The Influence of Granting Ngor Eggplant (Solanium Indicum) Extract inhibiting of Spermatogenesis in Mice (Mus Musculus)

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Abstract

The study about contraceptive for adult especially for men that the numbers were limited, so needed the ingredients contraceptive is derived from a plant. One of the plants that potentially becomes contraceptive is ngor eggplant because contains antifertility substances. The research has been done about granting of ngor eggplant extract inhibiting spermatogenesis in mice. The design of research used random post-test control group design by one group control, three groups by treatment and six times doing treatment again. \((T_0 = \text{control group}, T_1 = \text{granting extract 15\%}, T_2 = \text{granting extract 20\%}, T_3 = \text{granting extract 25\%})\). This treatment has been done in 36 days. The result of this research showed the granting ngor eggplant extract to \(T_1\) could reduce the number of cells spermatocyte primary pakheten and cells spermatids 7 & 19 by meaningful \((P<0.05)\). In \(T_2\) and \(T_3\) could reduce the number spermatogonia A cells, spermatocyte primary pakheten cells and spermatids 7 & 19 cells \((P<0.05)\).

Keywords

Spermatids 7 & 19; Spermatagonium A; Ngor Eggplant Extract; Spermatocyte Primary; Pakheten;

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1. Introduction

To solve the population growth, the central government of Indonesia implements the program family planning as a national program. One of the efforts that used is providing the contraceptive device but the most devoted to women. Besides the women, men should participate in the implementation of the family planning. According to the data of BKKBN, the role of man in the implementation of the family planning program is still relatively low is 5.6%. It shows there should have been a contraceptive device man as a support of the program (Arsyah, 1986).

Researches toward contraception man invention are a challenge for Andrology scientist. Eggplant (Solanum sp) is a kind of plant that is very useful to Indonesian people. Solanus is a plant as an antifertility source that classified of group “estrogenic agent”. Ngor eggplant (Solanum indicum) contains solasodine which is glycoalkaloid, the aglikol has steroid nucleus. Glikoalkaloid steroid framework based cholestane C27 as material for producing steroid hormones to contraception (Desai et al, 2011). Alkaloid Solanum spatially competitive against Follicle Stimulating Hormone receptors. FSH that comes out of the anterior pituitary bind with a receptor FSH into the Sertoli cell membrane, to stimulate Sertoli cells to increase synthesis and secretion Androgen Binding Protein (ABP). As a result of this solasodine bound with a receptor FSH so the formation of ABP in the Sertoli cells will be reduced. When Andogen Binding Protein (ABP) declined the process spermatogenesis is obstructed. Mechanism apoptosis compound solasodine held by means of disturbing a cell membrane by special relationship the branch of sugar bound and disturbing integrity cancer cells by means of change morphology cells and the DNA so as to cause apoptosis (Hsu et al.2016, Chang et al, 2008). The results of Yolanda’s research (2011) showed the provision of seeds extract ethanol eggplant pokak motility be able to decrease and increase the number of spermatozoa abnormal, and decrease the total spermatocyte primary.

2. Research Method

Material
Wistar mice adult male strains balb-c in the age 1.5 months with a weight 25 - 30 grams, mice feed, ethanol 96 % ngor fresh fruit eggplant, ether, formalin 10 %, paraffin.

Instrument
An instrument used the animal pet cage, sonde equipment, beker glass, weight electronic scale, small scissors, anatomy tweezers, sirurgik tweezers and the razor knife.

Method
The design of research used in this research is experimental design with randomized post-test only control group design.

How to Work
Making of ngor eggplant extract
Ngor eggplant fresh cutting small and dried after that blended then sifted with sifting equipment B 40. The powder extracted with a solution of ethanol 96 % using soxhlet. Extract evaporated to get ngor eggplant extract viscous. Then, make the concentration extract 15 %, 20 % and 25 %.

Treatment
The mice are divided into 4 group each group consists of 6 mice. The first is the control group and the others are the treatment. Every group treatment giving ngor eggplant ethanol extract with 15%, 20%, and 25% doses by 0,3 cc every day with orally.

