

Effect of Bitter Extract (*Andrographis paniculata* Nees.) to Epididymal Mice Sperm Quality

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ABSTRACT

Bitter (*Andrographis paniculata* Nees.) used to prevention of various diseases, such as anticancer, anti-inflammatory, and antifertility. Ones of the bitter content is Andrographolide. It has antimitotic agent cause decreased of mitotic cells. The aimed of this research to determine the effects of Bitter extract to the mice (*Mus musculus* L. Swiss Webster)-epididymal sperm quality. The research used Completely Randomized Design (CRD), 4 treatments and 6 replications as the control (T0) without bitter extract treatment, and the other were gave a bitter extract orally for 36 days with dosages of T1 0,2; T2 0.4; or T3 0.6 g/ml/day. After treatment, the mice were decapitated, dissected and taken the epididymal. The number of normal or abnormal spermatozoa counted by a Neubauer Improved method and Eocine Stained. Data were Analyzed using *Analysis of Varian* and Duncan's New Multiple Range Test. The results Showed the highest average number of sperm in T0 29.83 x 10⁵, comparison to T1 18.17 x 10⁵; T2 24.33 x 10⁵ and T3 14.17 x 10⁵. The avarage number of normal sperm increased as decreased of dosage. The number of abnormal sperm ncreased as the increased of dosage. It can conclude that the bitter extract Orally for 36 days can decrease of mice sperm quality and increase of abnormal sperm of mice.

Keywords: *andrographolide, bitter, epididymal, spermatozoa quality.*

1. INTRODUCTION

The use of herbs for traditional medicine and traditional medicine has long been practiced throughout the world, both in developing countries and developed countries, including Indonesia. According to WHO, about 65% of the population of developed countries and 80% of people in developing countries have been using herbs for traditional medicine. WHO support to the concept *back to nature* is evidenced by the recommendation to use plants as traditional medicine, including the use of Herball in the maintenance of public health and disease prevention, especially for chronic diseases, degenerative diseases and cancer [1].

Indonesia is one country with a market potential of plants as herbal remedies and phytopharmaca has approximately 30,000 species of plants and 940 species of which are medicinal plants. Until now there are 180 species that have been used by traditional herbal medicine industry. Some herbs that have medicinal properties are also used by the public as medicine for spacing. Examples of this are the Dayak community in South Kalimantan Mangkling drinking water soaking the roots bikat (*Gnetum gnemoides* Brongn.) as a birth control drug.

Until now, fertility regulation is directed at women, because the right contraceptive method for men is still not assured, and often side effects and constraints that accompany it. Various techniques to reduce fertility in men is done with the use of various compounds including

suspected antifertilitas can lower sperm count, hormone regulation, prevention of sperm maturation, and changing the structure of the sperm itself. Use of the compound antifertilitas in men has certain requirements, as can lower sperm count to reach azoospermia, safe for health, can be restored within a certain period, and the specific work [2]. Drug and Medicine of Indonesian Institute is currently working with several universities are researching medicinal plants 9 national seed up to the stage of clinical trials, one of which is bitter (*Andrographispaniculata* Nees.). Ethanol extract of bitter herbs cause damage to *the seminiferous tubules* which is a network-forming spermatozoon [3]. The increase in the dose given, exacerbating the damage *seminiferous* causedtubules. According [4] giving dried leaf powder *A. paniculata* on male albino mice orally at a dose of 20 mg of powder daily for 60 days resulted in a cessation of spermatogenesis and damage to the seminiferous tubules of the testes.

Since the latter half of the 20th century, various studies have been conducted most of his concentration to determine the composition, safety, efficacy and mechanism of action of bitter. In Indonesia, bitter marketed either in a single dosage in tablet form, which is still herbs [1]. The chemical constituents present in the extract of bitter are lactones and flavonoids [5]. The main content is a bitter plant andrographolide.

Andrographolide is the active substance in a bitter (*Andrographis paniculata* Nees.), Which works to prevent cell division (cytokinesis). In this case the process of gametogenesis in the proliferative phase has decreased

resulting in spermatogonia produced will also decrease, then the number of sperm that will be generated is reduced. One of the processes that occur in the body cytokinesis is the process of spermatogenesis. Spermatogenesis is affected by hormones. According to Halim [3] it did not affect the bitter extract mechanism LH and androgen hormones. This is similar results, which found that andrographolide is not related to androgen hormones, but probably related to the FSH produced by the anterior pituitary gland.

