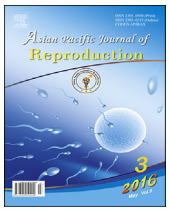
Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net



Original research http://dx.doi.org/10.1016/j.apjr.2016.04.008

Effects of honey to mobilize endogenous stem cells in efforts intestinal and ovarian tissue regeneration in rats with protein energy malnutrition

R. Heru Prasetyo[1*], Erma Safitri[2]

[1]*Departement of Parasitology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia*

[2]*Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia*

ARTICLE INFO

Article history:
Received 8 Sep 2015
Received in revised form 9 Dec 2015
Accepted 14 Jan 2016
Available online 16 Apr 2016

Keywords:
Honey
Malnutrition
Stem cells
Intestine
Ovary

ABSTRACT

Objective: Auto-regeneration of the intestinal and ovarian tissue are experiencing degenerative due to protein energy malnutrition (PEM) through of auto-mobilization, increase of immune response and differentiation of endogenous stem cells.
Methods: Female rat model of PEM obtained through fasting meals for 5 d and only to drink, causing malnutrition and damage of the intestinal and ovarian tissue. Furthermore, the therapy of honey with a dose of 30% water (T1) and 50% water (T2), respectively for 5 d and compared with the positive control, were fasted without being given honey (T0+) and negative control, not fasted and without being given honey (T0−). Observations on: auto-mobilization, increased immune response, differentiation of stem cells, and regeneration of the intestinal and ovarian tissue with HE staining.
Results: Auto-mobilization of stem cells based on the expression of CD34+ and CD45+, which is a marker of endogenous stem cells (hematopoietic stem cells/HSCs). Increased immune response is based on Hsp70 expression and PGE2 in intestinal tissue. Differentiation of stem cells into progenitor cells that expected based expression of growth differentiation factor-9 (GDF-9) by immunohistochemistry in ovarian tissue.
Conclusions: Expression of CD34+ and CD45+, which significantly different in treatment 2 (2). Furthermore, increase of immune response (decrease Hsp70 expression and increased PGE2) in intestinal tissue. Increased immune response causes expression of GDF-9 in ovarian tissue. Decreased of Hsp70 expression, increased PGE2 and increased GDF-9 followed the process of regeneration of the intestinal and ovarian tissue.

1. Introduction

Protein energy malnutrition (PEM) has become one of the causes of immune deficiency. Immune deficiency conditions PEM cause a decrease in the number of immune cells such as B-IgA+, population of T-CD5+, CD4+ cells, CD8α+, CD8β+, TCRαβ+, and TCRγδ in the lamina propria and intraepithelial villi intestine [1]. PEM can cause macrophage dysfunction [2]. Besides, the condition of protein energy malnutrition is the most common cause of secondary immune deficiency [3], thus opening incidence of opportunistic infections of intestinal parasites such as *Cryptosporidium* [4–9]. In the United States and Western Europe, the prevalence of cryptosporidiosis in patients with immune deficiency was about 10%–20%, and in the developing countries of Africa and Latin America it reached 50% [4].

Until now, malnutrition is still a health problem in Indonesia. The prevalence of protein energy malnutrition in Indonesia has shown an increase since 2000. Death of nutrient deficiency in children is more than 50% due to protein energy malnutrition, and the cause of death of nutrient deficiency in children increased mortality due to diarrheal diseases. The result of the Direktorat Bina Gizi Masyarakat Ministry of Health, East Java was included in the category of ten provinces with the highest protein energy malnutrition cases in 2005. In 2009, East Java occupied the top position of national cases of severe malnutrition. This year, the number of PEM patients under 5 years old in East Java reached 77 500, the figure reached 2.5% out of the 3.1 million. Even the number of nutrient deficiency of children under 5 years is 527 000 children, or 17% of the total children

*Corresponding author: R. Heru Prasetyo, Departement of Parasitology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia.
E-mail: rheru_prasetyo@yahoo.co.id

Peer review under responsibility of Hainan Medical College.
Foundation project: The study was supported by funding from the Directorate General of Higher Education (DIKTI) 2015.

under five year which is much higher [10]. In trial animals who have reached the age of puberty, protein energy malnutrition caused of the degeneration of the testes and ovaries cases so that the animals become infertile [11].

Interest in stem cell therapy today and the next few decades greatly increased sharply. This is because the potential of stem cell is very promising to be used as a treatment of various diseases. Stem cell transplantation provide new hope in the treatment of various diseases including immune deficiency diseases and infertility due degenerative conditions of the gonads can not be cured through treatment and operative measures [12–15].

