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The Effect of Administration of Extract from Areca Nut Seeds (Areca Catechu L) on the Estradiol and Estrus Cycle Balb/C Female Rats

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Abstract. Population control is very important for individual and national welfare. Although various synthetic contraceptive agents are available, their use is associated with severe side effects. Therefore, an approach is taken to identify new antifertility agents from natural sources. This study was conducted to determine the effect of areca nut extract on estradiol levels and the estrus cycle of female balb / c mice. The experimental animal models used were fifteen adult female balb / c mice, aged 8-12 weeks, weight 20-40 g and induced for 1 week with water extract of areca nut with a dose of 1 g / 200 g body weight of mice (K1), 2 g / 200 g of body weight of mice (K2) and distilled water in the control group (K0). The estrus cycle was identified by a daily assessment of the relative ratio of nucleated epithelial cells, cornified squamous epithelial cells and leukocytes found at the time of vaginal swabs for 15 days continuously. At the proestrus stage blood is taken from the tail. Serum estradiol levels were determined by ELISA technique. There was a decrease in serum estradiol levels in the treatment group 1 (K1) 1.9 times compared to the control group (K0) and decreased estradiol levels in the treatment group 2 (K2) 5.29 times compared to the control group (K0). Changes in the duration of the estrus cycle occurred in treatment group 1 (K1) to be elongated and treatment group 2 (K2) became non-estrus, while the control group (K0) did not change the duration of the estrus cycle (normal). The decrease in serum estradiol levels affects the estrus cycle of mice (P = 0,000). The administration of areca nut extract has the effect of reducing serum estradiol levels and disrupting the duration of the estrous cycle of female balb / c mice. This finding has important implications for the development of contraception in women.

1. Introduction

The explosion in population caused a number of adverse effects, such as environmental damage, global warming, famine, and disease development [1]. In 2025 there are estimated numbers in

Indonesia the population reached 273.65 million [2]. Overcoming this problem has been many modern contraceptive methods are used, but users of modern contraceptive methods worry about side effects that can affect their daily lives [3]. The development of science in the processing of natural ingredients has experienced a rapid increase. Nearly 80% of the world's population depends on traditional medicines for primary health care, most of which involve the use of plant extracts. [4]. Herbal preparations have also been used since time immemorial for their effects on reproductive health in particular to suppress fertility, regulate the menstrual cycle, eliminate dysmenorrhea, treat prostate enlargement, menopausal symptoms, breast pain and pain during and after childbirth [5]. Wrong one ingredient that is widely studied is betel nut (Areca catechu) [6] The main component contained in the areca nut is Arecoline [7]. There are four alkaloids playing in areca nut, namely arecoline (7.5 mg / g), arecaidine (1.5 mg / g) guvacoline (2.0 mg / g) and guvacine (2.9 mg /g) [8]. Giving areca can cause cytotoxicity [9], apoptosis [10], hepatoprotective, [11] hypoglycemic, [12] astringent, vermifugal, sialogogue, [13] antibacterial, antioxidant, antiseptic, bronchostimulant, euphorient, and wound healing properties. [14] In addition, it has abortifacient, anti-implantation and antifertility activities [15]. Research has revealed that betel nut causes morphological changes such as hormonogenesis stimulation and spermatogenesis disorders. [16] This certainly opens wider opportunities to study the effects of areca nut on estradiol levels and the estrus cycle of female mice in an effort to unmask the potential of areca nut as a candidate for antifertility in women.

2. Research Methods

The experimental procedure was carried out at the Laboratory of Molecular Biology and Immunology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. This research is an in vivo post-design experimental research conducted in the period from December 2016 to April 2017. The experiment was approved by the PT Health and Health Research Ethics Committee Faculty of Medicine Hasanuddin University Makassar Indonesia (Number: 1625 / H4.8.4.5.31 / PP36-COMETIC / 2016).

2.1. Ingredients for Areca Palm Seeds (Arecacatechu L).

The areca nuts that are used come from areca nuts that have all yellow skin. This type of areca nut is known to contain lower levels of arecoline than young areca nut. Areca nuts are selected in good condition, fresh, not rotten or moldy.

How to make water fraction of areca nut extract. Areca nut shelled, crushed and mashed using a hammer. Then weighed as much as 100 g and added 200 mL of distilled water, heated to the remaining 100 mL of areca nut extract, so that every 1 mL contains 2 g of areca nut extract [7].

