, catechin, naringenin, galangin, luteolin, kaempferol, apigenin, myricetin, and quercetin, two phenolic acids, cinnamic acid and caffeic acid, and one stilbene derivative, resveratrol, in propolis extract were determined by capillary zone electrophoresis [8]. Chrysin increase sperm concentration, boost testosterone and attenuate oxidative stress and apoptosis [9]. Propolis was found significantly increase testosterone level, body weight, the relative weight of epididymis, semen characteristics and seminal plasma enzymes and decreased the levels of free radicals and lactate dehydrogenase [10].

2. Materials and methods

This research used completely randomized design with five treatments and five replications. Po (control) were given 0.5 ml aquadest only, for propolis treatment groups were given propolis P1 1.6 mg/0.5 ml/day, P2 3.2 mg/0.5 ml/day, P3, 6.4 mg/0.5 ml/day and P4 12.8 mg/0.5 ml/day. Treatment was conduct in 14 days, furthermore the testis removed from mice's body to make histological specimen.

After completion of each step, spermatogenic cells number and diameter of seminiferous tubules were analyzed. Spermatogenic cells which counted are spermatogonial

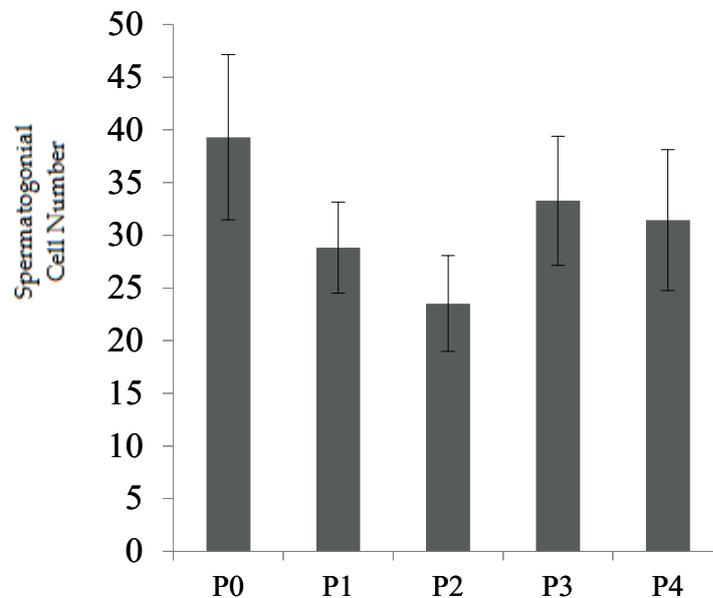


Figure 1: Mean \pm standard deviation of spermatogonial cells number of mice testis.

cells, primary spermatocytes, and spermatids. Spermatogenic cells and diameter of seminiferous tubules were examined using a light microscope and supporting feature Nikon Imaging Software.

Data were analyzed using one-way ANOVA to assess differences among treatments, followed by Duncan Multiple Range test with significant value 5% to determine the best treatment [11]. The result is presented as a mean \pm standard deviation.

3. Results

The results of propolis extract addition to spermatogenic cells number are presented in the charts below (Figure 1 - 3).

The results showed a decline in spermatogenic cells that can be seen from the reduced number of spermatogonial cells and primary spermatocytes in all groups which given propolis treatment compared with the control group. Significant differences can be seen in propolis dose of 1,6 mg/0,5 ml/day and 3,2 mg/0,5 ml/day while the number of spermatids increased with significant differences in propolis dose of 1,6 mg/0,5 ml/day, 6,4 mg/0,5 ml/day, and 12,8 mg/0,5 ml/day. The results of propolis extract addition to the diameter of seminiferous tubules are presented in **Fig 4**.

The result showed a decline in the size of seminiferous tubules diameter in each treatment that given propolis. The significant difference in diameter of the seminiferous tubules found in propolis dose of 3,2mg / 0,5ml/ day and 6,4mg / 0,5ml /day. The

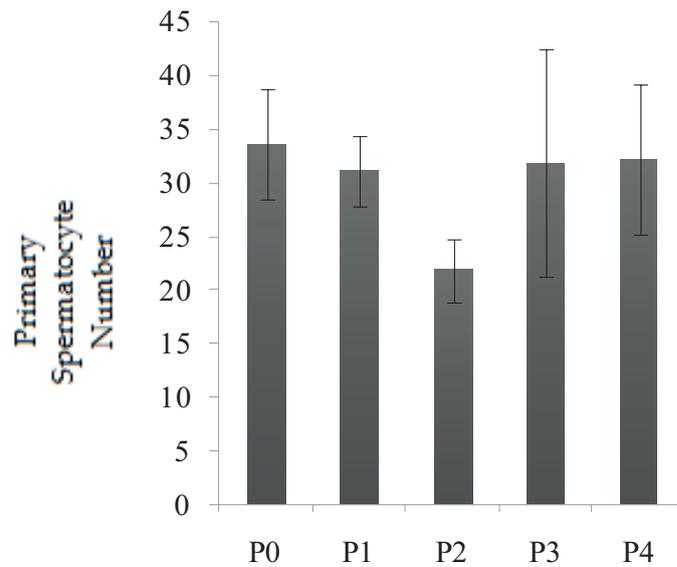


Figure 2: Mean ± standard deviation of primary spermatocyte number of mice testis.

