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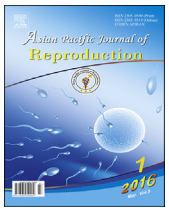

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Auto-regeneration of mice testicle seminiferous tubules due to malnutrition based on stem cells mobilization using honey

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ABSTRACT

Objective: To find out a novel therapy based on mobilization and differentiation of own body-derived stem cells using natural bee honey.

Methods: Testicle degeneration model of mouse is available through food fasting 5 days long that causes malnutrition and affects the testicle. Then administration of 30% (T1) and 50% bee honey (T2) each for five days long and compared to the positive control, fast without bee honey (T0+) and negative control, feed but without bee honey (T0−). Subsequently observation of Hematopoietic Stem Cells (HSCs) mobilization according to the CD34 and CD45 expressions performed using flowcytometry method was conducted. Besides, identification of differentiation is examined through the expression of spermatogonial stem cells (SSCs) using immunohistochemistry technique, while the seminiferous tubules cell regeneration through H&E staining microscopic examination.

Results: Stem cells mobilization based on the expression of CD34 and CD45, which is a marker of HSCs. Differentiation of stem cells into progenitor cells that expected based expression of SSCs in testicle tissue. SSCs increase causes regeneration testicle seminiferous tubules.

Conclusions: Results of this study revealed a significantly different of C34 and CD45 expressions between groups, also an increase SSCs expression and testicle seminiferous tubules cells regeneration as well.

1. Introduction

The regenerative-medicine in this decade could be a basis of many diseases treatment of the future, mainly the degenerative disease that cured through neither medical therapy nor surgery [1], especially testicle degeneration [2,3]. Testicular degeneration causes azoospermia. Azoospermia is a condition which is no spermatozoon produced by testicular seminiferous tubules, and makes infertility in the male and it means no offspring [3].

Testicular degeneration is the main cause of infertility in the male, the etiology varies such as genetic alteration, mechanical trauma, neoplastic changes and aging or senility. Current stem cell therapy usually is through stem cell transplantation which is cultured *in vitro* previously. This is obviously very expensive. Therefore, it is needed a novel therapy based on mobilization and differentiation of own body-derived stem cells using natural bee honey.

Rapidly mobilized stem cells in an adequate number towards defected tissue (testis), in turn will differentiate to be certain cells (seminiferous tubules) from a certain tissue (testis) and will replace the damaged and apoptotic cells due to degenerative diseases, in this case, the differentiation becoming seminiferous tubules, sertoli and interstitial cells of Leydig.

It is important to find out an innovation of therapy through auto-regeneration-induced of seminiferous tubule cells using beneficial of natural bee honey in reproducing of spermatozoa.

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Regeneration of seminiferous tubule and subsequently sertoli cells will provide support, nutrients and other environmental factors for young spermatozoa and male behavior to allow spermatogenesis and the ability of conception to happen.

Natural bee honey, a nutrient source from bee [4,5] has antibacterial and antioxidant potencies [5–7]. Antioxidant is an important substance in protecting individual against free radicals. An adequate antioxidant consumption can reduce the prevalence of cancers, cardiovascular disease, cataract, digestive tract disorder and other degenerative diseases [8] and in present study, the testicular degeneration. Bee honey consumption will improve the digestive system, has a good result in diarrhea therapy [5] and reproductive system disorder as well [9]. As conceptual solution, it needs further study to explore the beneficial of natural bee honey to induce auto-regeneration of testicular seminiferous tubule as a source of spermatozoa-producing tissue.

2. Material and methods

2.1. Testicular degeneration model and treatment

Testicular degeneration model of male mice is available through food fasting 5 days long, but drinking water is still administered [10,11]. The experiment animals used in this study are healthy 8–10 week-old Balb/C male mice and 20–25 g bodyweight each. They are placed in an individual plastic cage in Experimental Animal Laboratory at Veterinary Medicine Faculty, Airlangga University. They are divided into 4 groups of 8 mice each:

1. Negative control group (T0−): feed but without bee honey; 2. Positive control group (T0+): fast but without bee honey; 3. Trial group 1 (T1): fast with 30% bee honey for 5 days; 4. Trial group 2 (T2): fast with 50% bee honey for 5 days.