Making histology testis preparations
The testis that has been taken put into a fixative (formalin 10 %) for 24 hours. After that, in successive has done dehydration with alcohol. Then incorporated into the toluene to clearly and is blocked by paraffin. Seyatan histology made with the microtome and tinged with hematoxylin-eosin (HE).

Data Collection
The data was taken from the investigation 60 the seminiferous tubules (30 tubules in the testes left and 30 tubules in the testes right) for each sample. The qualitative data is obtained from the description of microscopic tests after treatment by determining the seminiferous tubules the damage caused by obstacles spermatogenesis in determining damage the seminiferous tubules used four categories are antrofi tubuler, necrosis tubuler, lost of intermedia cells, and reduced spermatogenesis. The quantitative data is obtained from the count of spermatogonium cells with the Abercrombie.

3. Results and Analysis
The Frame of Histology Testis
Category of tubules damage that occurs based on the criteria tubules damage. The level of tubules damage can be seen in the table below:
The level of damage the seminiferous tubules mice at 60 the seminiferous tubules to every sample.
The influence of granting ngor eggplant (Solanum indicum) extract inhibiting of spermatogenesis in mice (Mus musculus). International Journal of Health Sciences, 1(2), 12-19.

Table 1. The level of the seminiferous tubules damage mice at 60 the seminiferous tubules to every sample

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Level of seminiferous tubules damage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>T0 (Control)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T1 (15%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T2 (20%)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>T3 (25%)</td>
<td>6</td>
<td>11</td>
</tr>
</tbody>
</table>

Statistical testing by test with non-parametric correlation Spearman-Rank shows that the higher the concentration of fruit extract the eggplant ngor, so the level of damage tubulum seminiferous mouse has escalated (p<0.05) with correlation coefficient r = 1.

**Spermatogonia A**
The number of spermatogonia cells in every treatment group is shown in table 2

Table 2. The average of point in the number of spermatogonia after granting the ngor eggplant extract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Rate of Spermatogonia numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 (Kontrol)</td>
<td>6</td>
<td>2,363</td>
</tr>
<tr>
<td>T1 (15%)</td>
<td>6</td>
<td>2,230</td>
</tr>
<tr>
<td>T2 (20%)</td>
<td>6</td>
<td>2,133</td>
</tr>
<tr>
<td>T3 (25%)</td>
<td>6</td>
<td>1,463</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>2,0475</td>
</tr>
</tbody>
</table>

Based on normality test, the distributed normal data (p>0.05). The result of the statistic test with one way ANOVA test showed that granting ngor eggplant extract could reduce spermatogonia A cells numbers by meaningful (p>0.005). Decreasing of spermatogonia A cells numbers by meaningful happened by granting 20% and 25% ngor eggplant extract. While in 15% treatment doses, it did not happen to decrease by meaningful.

**Pakhiten Spermatocyte Primer**
The result of counting Spermatocyte primer pakhiten cells number in table 3.

Table 3. The average point of spermatocyte Pakhiten cells number

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>The rate of Spermatocyte Primer Pakhiten numberfissi</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 (Kontrol)</td>
<td>6</td>
<td>72,6483</td>
</tr>
<tr>
<td>T1 (15%)</td>
<td>6</td>
<td>43,0083</td>
</tr>
<tr>
<td>T2 (20%)</td>
<td>6</td>
<td>38,3767</td>
</tr>
<tr>
<td>T3 (25%)</td>
<td>6</td>
<td>28,0750</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>45,5271</td>
</tr>
</tbody>
</table>

The result of research showed that decreasing of spermatocyte primer pakhiten cells number by meaningful (p>0.05).
Spermatid 7 and 19
The results of counting spermatid cells number in table 4.

Table 4. The average point spermatid cell number

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>The Rate of Spermatid 7 and 19 Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 (Kontrol)</td>
<td>6</td>
<td>130,9817</td>
</tr>
<tr>
<td>T1 (15%)</td>
<td>6</td>
<td>97,8900</td>
</tr>
<tr>
<td>T2 (20%)</td>
<td>6</td>
<td>53,7683</td>
</tr>
<tr>
<td>T3 (25%)</td>
<td>6</td>
<td>39,2583</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>80,4746</td>
</tr>
</tbody>
</table>

Based on the normality test, it got the result of the distributed normal data (p>0,05) and Homogeneity test showed homogenous variant (p>0,0%). The result of the analysis showed that granting of ngor eggplant extract could reduce spermatid 7 and 19 cells number by meaningful (p<0,05).