Andrographolide be used in patients with cancer cells, can also affect the process of spermatogenesis in the seminiferous tubules and can impact on the quality of sperm produced. Andrographolide contained in the extract of bitter leaf allegedly able to reduce the number and quality of spermatozoa [6]. The bitter (*Andrographis paniculata* Nees.) extract decreases the quality of the ejaculated sperm of mice [7]. Based on these descriptions have been carried out a study entitled: The Effect of Bitter (*Andrographis paniculata* Nees.) Extract To The epididymal sperm quality of mice (*Mus musculus L.* Swiss Webster).

2. METHODS

2.1. Experiment Design

This research is an experiment using Completely Randomized Design (CRD), 4 treatments and 6 replications that control (T0); Treatment T1 were given extracts of bitter dose of 0,2g/ml/day; T2 treatment 0,4g/ml/day; and T3 0,6g/ml/day.

2.2. Procedure

Extract *Andrographis paniculata* Nees

Bitter taken from the city of Padang, West Sumatra. Bitter taken by revoking all parts of the plant. Bitter taken was bitter that are flowering. The part that will be used are the leaves and ramifications alone. The leaves and stems of bitter cut along the 1-2 cm and then dried in the sun to dry (dark brown). Once dried, then crushed using a mortar and pestle to form a dry powder. To manufacture paniculata extract made with maceration method. The filtrate was evaporated in a water bath at 500 C until evaporate the solvent depleted so that the resulting concentrated extract. Aquabides used to dissolve extracts as much as 100 ml and then dissolved in a solution of 2% CMC. Preparation test animals

Animal used were male mice (*Mus musculus L.*) Swiss Webster with adults aged 11-12 weeks were obtained from the laboratory of Zoology Department of Biology, Faculty of Mathematic and Natural Science of Universitas Negeri Padang. Mice were placed in plastic basin rectangular shape cages with a size of 30 cm (w) x 20 cm (l) x 10 cm (t) are covered with wire and were given the rest of the

board timber waste are replaced twice a week. Feed and water were given *ad libitum*.

Dosage

The administered dosage refers to a dosage of bitter (*Andrographis paniculata* Nees.) that has been used for human consumption and converted to 0.511 g to mice. So to see the antifertility effects on male mice. So that the doses used in this study was 0 g (T0), 0.2 g (T1), 0.4 g (T2), and 0.6 g (T3).

2.3. Treatment

Extract Treatment

Extract *Andrographis paniculata* Nees. orally with a tuberculin syringe and needle gavage to test animals as much as 0,5 cc/mice every day for 35 days refers to spermatogenesis cycle time of 35.5 days [8]

Animal Surgery

Surgery performed on mice scrotum after deep slept the mice by diazepam.

Sperm counts

Cutting epididymal left and right parts, placed in Petri dishes filled with PBS solution. Sort the epididymis using small tweezers and then aspirated cement as much as 10 microns using mikropipeter. Cement is inserted into the micro tube was then added 90 microns PBS solution and vibrated by hand to homogeneous. Cement sucked as much as 20 microns and then dripped on the Improved Neubauer, then observed brought microscope. Sperm calculated in 5 boxes, namely a, b, c, d and e are calculated by using magnification of 400 times The results of calculation of the amount of sperm and then inserted into the formula for determining the amount of sperm/ml suspension. Furthermore, cement stocks sucked or pipeting as much as 20 microns and by staining with eosin to view sperm morphology.

2.4. Data Analysis Techniques

The data were presented in the form of qualitative and quantitative data were analyzed by *Analysis of Variance*. The results of the data analysis show the effect of the test conducted on the degree of a $p < 0.05$ at further DNMRT [9].

3. RESULT AND DISCUSSION

3.1. Result

The results of this study can be seen the effect of extracts of bitter (*Andrographis paniculata* Nees.) On the quality of sperm in mice (*Mus musculus L.*) Swiss Webster is in Figure 1 as follows.

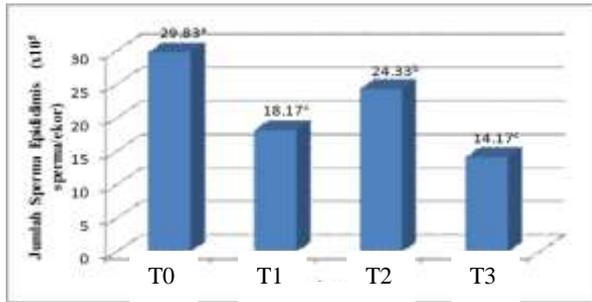


Figure 1. Histogram Number epididymis sperm of mice (*Mus musculus L.*) in Different Treatments Bitter extract T. Number on the histogram which followed the same lowercase, no significant difference in the level of $p < 0.05$ DNMR test

Data obtained from the sperm count semen obtained in mice epididymis shows the influence of extract of bitter (Figure 1) to the number of sperm. The fewest amount obtained in the treatment of 0.6 g/ml/day which is 14.17 x 10⁵.

