In vivo test of bitter (andrographis paniculata nees.) extract to ejaculated sperm quality

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Abstract. Sambiloto or Bitter (Andrographis paniculata Nees.), are often used to treat various diseases, such as influenza, cancer, anti-inflammation, anti-HIV, anti-mitotic and anti-fertility. This study aimed to determine the effects of the bitter (Andrographis paniculata Nees.) extract to ejaculated sperm mice quality (Mus musculus L. Swiss Webster). This research was conducted using Completely Randomized Design with 4 treatments, which are 0.0 g/b.w., (P0), 0.2 g/b.w., (P1), 0.4 g/b.w., (P3), or 0.6 g/b.w., (P4) bitter extract orally for 36 days. After treatment, the mice decapitated, dissected and collected the sperm from vas deferens. Then, the number of sperm counted by using the improved Neubauer and then stained by Eosin to count the abnormal sperm. Data analyzed by ANOVA (Analysis of Variance) then DNMRT. The results showed that the average numbers of sperm are 28.80 x 10⁵ (P0), 19.50 x 10⁵ (P1), 12.50 x10⁵ (P3) and 9.50 x 10⁵ (P3). The average abnormal sperm numbers are 18.33 x 10⁵ (P0), 22.50 x 10⁵ (P1), 31.50 x10⁵ (P3) and 39.33 x 10⁵ (P3). It showed that the effective treatment to decrease sperm number was 0.2 g/b.w., of bitter extract. It can conclude that the bitter (Andrographis paniculata Nees.) extract decreases the quality of the ejaculated sperm of mice (Mus musculus L.)

Keywords: andrographolide, bitter, ejaculated spermatozoa

1. Introduction
Family Planning Program has been announced by the government of the Republic of Indonesia as a national program. One of the efforts that have implemented in the program is the provision of contraceptives. Use of contraception in principle is to prevent fertilization or fusion of the sperm cell and an ovum cell. Means of contraception is more aimed at women, while men are still limited, so that the development of the male contraceptives is less than to the female contraceptives [1]

One of the plants that are expected to be anti-fertility is bitter (Andrographis paniculata Nees.). Bitter widely used as a medicinal plant that has many properties in various countries, among others, as an anti-oxidant, anti-diabetic, anti-fertility, anti-HIV, anti-flu, anti-malaria, anti-diarrhea and others [2].
Chemically this plant contains diterpenoid lactone. In the lactone, components are andrographolide, which is the main active ingredient of this plant [3]. Besides other content found in this plant are pani kulin and flavonoids. From various studies, the content of which are believed to fight disease is andrographolide. Besides, the bitter leaf contains saponins, alkaloids, and tannins [4].

Of the many chemical compounds found in bitter (Andrographis paniculata Nees.) Compound that acts as an anti-fertility is andrographolide. According to [5] andrographolide is the active substance contained in bitter that works to prevent cell division (cytokinesis). Cells can affect spermatogenesis occurs in the seminiferous tubules. Then [6] find that bitter extract still reveals the abnormality to development of mice fetuses.

According to Bardin [7], bitter extract in male mice given orally can decrease the number of epididymal spermatozoa. As the result of the declining number of spermatozoa found in the epididymis and disruption of the maturation of sperm cells, it can be expected to affect the ejaculated sperm contained in the vas deferens. To determine how it affects, then conducted a study entitled In Vivo Test of Bitter (Andrographis paniculata Nees.) Extract On the Quality of ejaculated spermatozoa of mice (Mus musculus L. Swiss Webster).

2. Materials and Methods
2.1 Materials
This study used a test animal mice Mus musculus L. Swiss Webster, 11-12 weeks, weighing 20-30 grams were obtained from the Laboratory of Zoology Department of Biological Science Universitas Negeri Padang. Extracts of bitter (Andrographis paniculata Nees.) obtained from various processes performed at the Laboratory of Plant Physiology FMIPA Universitas Negeri Padang. The bitter plant obtained from the city of Padang, West Sumatra in conditions that are in bloom.