Discussion
The eggplants group (Solanum sp.) is a plant that contains metabolite secondary is called solasodine. Solasodine is glycoalkaloid steroids that have basic of Cholestan C27 framework (Desai et al, 2011). Solasodine could be easier converted to 16 dihydro pregnenolone. A key intermediate in synthesis steroid drugs such as progesterone and cortisone. Solasodine obtained by the hydrolysis chemical or microbes solamargine. This is a passage that potential to be used as a substitute for diosgenin in the production of semi-synthetic of steroid hormones in pharmacy. Therefore, of steroid glycoalkaloid, night shade plant has become increasingly important as the initial material for the production of steroid hormones (Patel et al. 2013). Based on table 1, there was a decrease in the number of spermatogonia on the treatment. Yolanda's research (2011) in Rattus norvegicus adult male mice were 24 a decline in testosterone levels of blood on variations solasodin gave doses. This decline is the effect solasodine that affects testosterone in the blood. Setiati (2011) did the research on the Quality of Spermatozoa Mice (Mus musculus L.) Strains BALB/C after granting the extract of pokak ethanolic eggplant seeds (Solanum torvum Swartz). The result showed that the extra of ethanolic pokak eggplant seeds can be lowered motility and increases the number of spermatozoa abnormal, and decreases the total primary spermatocyte. A decline in blood levels of testosterone in adult male mice due to the eggplant (Solanum Khasianum) is the effect posed by solasodine that affects testosterone in the blood. Solasodine that is estrogencic this may be could inhibit of gonadotropin hormone balance by the hypothalamus and could inhibit secretion LH and FSH by the anterior pituitary. Decreasing of spermatogonia A cells numbers by meaningful happened by granting 20% and 25% ngor eggplant extract. While in 15% treatment doses, it did not happen to decrease by meaningful. It was because of nutrition supply from vascular testis system was not good. It was toxic effect by granting ngor eggplant extract. It occurs congestion that caused disorder nutrition supply by vascular tubulus seminiferus testis system. The decline in the number of spermatogonia A could be caused by this secondary metabolite that contained an extract of ethanol ngor eggplant was alkaloids and saponin. This secondary metabolite was assumed the antifertility (Aberoumand, 2012). Decreasing of cells number itself because of disorder of process of meiosis besides of disorder nutrition supply to tubulus seminiferus. The disorder of the early spermatogenesis process because of cell off spermatid to lumen tubulus and disorder food supply to tubulus seminiferus. Testosterone has a role in meiosis spermatocyte primer fission becomes secunder spermatocyte, especially for the first meiosis is a diakinesis phase and after that produce spermatid (Weinbauerer et al, 2010). Solasodin were competitives against receptors inhibitor LH and FSH. Solasodin was inhibiting the spermatogenesis and decreasing testosterone. FSH has spurred synthesis ABP (Androgen Binding Protein) on the sertoli cells.
When ABP declined and spermatogenesis will be stunted of the process. LH roles in accelerated cells Leydig to produce testosterone. When LH declined and testosterone is produced by Leydig cells declined also.

4. Conclusion
Based on the result of research could be concluded that ngor eggplant extract could reduce spermatogonium A, spermatocyte primer and spermatid 7 and 19 cells with value P<0.005.

Suggestion
It is needed to do further research to know the impact of ngor eggplant extract with the level of testosterone, LH and FSH in blood serum.

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Our deep and sincere gratitude were presented to God for having granted us the ability and the opportunity to complete this paper. As well as, We have much appreciated to our friends for their support, suggestion, contribution in finishing this research. We would like thanking Suryasa that has given me a good advisement. Last but not least, we dedicated our dreadful thank to our friend who those as editor in SS of International Journal.
References
Arsyad, K.M.1986, Possible Development of Male Contraceptives, MKI