Effect of Lamun Leaves [Enhalus acoroides (L.f.) Royle] on Leydig Cell Morphology

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Abstract

The ageing male process lead deterioration fertilization ability that caused of Leydig cell degeneration by an accumulation of oxidative stress with decreased testosterone levels. Enhalus acoroides contains antioxidants and phytosterols which can be converted into testosterone. The aim of this study to investigate the extract of Enhalus acoroides effect on Leydig cell morphology in male mice. Male mice was divided into 8 groups: adult mice no treatment (AG1), adult mice with olive oil (AG2), adult mice with extract of Enhalus acoroides at dose 25 mgKg-1 (AG3), adult mice with extract of Enhalus acoroides at dose 50 mgKg-1 (AG4), old mice no treatment (OG1), old mice with olive oil (OG2), old mice with extract of Enhalus acoroides at dose 25 mg Kg-1 (OG3), and at dose 50 mgKg-1 (OG4). All groups were repeated for 14 days. There was a significantly increased number of old Leydig cells in old mice group and adult mice group at dose 25 mgKg-1 compared without treatment. There is no difference in the observation of adult Leydig cell degeneration.

Keywords: Enhalus acoroides, Male Aging, Leydig Cell.

A. INTRODUCTION

A man who is undergoing the ageing process occurs degenerative changes, Leydig cell particularly, reduce of 50 % steroidogenic capacity known as Testosterone Deficiency Syndrome (TDS). TDS showed a decreased quality of life and caused by male infertility [1]. Addition of exogenous testosterone such as hormone replacement therapy (HRT) will effect in testis such as testicular atrophy and infertility. Also, long term treatment of HRT causing coronary heart disease, fluid retention and prostate cancer [2]. In the ageing male, the testicular function is decline and influence of quality and quantity in sperm analysis [3]. This condition is correlated with enhancing the formation of reactive oxygen species (ROS) and a decrease in antioxidant capacity thus causing oxidative stress [4].
Enhalus acoroides is one type of lamun (seagrass) who lives immersed in the sea grows spread almost all over shallow marine waters and coastal beaches in Indonesia. Lamun is reported to contain antioxidant, phytosterol (stigmasterol and sitosterol) [5]. Phytosterol through progesterone can be converted into testosterone [6]. In developed countries seagrass is used as well as medicines, one of them to prevent many degenerative diseases and as antioxidant [5]. Wakano (2013) reported that 100% of the people in Maluku Indonesia have been used to improve the body’s resistance against degenerative diseases.

B. METHODS

Animals

This study has to get permission in writing from a health research ethics committees FKUI-RSCM number 80/UN2.F1/ETIK/2016. Twelve weeks of adult mice and fourteen months old mice [7] (mus Musculus) strain Deuch Democratic Yokohama (DDY) obtained from the Laboratory of Pathology, Bogor Agricultural University. Acclimatization has been done for 1 week. Mice were given standard food and drink ad libitum. Handling of experimental animals accordance to the commission of ethics. Total of 32 mice (mus Musculus) strain DDY male was divided into 8 groups, adult mice: AG1 (no treatment), AG2 (olive oil), AG3 (25 mgKg-1 of extract), AG4 (50 mgKg-1 of extract), old mice: OG1 (no treatment), OG2 (olive oil), OG3 (25 mgKg-1 of extract) and OG4 (50 mgKg-1 of extract). All groups were repeated for 14 days.

Chemicals

This study has to get permission in writing from the Indonesian Institute of Sciences’ Oceanographic Development Centre (LIPI, Lembaga Ilmu Pengetahuan Indonesia) number 228/IPK. 12/UM/VII/2015. Enhalus acoroides leaves obtained from Pari Island, Thousand Islands Jakarta, Indonesia. 25 mgKg-1 and 50 mgKg-1 of extract Enhalus acoroides were prepared in ethyl acetate, dissolved in 10 ml of olive oil and was administrated per oral for 14 days [8], [9]. Semen was analyzed with eosin Y (viability of sperm), George solution (concentration of sperm), Giemsa (morphological of sperm), 10% buffer formalin solution was used to testis tissue fixation and blocked with paraffin before haematoxycilin eosin (HE) dyes.

