THE EFFECT OF ADMINISTRATION OF EXTRACT FROM ARECA NUT SEEDS (ARECA CATECHU L) ON THE FSH AND LH IN PROESTRUS PHASE CYCLE BALB/C FEMALE RATS

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ABSTRACT

Objective: This research was conducted to determine the effect of areca nut seed extract on follicle stimulating hormone (FSH), Luteinizing hormone (LH) phase of proestrus Balb/c female rats.

Methods: The experimental animal models used were fifteen adult female Balb/c mice, 8 to 12 weeks old, 20-40 g weight and induced for 1 week with water extract of areca nut seed at dose 1, 2 g / 200g body weight of mice and placebo in the control group. FSH and LH levels were determined by ELISA technique.

Results: The results showed a decrease in serum FSH and LH levels in the proestrus phase in the treatment group 1 (K1) and the treatment group 2 (K2) compared to the control group (K0) after giving the areca nut extract at 1,2 g/ 200 g and control group given placebo.

Conclusion: This allows the extract of areca nut seed to give anti ovulation effect.

Keywords: Areca nut extract (Areca catechu L), follicle stimulating hormone (FSH), Luteinizing hormone (LH), proestrus

Introduction

Population control is of immense importance for individual and national

welfare. Although a variety of synthetic contraceptive agents are available, their use is associated with severe side-effects.[1] Hence, an approach was pursued to identify new antifertility agents from natural sources. The use of areca nut (Areca catechu L), as a traditional medicine, has been used widely since hundreds of years ago. It is estimated that the population of betel nut users periodically in various dosage forms reaches about 500 million people. There are about 200-600 million people in the world consume Umang areca nut during their lifetime [2]. About 10% of the world's people consume areca nut continuously [3]. The Areca catechu L is a potential source of natural antioxidant which may be helpful for the prevention or progression of diseases related to oxidative stress or may be a potent alternative to synthetic antioxidants and the nutraceuticals to extend its health benefit to the human being [4]. The areca nut contains arecoline, the active alkaloid compound [5]. In addition to arecoline, areca also contains, are cuisine, arcane, arecoline, gasoline, isoguvacine, and choline [6]. The effect of areca nut is mainly caused by its active ingredient, arecoline [7]. The results suggest that the effects of arecoline cause cytotoxicity in various mammalian cells, [8] have antiproliferative effects by inhibiting growth and spur cell apoptosis [9] in accordance with the results of Sinha and Rao (1985) research that arecoline as the main seed alkaloids areca can cause germ cell-cell DNA damage [10]. This allows the extract of areca nut seed to give anti ovulation effect [12]. The anovulatory cycle is a menstrual cycle characterized by varying levels of menstrual intervals and the absence of ovulation and luteal phase. Without ovulation, there will be infertility. However, some studies have not been able to answer whether the areca nut extract affects the reproductive endocrine system. This study was conducted to determine the effect of exposure of areca nut seed extract to FSH and LH levels of proestrus phase in female Balb/c mice.

MATERIALS AND METHODS

The experimental procedure was performed at the Laboratory of Molecular Biology and Immunology, Faculty of Medicine, Hasanuddin University Makassar Indonesia. This study is an experimental in vivo post-design study conducted in the period from December 2016 to April 2017. The experiment was approved by PT Committee on Health and Medical Research Ethics Faculty of Medicine, Hasanuddin University

Makassar Indonesia (Number: 1625/H4.8.4.5.31/PP36- KOM E ICT/2016).

Materials for the Freshwater Fraction of areca nut Beans (Areca catechu L).

The areca nut is used for the areca nut which has yellow all. This type of area is known to contain lower arecoline levels than young areca nut. The areca nut selected in good condition, fresh, not rotten or moldy.

How to manufacture water fraction of areca nut extract as follows:

Water fraction of areca seed extract as follows: Peel peeled, crushed and mashed by using a hammer. Further weighed as much as 100 g and 200 mL added water, heated until the remaining 100 mL of areca nut seed extract so that every 1 mL contains 2 g of areca nut seed extract [11]

Animal Experiments and Treatment

The experimental animals used in this study were fifteen Balb/c female rats (8-12 weeks, 20-40 g), divided into 3 groups, each group consists of 5 mice. Mice were adapted for 1 week in the laboratory and fed standard. Mice were stuck at normal ambient temperature with 12 hours and 12-hour curfew cycles and feed and drinking in ad libitum (acidified water). Provision of pinang seed extract is done by direct feeding into the stomach by using a modified 5 ml syringe. Determination of dosage of pinang seed extract according to research result Akmal M., et al. (2010) [13]. Group: K0 = given placebo, K1 = given grape seed extract 1 g / 200 g body weight of mice, K2 = grated 2 g/200 g body weight of mice mole seed extract, each given for 7 days.