2.2. Experimental Animals and Treatment

The experimental animals used in this study were adult female balb / c mice. Age 8 - 12 weeks and weight of experimental animals 20-40 g. The chosen strain is balb / c. The total number of Balb / C mice used was 15 divided into 3 groups, each group consisted of 5 mice. Mice are adapted for 1 week in the laboratory and given standard feed. Mice are grounded at ordinary ambient temperatures with a cycle of 12 hours a day and 12 hours a night and are given food and drink ad libitum (acidified water). The administration of areca nut extract is done by force-feeding directly to the stomach using a 5 ml syringe that has been modified. Determination of the dosage of areca nut extract according to the results of the study [17] Group: K0 = given distilled water, K1 = given areca nut extract 1 g / 200g body weight of mice, K2 = given areca nut extract 2 g / 200gr body weight of mice, each given for 7 days.

2.3. How to take blood

Blood sampling in the tail vein was 0.1 mL using microhemactocrit syringe. The time for taking balb / c blood serum is done when the estrus cycle is at the proestrus stage. Proestrus stage determination is done by examining vaginal cytology. Blood is collected and centrifuged to get serum. Serum is stored

in a sterile tube and placed in a refrigerator temperature of -20 ° C. P emeriksaan Estradiol levels using Elisa Kit.

2.4. Vaginal cytology method

Examination of vaginal reviews is done to examine the epithelial cell image in the vagina of the mouse so that it can determine each stage of the estrus cycle. Vaginal cells were collected using a pipette filled with 0.2 ml of physiological salt water and inserted into the vagina of the mouse and sucked back (2-3 x). Vaginal fluid is transferred to a dry glass slide by dripping and making 2 cultures in one slide. The slides were dried with bunsen fire and air, then stained with giemsa liquid in 45 seconds. The slide is rinsed with physiological fluid, covered with coverslip, and viewed directly at 400 magnification under bright field lighting. Identification of each stage of the estrus cycle from the daily assessment of the relative ratio of nucleated epithelial cells, cornified squamous epithelial cells and leukocytes found at the time of vaginal swab. The proestrus stage is determined by finding a group of round cells, nucleated epithelial cells [18]. Ulas vagina is carried out every day from 9:00 a.m. to 10:00 a.m. for 15 days continuously. Vaginal cytology examination starting from day 1 (first) after intervention treatment of areca nut fraction was completed.

2.5. Statistical Analysis

The data obtained were tested for normality with the Kolmogorv-Smirnov test and continued with a homogeneity test. The test results show that all data are normally distributed and homogeneous. The data was then tested using parametric analysis, namely paired T test (Pair T - Test). To see the estradiol levels, Anova was tested

3. Result and Discussion

Research on the effect of giving Pinang fruit seed extract (Areca catechu. L) to estradiol (E2) and estrous cycles in In Vitro results obtained as follows

Table 1. Effect of areca nut extract on estradiol levels

Variable	Group	Mean	Elementary school	Sig
Estradiol levels	K0 (n = 5)	626,691	31,833	.000
	K1 (n = 5)	329,421	46,469	
	K2 (n = 5)	118,475	49,764	

Value is expressed in the mean, SD = Std. Deviation, sig = significant, n = 5 each group; P = 0.000 compared to control

Analysis

In this study there was a decrease in serum estradiol (E2) levels in the treatment group 1 (K1) 1.9 times compared to the control group (K0) and decreased estradiol levels in the treatment group 2 (K2) 5.29 times compared to the control group (K0) after being given areca nut extract for 7 days. Giving betel extract has an effect on decreasing serum estradiol hormone levels

Table 2. Effect of Pinang seed extract on the duration of the estrus cycle

Group	Estrus cycle	Mean	Elementary school

K0 (n = 5)	Normal	626,691	31,833
K1 (n = 5)			
K2 (n = 5)	Lengthen	239,862	121,317
	Not estrus	186,815	133,363

Value is expressed in the mean, SD = Std. Deviation, n = 5 each group .