Data Analysis

Mean of the results of the normality test and subsequently test Levene One Way ANOVA parametric test for numerical data more than two unpaired groups and to see the difference in the effect of sea grass extract in mice. Followed by Least Significant Difference test (LSD) or multiple comparison test. But if it does not
qualify then the parametric test Kruskal Wallis test. If the Kruskal Wallis test results meaningful then followed by Mann Whitney test to determine which groups of mice were significantly different. Differences were considered significant when P < 0.05.

C. RESULTS AND DISCUSSION

The following is presented Leydig Cell Morphology based on the test results in table 1 below:

<table>
<thead>
<tr>
<th>Variable</th>
<th>AG1 mean ± SD</th>
<th>AG2 mean ± SD</th>
<th>AG3 mean ± SD</th>
<th>AG4 mean ± SD</th>
<th>OG1 mean ± SD</th>
<th>OG2 mean ± SD</th>
<th>OG3 mean ± SD</th>
<th>OG4 mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oval nucleated cells</td>
<td>2.2 ± 0.8</td>
<td>1.6 ± 1.0</td>
<td>1.4 ± 1.4</td>
<td>1.8 ± 2.8</td>
<td>2.8 ± 1.4</td>
<td>2.8 ± 1.5</td>
<td>2.8 ± 1.5</td>
<td>2.8 ± 1.5</td>
</tr>
<tr>
<td>Total</td>
<td>22.6 ± 20.2</td>
<td>28.8 ± 24.4</td>
<td>24.8 ± 20.3</td>
<td>28.6 ± 22.2</td>
<td>7.2 ± 7.4</td>
<td>8.4 ± 7.3</td>
<td>8.4 ± 7.3</td>
<td>8.4 ± 7.3</td>
</tr>
<tr>
<td>Immature</td>
<td>7.2 ± 4.3</td>
<td>7.25 ± 6.8</td>
<td>6.4 ± 7.3</td>
<td>8.4 ± 5.6</td>
<td>2.2 ± 1.7</td>
<td>1.5 ± 1.5</td>
<td>2.2 ± 1.5</td>
<td>2.2 ± 1.5</td>
</tr>
<tr>
<td>Adult</td>
<td>11.4 ± 6.8</td>
<td>12.0 ± 12.2</td>
<td>12.6 ± 6.3</td>
<td>9.2 ± 11.0</td>
<td>2.7 ± 1.6</td>
<td>4.1 ± 2.7</td>
<td>4.1 ± 2.7</td>
<td>4.1 ± 2.7</td>
</tr>
<tr>
<td>Old</td>
<td>4.0 ± 6.0</td>
<td>9.5 ± 6.2</td>
<td>6.8 ± 6.8</td>
<td>11.0 ± 5.6</td>
<td>0.7 ± 1.9</td>
<td>1.7 ± 1.8*</td>
<td>2.4 ± 3.2*</td>
<td>1.1 ± 3.2*</td>
</tr>
</tbody>
</table>

*Significant p < 0.05

Oval Nucleated Cells of Seminiferous Tubules Interstitial Regions

There was no significant difference in both group adult and old mice. However, the trend of increased oval nucleated cells area interstitial tubule seminiferous in old mice with a dose of 25 mgKg\(^{-1}\) although not significantly.

The number of Leydig Cells Total

There was no significant difference in both groups of adult and old mice. But there was a tendency of increase in the number of Leydig cells in old mice with a dose of 25 mgKg-1, although not significantly.

Immature Leydig Cells

There was no significant difference in both groups of adult and old mice. But the tendency of increase in the Leydig cells of immature was showed in old mice group with a dose of 25 mgKg\(^{-1}\), although not significantly.
Adults Leydig Cells

There was no significant difference between the group given the extract with the untreated group. But having the downward trend mean Leydig cells grown in old mice compared with mice without treatment.

Old Leydig Cells

There was a significantly increased number of old Leydig cells in old mice group and adult mice group at dose 25 mgKg\(^{-1}\) compared without treatment in both group (p < 0.05).

Pytochemical Test

In this study, *Enhalus acoroides* leaves was extracted with resulted phytochemical that contains saponins, tannins, alkaloids, steroids and glycosides. This is presumably due to the location of different sampling and solvent usage during different extraction will produce secondary metabolites, which are different.