2.2 Methods
Mice were grouping into one control group and three treatment groups. Each group consisted of 6 mice. The control (P0) group was gave only the solvent of extract, and the food was the same as the treatment group. Group P1, P2, and P3 are the groups treated with the extract of bitter orally for 36 days with a dose of 0.2 g, 0.4 g and 0.6 g. After the test of completion in each group, the mice were decapitated, dissected and cut the vas deferens of the left and right, place it in Petri dishes filled with 10 micron PBS (Phosphate Buffered Saline). To calculate the number of spermatozoa, it were counted using the Improved Neubauer. As for seeing and counting the abnormal number of sperm (the morphology of spermatozoa), using Eosin staining method. The data analyzed by ANOVA (Analysis Of Variance) and Duncan New Multiple Range Test.

3. Results and Discussion
From this research, it can be seen the effect of extracts of bitter (Andrographis paniculata Nees.) to the ejaculated spermatozoa number in mice (Mus musculus L.) is as Figure 1.
Figure 1. The number of ejaculated sperm of Mice on the Different Treatment of Extract Bitter. The number followed by different small letters, significantly p<0.05

After the extract bitter (Andrographis paniculata Nees.) With dosage 0.2 g, 0.4 g and 0.6 g in mice (Mus musculus L.) for 36 days there is decreases in the number of ejaculated spermatozoa. According to Pallav et al. [3], the number of spermatozoa produced is very dependent on the direct process that occurs during the process of spermatogenesis in the seminiferous tubules. When normal spermatogenesis takes place, it will produce normal sperm count as well. Conversely, if an interruption occurs during spermatogenesis process, the development will affect the number of spermatogonial cells formed spermatozoa. So with the division during spermatogenesis disorder, it is expected to lead to changes or damage to spermatozoa produced thus might change, and the damage will effect to decrease in the number of ejaculated spermatozoa.

Based on the test results can be seen that the use of 0.2 g dose was the most in reducing the number of ejaculated spermatozoa, and bias used as an anti-fertilizer dose. At a dose of 0.2 g is obtained an average of 12.00×10^5 sperm count and has seen a significant decrease in the number of spermatozoa compared with controls who had 28.08×10^5.

Figure 2. The number of normal spermatozoa of mice in various treatments of bitter extract. Histogram followed by superscript letters are different, significantly at p<0.05

The number of normal spermatozoa morphology examination (Figure 2) in the vas deferens mice (Mus musculus L.) obtained an average percentage of normal sperm morphology dose of 0.2 g/b.w., 0.4 g/b.w., and 0.6 g/b.w., lower than compared with the control. After
further test was statistically, there are significant due to the extract of bitter (*Andrographis paniculata* Nees.). In this study, the higher dose given, the fewer number of normal spermatozoa. The dose that can using as an anti-fertilizer is on treatment with a dose 0.2 g/b.w., because it can inhibit or reduce the number of normal sperm.

Based on Figure 3, it can be seen that the extract of bitter (*Andrographis paniculata* Nees.) also, cause abnormal spermatozoa. Abnormalities in spermatozoa of mice (*Mus musculus* L.) allegedly because of interference maturation phase of spermiogenesis spermatozoa in this case by the theory put forward in the seminiferous tubules, androgen function in controlling the process of spermatogenesis in meiosis and spermiogenesis process. In this study suspected antimitotic compounds contained in andrographolide has inhibited androgen produced, so that an interruption in the phase of spermiogenesis. So if the hormone androgen is a hormone that plays a role in spermatogenesis process when availability is a little, it will cause the process was interrupted and could result in abnormalities in sperm during spermiogenesis.

4. Conclusions

Based on the results obtained can be concluded that the extract of bitter (*Andrographis paniculata* Nees.) decreased the number of ejaculated spermatozoa of mice (*Mus musculus* L.) and decreased the number of normal sperm. In other words, the extracts of bitter (*Andrographis paniculata* Nees.) decreased the quality of the ejaculated spermatozoa of mice (*Mus musculus* L.).
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