Analysis

Changes in the estrus cycle occurred in treatment group 1 (K1) to be elongated and treatment group 2 (K2) became non-estrus after giving betel seed extract for 7 days, while the control group (K0) did not experience a change in the estrus cycle. The length of observation is 15 continuously

Table 3. Effect of Estradiol hormones on the estrus cycle

		Sum of Squares	df	Mean Square	F	Sig.
E2pgpermL	Between Groups	546579,837	2		25,634	.000
	Within Groups	127933.508	12	10661.126		
	Total	674513.344	14			

n = 5 each group, sig 0.000

Analysis

Decrease in serum estradiol hormone levels has a significant effect (sig 0.000) on changes in the duration of the estrus mice cycle. where estrus status of treatment group 1 (K1) is elongated and treatment group 2 (K2) is not found in estrus phase

The results of this study indicate that after 7 days the administration of areca nut extract has a reduced effect on estradiol levels (E2) at the proestrus stage. Serum estradiol levels decrease with increasing doses of areca nut extract. Ovarian hormones are produced by various types of ovarian cells such as granulosa cells from folic el mature and corpus luteum. [19] The lack i ni hormone imbalance causing irregularity of ovarian function and the duration of the estrous cycle [20]. This study is different from that conveyed by research sebelimnya wherein at p roestrus phase serum estradiol concentration is higher than all other estrus phase [21]. Decreased serum estradiol levels after administration of the host p seed extract (arecacatechu L.) in animals caused by arecoline content contained in the betel nut causes hypoglycemic, hipolipidnemia and the onset of cytotoxicity that spur apoptosis of germ cells [22]-[24]. Supplementation of arecanut extract significantly reduced triglyceride absorption and plasma lipid concentration [25]. Absorption of free cholesterol in the intestine and small intestine pCEase activity is significantly reduced when supplementing areca nut extract [26]. Cholesterol is a precursor for steroidogenesis of ovarian endocrine tissues, estrogens, progestins, and androgens. [27]. Most species specifically use LDL cholesterol as a precursor to ovarian steroid synthesis. A da positive relationship between HDL content of apolipoprotein E and the importance of HDL cholesterol as a precursor for steroidogenesis. Cholesterol used for steroid synthesis by ovarian tissue can originate from cellular uptake of lipoprotein cholesterol [25]. In addition arecoline found in Areca catechu has hypoglycemic activity and becomes alloxanize, [12], has potent α-glucosidase for inhibitors and is effective in suppressing rat blood glucose elevation [28]. Changes in the use of glucose by steroid-sensitive reproductive tract tissues can affect the disruption of reproductive ability, [29]. Insulin is the key that opens the door to tissue cells, inserts sugar in the cell and closes the door again. Insulin stimulates ovarian androgen production, [30] There was a significant correlation between basal levels of plasma insulin and androstenedione found between regions of plasma insulin response. The results of the study show that hydrogenation is correlated with hyperinsulinism [31]. Cell type changes during the estrus cycle are indicative of endocrine events in mice [18] Histologic

changes and cytology of vaginal mucosa depend on sex steroid hormones. The main hormone that induces changes in the vaginal mucosa is estradiol [32]. During proestrus, vaginal papules contain many nucleated epithelial cells and several leukocytes [33]. In this study hormone levels decreased significantly (0.000 sig) to estrous mouse cycles, where the duration of the estrus cycle treatment group 1 (K1) became elongated (> 5 days) and treatment group 2 (K2) was not found estrus phase (no experiencing estrus stage) while the duration of the control group is normal (4-5 days). This condition occurs because during the observation of 15 consecutive days found leukocytes that dominated the field of view (diestrus) which lasted more than 4 days in the treatment group. If the majority of cells are leukocytes, then it is labeled as a diestrus phase. [34] Previous studies that revealed the estrous cycle in mice lasted for 5 days [35]. Each phase in the cycle is determined based on the shape of the epithelial cell there is a vaginal cytology observation. Diestrus is the longest stage that lasts more than 2 days [36]. In the diestrus phase the vaginal contents are consistently deficient in cornified cells and leukocytes predominate in the smear. The frequency of cornified epithelial cells is reduced and nucleated epithelial cells begin to be detected only before the transition to proestrus [33]. The estrus cycle consists of 4 phases, namely proestrus, estrus, metestrus and diestrus [36]. This proves that the decreased estradiol level after giving areca nut extract results in a change in the duration of the estrus cycle. Lengthening of the diestrus phase indicates the absence of mature graafian follicles or nonsecondary follicular maturation. Estrogen positive feedback that stimulates de novo neuroprogesterone synthesis to trigger important luteinizing surge hormone for ovulation [37]. At the stage of diestrus, the serum estradiol concentration is lower than proestrus [21]. In this study, the pathway from estradiol cannot be ascertained due to the administration of areca nut water extract

4. Conclusion

The parameters in this study indicate that areca nut extract (A. Catechu) has a reduced effect on estradiol levels and disruption in the duration of the estrus cycle. Serum estradiol levels decrease with increasing doses of areca nut extract. Decreased estradiol levels are associated with changes in the duration of the estrus cycle. This finding has important implications for the development of natural contraception in women

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