It is supported by Rumiatin in 2011 stating that the leaf extract Enhalus acoroides taken from Pramuka Island Thousand Islands Jakarta Indonesia has phenolic content which is very strong in a row of the extract obtained using methanol, ethyl acetate and n-hexane. Seagrass extraction using methanol contains bioactive compounds that more than extract ethyl acetate. Bioactive components contained in the methanol extract includes flavonoids, phenols hydroquinone, steroids, tannins and saponins. Bioactive compounds contained in extracts of ethyl acetate and n-hexane extract includes flavonoids, phenols hydroquinone, steroids and triterpenoids [10].

Oval Nucleated Cells of Seminiferous Tubules Interstitial Regions

It is anticipated by the old mice decreased testosterone levels and antioxidants in the body resulting in a decrease in the number of oval nucleated cells seminiferous tubules interstitial regions. In old mice who were given the leaf extract Enhalus acoroides 25 mgKg\(^{-1}\) expected to increase testosterone levels and antioxidants in the body so that it can stimulate the development of stem Leydig cells into progenitor cells Leydig cells.

The oval nucleated cell is alleged is mesenchymal which differentiate into progenitor cells of cells Leydig. Leydig progenitor cells have a characteristic oval-shaped, dark core and are centrally located in the seminiferous tubules interstitial peritubular away, through the process of proliferation and differentiation of Leydig cells become influenced by several factors one of which is androgen [11]. In older men, physiological changes hormonal increased secretion of LH and downs of
testosterone [12]. Role of testosterone in Leydig cell differentiation and development in general, namely: to stimulate the differentiation and development of progenitor cells to become mature Leydig cells. Without the presence of testosterone, immature Leydig cell is still able to differentiate but will fail to develop into Leydig cells according to their morphological characteristics [13].

The Number of Leydig Cells Total

Presence of a significantly increased number adult Leydig cells adults in old mice with leaf extract Enhalus acoroides dose of 50 mgKg-1 compared with mice given control of olive oil as a solvent. But having a downward trend in the number of Leydig cells in old mice adults when compared with untreated mice. It is presumed granting of olive oil affects the proliferation and differentiation of Leydig cells and extract dose of 50 mgKg-1 allegedly doses can increase testosterone levels and antioxidants in the body, so the stimulus differentiation of Leydig cells early stage into the Leydig cells grown awake / can delay ageing Leydig cells.

In older men increased secretion of LH and the decline in testosterone production [12]. While testosterone plays an important role in the differentiation and development of Leydig cells, among others: stimulates the differentiation and development of progenitor cells to become Leydig cells mature, keeping the development process of cell morphology immature Leydig Leydig cells become mature, adult stimulates the Leydig cell movement into the middle of the interstitial space and inhibits differentiation of precursor cells to keep the number of adult Leydig cells remain constant [13]. Without the presence of immature Leydig cell, testosterone are still able to differentiate but will fail to develop into Leydig cells according to their morphological characteristics [14]. However, the proliferation and differentiation of Leydig cells are also affected by FSH, LH, thyroid hormones, Anti Mulierian hormone, cytokines produced by macrophages, growth factors and factors of differentiation of the Sertoli cells [11].

Old Leydig Cells

There is a significantly increased aged Leydig cell in old mice by administration leaf extract Enhalus acoroides dose of 25 mgKg-1 and a significant reduction in old mice that were given the leaf extract Enhalus acoroides dose of 50 mgKg-1. It is suspected of an extract of leaves of a dose of 50 mgKg-1 is the dose that can delay ageing Leydig cells that mature Leydig cells can be maintained in its development. In older men increased secretion of LH and decline in producing testosterone [12]. While the testosterone role of stimulating immature Leydig cell differentiation into mature Leydig cells and plays an important role in keeping immature Leydig cell morphology that is at a development stage [15].
Based on this study it can be concluded that the effect of leaf *Enhalus acoroides* extract containing saponins, tannins, alkaloids, steroids and glycosides. But there is no difference in the observation of adult Leydig cell degeneration.

**D. ACKNOWLEDGEMENTS**

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