How to take blood

Blood intake in the vein of the tail section of 0.1 mL using a microhematocrit syringe. The taking time for serum blood serum Balb/c is performed when the estrous cycle is at the proestrus stage. Determination of Proestrus stage is done by examination of vaginal cytology. Blood is collected and centrifuged to obtain serum. The Serum is stored in a sterile tube and stored refrigerated temperature of -20°C. Examination of FSH and LH levels using Elisa Kit.

How vaginal cytology:

An examination of the vaginal review is done to examine the epithelial cell image in the vagina of the mice so as to determine the proestrus stage of the estrous cycle. The vaginal cells are collected by using a pipette filled with 0.2 mL physiological salt and inserted into the vagina of the mice and in suction again (2-3 x). The vaginal fluid is transferred to a dry glass slide by dripping and making 2 cultures in one slide. The

slides are dried with Bunsen and air fire, then stained with Giemsa fluid within 45 seconds. Slides were rinsed with physiological fluids, overlaid with a coverslip and viewed directly on 400 di magnification under bright field illumination. The colored vaginal preparation is determined by the estrous cycle stage by identification of epithelial cell morphology. Proestrus stage is determined by in group of cells are round, nucleated epithelial cells. [14] Vaginal review is done every day from 09.00 - 10.00 With for 3 x normal cycle (15 days). The vaginal cytology at the start of day 1 (first) after treatment intervention fraction of betel nut finish.

Statistical Analysis: The data obtained was tested the normality with *Kolmogorov-Smirnov test* and with homogeneity test. The test results show that all data is normally distributed and homogeneous. The data were then tested by using parametric analysis, ie T-test in pairs (Pair T-Test). To see the level of FSH, and LH conducted ANOVA test.

RESULTS

TABLE 1. FSH LEVELS AFTER TREATMENT

Variables	Group	Mean	SD
FSH levels	K0	50.259	2,705
	K1	26.238	3,748
	K2	9,511	3.927

divided into 3 groups, each group consists of 5 mice: K0: 5 mice, K1: 5 mice, K2: 5 mice

Analysis

From the table above can be in the know that the treatment group 1 (K1) decreased levels of FSH as much as 1.92 times after a given fraction betel nut 1 g / 200 g body weight of mice for 7 days and the treatment group 2 (K2) decreased levels of FSH 5.28 times (2 g / 200 g body weight of mice for 7 days) compared with the control group (placebo).

The results of statistical analysis show the existence a significant effect (P = 0.000) based on variations in dose to the decline in FSH levels after a given fraction of betel nut with a dose of 1 g / 200 g body weight of mice (K1) and a dose of 2 g / 200 g body weight of mice (K2) for 7 days compared to the control group (K0) which is given placebo.

TABLE 2. LH LEVELS AFTER TREATMENT

Variables	Group	Mean	SD
LH levels	K0	17.015	0.960
	K1	8.756	1.310
	K2	2.992	1.353

divided into 3 groups, each group consists of 5 mice: K0: 5 mice, K1: 5 mice, K2: 5 mice

Analysis

From the above table it can be seen that in the treatment group 1 (K1) there was a decrease of LH level 1.94 times after giving of pinang seed 1g / 200 g body weight of mice for 7 days and treatment group 2 (K2) was decreased LH 5,69 times (fraction of pin nut 2 g / 200 g body weight of mice for 7 days) compared to the control group (placebo). The result of statistical analysis showed that there was a significant influence (P = 0.000) based on dose variation on decrease of LH concentration after graded pinang extract with dosage 1g / 200 g body weight of mice (K1) and 2 g / 200 g body weight of mice (K2) dose for 7 days compared control group (K0) given placebo.

DISCUSSION

Decrease FSH and LH levels of experimental animals in the proestrus phase occurred after administration of pinang seed extract with a dose of 1, 2 g / 200 g body weight of mice for 7 days. The decrease of FSH and LH levels in treatment group 1 (K1) occurred 1- 2 times and 4-5 times in treatment group 2 (K2) in comparison with control group (K0) given placebo. The decrease of FSH and LH levels of proestrus phase in treatment group 1 (K1) and treatment group 2 (K2) was due to the activity of alkaloid substance of areca nut, arecoline. The results of this study differ from those of Calogero et al (1989) and Shyi-Wu et al (2008) which shows that cholinergic agonists arecoline stimulates the hypothalamus-pituitary-adrenal (HPA) in mice and arecoline does not alter GnRH-induced LH secretion in vitro[15,16]. The results presented by Gal Arnon, et. al. (2014) and Spotnitz et al.,(1999); Smith et al., (1975) showed that the concentrations of FSH and LH tended to be higher during proestrus and low first cycle of estrus. [17,18,19] This indicates that the areca seed extract provides an antiovulatory effect through its effect on the decrease in FSH and LH levels in the proestrus phase.