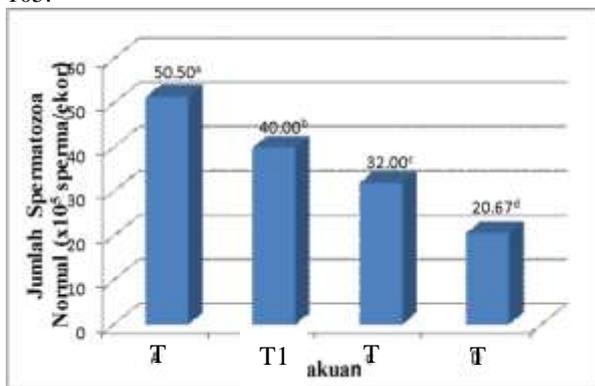


Figure 2. Histogram Number of the Abnormal Epididymis sperm of mice (*Mus musculus L.*) Treatment Various Bitter extract. The numbered followed the same lowercase, no significant difference $p < 0.05$ DNMR test

Fewest amounts of abnormal spermatozoa was found in control, which is 22.00 x 10⁵ and was highest in the treatment of 0.6 g/ml/day which is 42.00 x 10⁵. The higher dose of the treatment the higher the number of abnormal sperm were found (Figure 2).

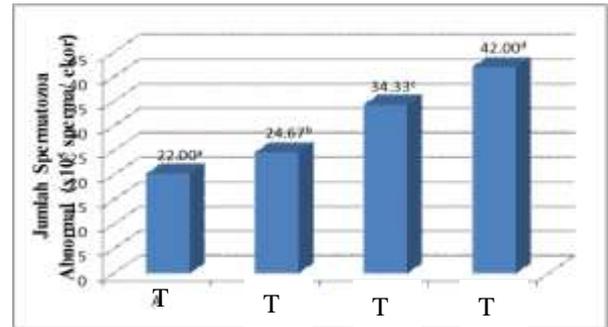


Figure 3. Histogram Normal Sperm number of epididymis of mice (*Mus musculus L.*) in Different Treatments Bitter extract T. Figures on the histogram which followed the same lowercase, no significant difference $p < 0.05$ DNMR test.

The highest number of normal spermatozoa in the control treatment that is 50.50 x 10⁵ (Figure 3) and the lowest at 0.6 treatment that is 20.67 x 10⁵. The higher dose of treatment cause the fewer number of normal sperm obtained.

3.2. Discussion

Based on the observations showed sperm counts in all treatments were given extracts of bitter. Based on the results of statistical analysis that the extract of bitter in various dosage effect on the number of epididymal spermatozoa. Pemberian higher dosages cause diminishing sperm counts (Figure 1).

The active substance contained in paniculata extract can prevent cell division (cytokinesis), so the process of gametogenesis in the proliferation phase of decline and resulted in the number of sperm produced is also reduced. According to Halim[3], the decrease of sperm count can be caused by delays in the process of mitosis in the seminiferous tubules by antimetabolic compound.

Best dose that can be used as a drug antifertilitas is at 0.2 g/ml/day for showing the reduction of sperm. However, at a dose of 0.4g/ml/day average number of sperm was higher when compared with the treatment of 0.2 g and 0.6 g. Increasing the number of sperm in the treatment of 0.4 g allegedly due to test animals that have variations in the physiological resilience of the dose given, but the amount does not exceed the control sperm.

From the results of the examination of abnormal spermatozoa (Figure 2) in the epididymis which gained an average of abnormal sperm in a dose of 0.2 g/ml/day, 0.4 g/ml/day, 0.6 g/ml/day higher than the control. After the extract was analyzed bitter (*Andrographis paniculata* Nees.) Affects the number of abnormal sperm. This is due to the maturation of spermatozoa in the phase disturbance spermiogenesis. Spermatozoa maturation process while in the epididymal duct is influenced by androgen hormones and secreted by the epithelial cells of the epididymis. This is consistent with the

theory put forward by Sumarmin[10] that in the seminiferous tubules, the androgen have function to control the process of spermiogenesis of meiosis process. Disturbances in the maturation of spermatozoa resulting in abnormal sperm just as tail curled, head without a tail, head without a hook, and a broken neck. 0.2 g dose treatment is the best that can be used as a drug antifertility for showing a reduction in the number of abnormal sperm. Normal sperm counts (Figure 3) in mice epididymis obtained on all treatments given paniculata extract

4. CONCLUSION

Based on the results it can be concluded that the extract of bitter (*Andrographis paniculata* Nees.), can reduce the quality of epididymal sperm in mice (*Mus musculus L.*) and increase sperm abnormalities.

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