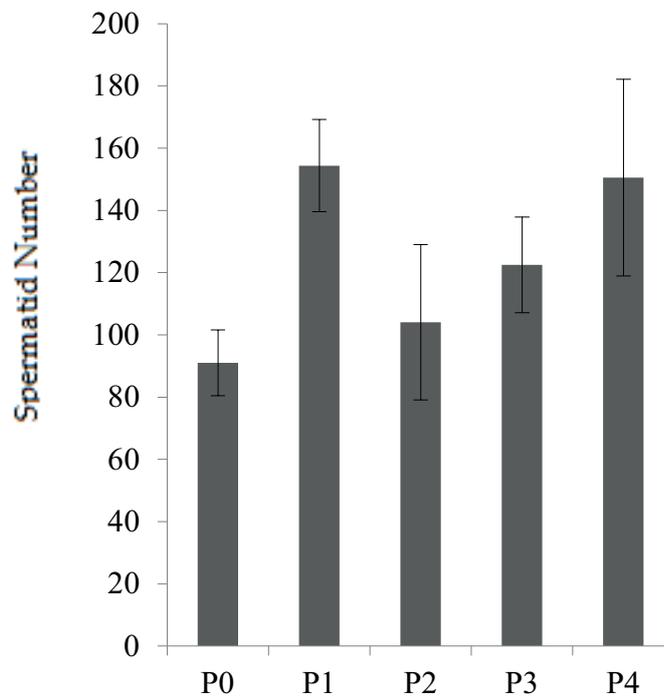


Figure 3: Mean ± standard deviation of spermatids number of mice testis. Mean with different alphabetical superscripts in the same column represent a significant difference ($p < 0.05$); P0 = control; P1 = propolis 1.6 mg/0.5 ml/day; P2 = propolis 3.2 mg/0.5 ml/day; P3 = propolis 6.4 mg/0.5 ml/day; P4 = propolis 12.8 mg/0.5 ml/day.

lowest size of seminiferous tubules diameter was at propolis dose of 3,2mg/0,5ml/day which also shows the lowest amount of spermatogenic cells.

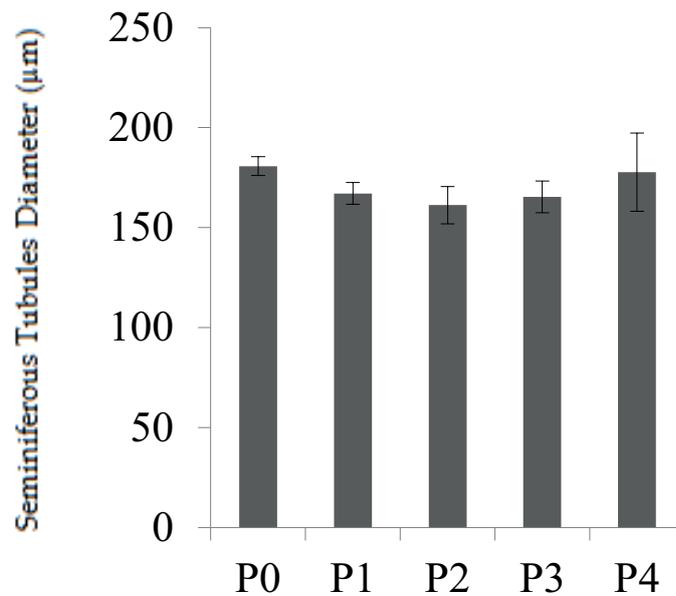


Figure 4: Mean \pm standard deviation of diameter of seminiferous tubules of mice testis. Mean with different alphabetical superscripts in the same column represent a significant difference ($p < 0.05$); P0 = control; P1 = propolis 1.6 mg/0.5 ml/day; P2 = propolis 3.2 mg/0.5 ml/day; P3 = propolis 6.4 mg/0.5 ml/day; P4 = propolis 12.8 mg/0.5 ml/day.

Administration of low doses of propolis in mice can increase the number of spermatogenic cells, but when the dose was increased it could reduce the number of spermatogenic cells [12]. Decreased number of spermatogenic cell was due to the increased work of these cells resulting excessive cell metabolism and increases the production of free radicals which compounds are contained one or more free electron so that the cells are not stable [13, 14]. Spermatozoa's plasma membrane contains a high amount of unsaturated fatty acids which susceptible to peroxidative damage, and ROS is one of a free radical synthesized by the testis and have the ability to stimulate oxidation and damage cells DNA [3, 15-17]. ROS exposure that occurs continuously would trigger apoptosis mechanism [18].

Increased number of spermatids in the present study showed there was an increase in spermatogenesis in meiosis stage. Although the present study conducted using local propolis, the results obtained were similar to research which used honey [19]. The proper dose of honey can boost the third stage of spermatogenesis in mice [20]. Chrysin contained in propolis increased testosterone which plays important role not only for proliferation but also for spermatogenic cell differentiation and at the stage of spermiogenesis [21].

Although the present study used propolis, the fluctuations of seminiferous tubule diameter are similar to the research which used honey [19]. Either the low production

of spermatozoa or no production of spermatozoa in the testis can decrease the diameter of seminiferous tubules, which means the production of spermatozoa affect the size of the diameter of seminiferous tubules [22].

Provision of propolis on male mice (*Mus musculus*) for 14 days at certain doses can reduce spermatogenesis process which evidenced by a decrease in the number of spermatogenic cells (spermatogonial cells and primary spermatocyte), increase spermatid formation and can reduce the diameter of seminiferous tubules in male mice.

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