The results of Shrestha J, Tara Shanbhag, Smita Shenoy, et al (2010) found that the ethanolic extract of Areca catechu (betel nut) has antiovulatory and abortifacient effects [20], this adds to our belief that areca nut can be an alternative new antifertility of natural materials for women [21] Ovulation

occurs from the beginning of the proestrus to the end of estrus [22,23]. Ovulation occurs after stimulation of FSH and LH in the secretion of anterior pituitary cells into the ovaries because the FSH receptor is expressed only in ovarian granulosa cells [24]. Maximum FSH is the best predictor of the FSH-based ovarian reserve. (Max FSH is the best FSH-based predictor of ovarian reserve) [25]. Synthesis and secretion of FSH and LH influenced by GnRH. The synthesis and secretion of LH and FSH are regulated either positively or negatively by steroids and gonads of peptides [26]. FSH and LH function to stimulate growth and development of ovarian follicles and formation of hormone-producing corpus luteum in the ovaries after ovulation and regulate estrogen and progesterone hormones [27]. Decreased levels of serum FSH and LH proestrus phase after the administration of the host pinang seed extract (Areca catechu L) in animals caused by arecoline content contained in the betel nut causes hypoglycemic, hypolipidemic and the cytotoxicity me Macau apoptotic germ cells [8,9,28]. The germ cells of the reproductive apparatus are called oogonium. Oogonium undergoes meiosis repeatedly and forms oocytes. Arekolin was able to increase p53 expression as well as increase the expression of p21WAF1 encoding gene [29]. Protein p53 is a major mediator of cellular apoptosis and growth retention after exposure to agents that damage DNA. Supplementation of the areca nut extract significantly decreased the absorption of triglycerides and plasma lipid concentrations [30]. Absorption of free cholesterol in the intestine and small intestinal please activity was significantly decreased as a nutmeg extract supplement [31]. Cholesterol is used for the synthesis of steroids by ovarian tissue. Most species specifically use LDL cholesterol as a precursor of ovarian steroid synthesis. A positive relationship between HDL content of apolipoprotein E and the importance of HDL cholesterol as a precursor for steroidogenesis. Cholesterol used for the synthesis of steroids by ovarian tissue can be derived from the synthesis of cellular uptake of lipoprotein cholesterol [33] In addition, the arecoline found in areca catechu has glycemic hypoactivity and becomes alloxanized [32], has a potent α-glucosidase for inhibitors and is effective in rat glucose elevation enzyme [33]. Changes in the use of glucose by the steroid-sensitive tissue reproductive tract may underlie the viability of reproductive capacity [34]. Insulin is the key that opens the door of a tissue cell, puts the sugar in the cell and

closes the door back. Insulin is the production of androgen of the ovaries [35]. There was a significant correlation between basal levels of insulin plasma and androstenedione found between plasma insulin response regions. The study shows that hyperandrogenism with hyperinsulinism [36]. In this study can not yet as certain which pathway of FSH and LH are disturbed due to the provision of water extract of areca nut seeds. This certainly opens a wider opportunity to study the effects of betel nut on the process of ovulation at the molecular level, in an effort to unfold the potential of betel nut as an antifertility candidate in females. It is understood that the areca seed water extract is potential to be used as an antifertility candidate in females. This is based on its activity in lowering FSH and LH levels of the proestrus phase, potentially affecting the pituitary gland in releasing FSH and LH to stimulate the ovaries. Cell-type changes during the estrous cycle are indicative of the endocrine event in mice [37]. During the proestrus, the vaginal pap contains many epithelial cells and multiple epithelial leucocytes [38,39,40]. In this study, the previous epithelial epithelial cells in the treatment group 1 (K1) and the treatment group 2 (K2) were smaller than the core epithelial cells of the control group (K0) after grated areca nut seed extract at 1g / 200 g body weight of mice (K1) and dose 2 g / 200 g body weight of mice (K2) for 7 days .The results of this study also prove decreased levels of FSH and LH affect the size of proestrus epithelial cells.

CONCLUSION

Provision of betel nut extract for 7 days with a dose of 1 g and 2 g / 200 g body weight of mice affect the decrease in levels of FSH and LH in the blood in the proestrus phase and potent water seed extract used as an antifertility candidate in females. Treatment based on synthetic/allopathic drugs is effective in the prevention and treatment of diseases, but such a type of treatment is expensive and adverse effects. The use of natural products in the prevention of diseases and treatment in the developing countries, due to their affordability and fewer side effects [41]

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AUTHOR CONTRIBUTIONS

Concept and design: TRA, RN, AWS, MNM; Drafting the manuscript: TRA, MH, EK.; Final approval of the version to be published: TRA, MH, EK: All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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