2.2. Flowcytometry observation of hscs mobilization based on expressions of CD34 and CD45

After the treatment, whole blood collected from cardiac puncture placed in heparin tube to prevent coagulate. Flowcytometry observation reveals the expressions of CD34 and CD45.

Flowcytometry method begins with whole blood centrifugation in 4 °C temperature, 6000 rpm for 15 min. Cellular precipitation as a result of centrifugation then mixed with cytoperm/cytofix in amount of 2 times of obtained cell number. This mixture then centrifuged again and formed supernatant and pellet. BD wash added to the pellet in the amount of 4 times of obtained cell number from the first centrifugation. Centrifuge the mixture then added lysis buffer in the amount of 2 times of the first obtained cell number. Subsequently labeled antibody added to each sample, five tubes are arranged and processed parallel. (1) Single staining with CD34 PE added to the *wash tube*. (2) Double staining with CD34 PE and CD45 PerCP. (3) Double staining with CD34 PE and CD45 PerCP and CD105 FITC-*trucount tube*. All the samples were then stored in 4 °C and dark room and were analyzed with flowcytometry for 1 h [12].

2.3. Immunohistochemical (IHC) technique observation of SSCs

Immunohistochemical observation was performed to determine the expression of SSCs. Before to IHC methods were made histological preparation, by way of an incision is made transversely testicular tissue from paraffin blocks. Further examination through immunohistochemical technique using a monoclonal antibodies (monoclonal antibodies SSCs) was conducted. This is done to determine the expression of SSCs. Observations of SSCs were made with a 40× microscope objective and the expression of each variable is indicated by the number of cells with brownish chromogen discoloration in each incision [13].

2.4. Histopathology observation of seminiferous tubules

Regeneration identification of seminiferous tubule cells of the testes through histopathological examination begins with the making of histological preparations. Histological preparations such as the following: mice testicular fixation in 10% formalin, 1 h later injected formalin 10% in mid-testis. Subsequently mice testes dehydrated in alcohol solution with a higher concentration gradually, *i.e.* from 70%, 80%, 90%, 96% (absolute). Then do the clearing in the testes of mice in xylol solution or chloroform or benzene. Furthermore performed embedding using liquid paraffin and mice testes were put into molds containing liquid paraffin. Before stained and sectioning performed, an incision using a microtome and mounted on glass objects. Furthermore the staining was done by removing of paraffin with xylol then put into a solution of alcohol with decreased concentration and then put it into H&E staining procedure. The last stage after stained is done mounting, put into water or alcohol to remove excess stain. Then it was put into a solution of alcohol with increasing concentration, and then put into xylol. Preparations then covered with a cover glass and mounted with Canada balsam or Entellan [14].

Histopathological examination is done using a light microscope with a magnification of 400 times. Observations and identification of seminiferous tubules regeneration is based on the existing histological description.

2.5. Statistical analysis

Expressions of CD34 and CD45 and SSCs were statistically analyzed using SPSS 15 for Windows XP with the level of significance 0.01 (P = 0.01) and the confidence level 99% (α = 0.01). Steps of comparative hypothesis testing are as follows: Test data normality with the Kolmogorov Smirnov test, homogeneity of variance test, Analysis of variance (ANOVA) factorial, Post hoc test (Least Significant Difference test) using the Tukey HSD 5%.

3. Results

Data collected from 32 male mice were divided into 4 trials: negative control group (T0−) is normal testis without bee honey; positive control group (T0+) is degenerative testis without bee honey; (T1) group is degenerative testis +30% bee honey in drinking water for 5 days; (T2) group is degenerative testis +50% bee honey in drinking water for 5 days. In detail, the results of the study are as follows: The effectively of bee honey was based on: 1. Mobilization of HSCs, 2. SSCs formation, 3. Regeneration of testicular tissue (such as: intact of seminiferous tubule tissue).