However, due to the complexity of the method of isolation, culture *in vitro* and transplant process with the high cost of transplantation of stem cells, it would require an innovation in an effort to auto-mobilize and increase immune response is accompanied by differentiation of endogenous stem cells without going through the transplant process. Auto-mobilization and increased immune response accompanied differentiation was achieved through the provision of food or beverages derived from natural materials [14].

In this research through the provision of honey, it is expected there will be an auto-mobilization and increased immune response and differentiation of the patient's with degeneration of intestine and ovary [16]. The presence of auto-mobilization and increased immune response and differentiation of stem cells is accompanied sourced from the body itself will take place regeneration of lamina propria and epithelial intestinal villi and follicle and corpus luteum of the ovary.

The regeneration can be proven both histopathological and molecular. The histopathologically will occur regeneration of intestinal and ovarian tissue. Molecularly proven through several expressions such as expression of CD34+ and CD45+ of hematopoietic stem cells (HSCs), Hsp70 and PGE2 intestinal tissue and growth differentiation factor-9 (GDF-9) of the ovary [17].

2. Material and methods

2.1. PEM modeling causes intestinal and ovarian degeneration

This study begins by modeling PEM causes the intestine and ovaries degeneration in female mice were fasted for 5 d without food, only water to drink every 8 h per sonde [10]. Animals used in this study were Wistar strain female rats, aged 10–12 weeks with a weight of 200–250 g, in a healthy condition characterized by active movement. Mice kept in a plastic cage space per individual in laboratory animals experiments in Veterinary Medicine Faculty Airlangga University with adequate ventilation.

2.2. Treatment

The study were divided into 4 groups, each containing 6 replicates: The control group– (T0–): rats not fasted and without honey; The control group+ (T0+): rats were fasted for 5 d and without honey; The treatment group (T1): rats were fasted for 5 d, then given a 30% (v/v) honey in the drinking water for the next 5 d; The treatment group (T2): mice were fasted for 5 d, then given a 50% (v/v) honey in the drinking water for the next 5 d.

2.3. Flowcytometric observation of HSCs mobilization based on expression of CD34+ and CD45+

After the rats were treated, further examination of whole blood as a sample is taken via cardiac puncture and inserted into the tube heparin to prevent coagulation. Further observations were done on the expression of CD34+ and CD45+ by flowcytometry.

Flowcytometric method, starting with the preparation of whole blood centrifugation in a temperature of 4 °C, with a speed of 6000 r/min for 15 min. Results centrifuging the cell in the form of sludge mixed with cytoperm/cytofix amount of 2 times the number of cells are obtained. A mixture of cells and cytoperm/cytofix centrifuged to obtain a supernatant and a pellet. BD then added to wash the pellet amount 4 times the number of cells obtained in the first centrifugation.

Furthermore add lysis buffer amounting to 2 times the amount of the initial cells were obtained. After that add labeled antibody conjugate to each sample, five tubes are prepared and processed in parallel. (1) Single staining with CD34 PE added to the wash tube. (2) Double staining with CD34 PE and CD45 PerCP and CD44 FITC wash tube. (3) Double staining with CD34 PE and CD45 PerCP trucount tube. The entire sample was then stored at 4 °C in the dark and analyzed using flowcytometry for 1 h [17].

2.4. Immunohistochemical (IHC) methods observation of HSP70, PGE2 and GDF-9

Immunohistochemical observation was performed to determine the expression of HSP70, PGE2 dan GDF-9. Before to IHC methods were made histological preparation, by way of an incision is made transversely intestine and ovarium tissue from paraffin blocks. Further examination was performed by making outward through immunohistochemical techniques using monoclonal antibodies. This is done to determine the expression of HSP70, PGE2 and GDF-9. Observations of HSP70, PGE2 and GDF-9 were made using a light microscope with a magnification of 200 times and the expression of each variable is indicated by the number of cells with brownish discoloration chromogen in each incision [18].

2.5. Histopathology anatomy observation of ovarium

Regeneration identified of intestine and ovarium tissue through histopathological examination begins with the making of histological preparations. Histological preparations such as the following: rat intestine and ovarium fixation in 10% buffer formalin. Subsequently, rat intestine and ovarium dehydrated in alcohol solution with a higher concentration, i.e., from 70%, 80%, 90%, 96% (absolute). Then do the clearing in the intestine and ovarium of rat in xylol solution or chloroform or benzene. Furthermore performed embedding using liquid paraffin and rat ovarium were put into molds containing liquid paraffin. Before stained and sectioning performed, an incision using a microtome and mounted on glass objects. Furthermore is done the staining by removing of paraffin with xylol then put into a solution of alcohol with decreased concentration and then put into stain matter. The last stage after stained is done mounting, put into water or alcohol to remove excess stain. Then put into a solution of alcohol with increasing concentration, and then put into xylol.