

7

by LPPM STIKesMI

Submission date: 02-Apr-2023 10:23PM (UTC-0700)

Submission ID: 2054317777

File name: Journal_FSH,_LH.pdf (135.5K)

Word count: 4006

Character count: 20841

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

TETTY RINA ARITONANG¹, ROSDIANA NATZIR², ANDI WARDIHAN SINRANG³, MUH NASRUM MASSI⁴, MOCHAMMAD HATTA⁴, EMMA KAMELIA⁵

¹Midwifery Program of Medistra Health Higher School 17114. Jakarta, Indonesia.

²Department of Biochemistry, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia. ³Department of Physiology, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia.

⁴Molecular Biology and Immunology Laboratory for Infections Diseases, Faculty of Medicine, Hasanuddin University, Makassar 90245. ⁵Dental Health Study Program, Tasikmalaya Health Polytechnic 46196. West Java, Indonesia.

*Email : tetty.rina.2109@gmail.com

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.³⁴ 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].²⁰ 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.² 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.

² 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 x 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 antioviulatory 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 antioviulatory 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. Arecolin 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 1 g / 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]

ACKNOWLEDGMENT

The authors would like to appreciate Mr.Romi Usman, Mr.Mus, and Marwani in the Molecular Microbiology and Immunology Laboratory, Medical Faculty, University of Hasanuddin, Makassar, Indonesia, who helped our research

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

Reference

1. Vaidya P, Padmashali S, Vagdevi HM, Sathyanarayana ND. Antifertility effect of the plant *Balanites roxburghii* (Balanitaceae) in female rats. *Indian J Pharm Sci.* 2006;3:347–51.
2. Giri S, Jeffrey RI, Chi-Chen T, Mark Z, Kristopher WK, and Gonzalez FJ. A metabolomic approach to the metabolism of the areca nut alkaloids Arecoline and aracaidine in the mouse. *J. Chem Res Toxicol.* 2006.19 (6): 818-827 .
3. Sahelian, RMD. Betel nut: Information on chewing areca catechu betel nut. 2004. <http://www.raysahelian.com/betelnut.html>.
4. Abdul H, Saumen K and Tapan KC. A Comparitive study of in-vitro antioxidant activity of different extracts of areca seed collected from areca catechu plant grown in assam. *Int J Pharm Pharm Sci, Vol 4, Issue 2, 420-427, 2012*
5. Agusta, A. Look out for the hazards of plants drug. *Phytochemical Laboratory, PuslitbangBiologi-LIPI, Bogor . 2001 / Feb / danger% 20tumb% 20obat.htm.* <http://www.indonesia.com/intisari/>
6. Anonymous. Pinang. 2001. <http://www.pnm.my/sirihpinang/sp-pinang.htm> .
7. Sundqvist, H., Y. Liu, J. Nair, CH Bartsch, H. Arvidson, and RC Grafstrom. Cytotoxic and genotoxic effects of areca nut-related compounds in cultured human buccal epithelial cells. *Cancer Res.* 1989. 49: 5294-5298.
8. Jeng JH, Chang MC, and Hahn LJ. Role of areca nut in betel quid-associated chemical carcinogenesis: Current awareness and future perspectives. *Oral. Oncol. Pathol. Med , 2001; 28: 64-71.*
9. Meiyanto E. et al. Ethanolic Extract of Pinang Fruit Beans (Areca catechu L.) able to inhibit proliferation and spur apoptosis of MCF-7 cells.

- Pharmaceutical Magazine Indonesia, 2008.19 (1), 12 - 19
10. Sinha, A. and AR Rao. Induction of shape abnormality and unscheduled DNA synthesis by arecoline in the germ cells of mice. *Mutat. Res.* 1985.158: 189-192.
 11. Circosta C, Sanogo R. Occhiutoprocera on estrous cycle and on estrogenic functionality in rats. *Farmaco.* 2001; 56: 373-8. [PubMed]
 12. Aulanni'am ,Akmal, M, Rosmaidar. Effect of Anfertility of Water Fungus of Areca Catechu As Agent of Apoptosis on Testis Cell Network of Rattusnorvegicus . *Media Journal of Veterinary Medicine.* 2007. 23 (3): 179-183
 13. Akmal M, Chanif Mahdi, Aulanni'am . Increased Testosterone Concentrations in Mice Due to Exposure to Pinang Bean Extract, University of Syiah Kuala, Banda Aceh, *Journal Veterinary* December 2010, Vol. 11 No. 4: 244-250 .
 14. M cLean , AC Nicolas Valenzuela, Stephen Fai, Steffany AL Bennett. Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification. 2012. URL: <http://www.jove.com/video/4389> . DOI: doi: 10.3791 / 4389
 15. Calogero AE, Kamilaris TC, Gomez MT, Johnson EO, Tartaglia ME, Gold PW, Chrousos GP. The muscarinic cholinergic agonist arecoline stimulates the rat hypothalamic-pituitary-adrenal axis through a centrallymediatedcorticotropin-releasing hormone-dependent mechanism. *Endocrinology* 1989.125: 2445-2453,
 16. Shyi-Wu Wang, Guey-Shyang Hwang, Te-Jung Chen, and Paulus S. Wang . Effects of arecoline on testosterone release in rats . Submitted 23 January 2008; accepted in final form 16 June 2008
 17. Gal Arnon, Lin Po-Ching, Barger Anne M, MacNeill Amy L, and KoCheMyong, Vaginal fold histology reduces the variability introduced by vaginal exfoliative cytology in the classification of mouse estrous cycle stages Published in final edited form as: *ToxicolPathol.* 42 (8): 1212-1220, 2014 (doi: 10.1177 / 0192623314526321)
 18. Spornit , UM, Socin and AA Dravid CD. Estrous Stage Determination in Rats by Means of Scanning Electron

- Microscopic Images of Uterine Surface Epithelium. *The Anatomical Record*, 254: 116-126, 1999
19. Smith S, Freeman ME & Neil JD, The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. 1975. *Endocrinology*, 96: 219-226.
 20. Shrestha J, Tara Shanbhag, Smita Shenoy, et al. Antioviulatory and abortifacient effects of Areca catechu (betel nut) in female rats. *Indian J Pharmacol*. 2010 Oct; 42(5): 306–311. doi: 10.4103/0253-7613.70350
 21. Ansari A.S., Nirmal Kumar Lohiya, Irshad Mohammad Mewati. Plants for Female Fertility Regulation: A Review. Article in *Journal of Pharmacology and Toxicology* · March 2017
DOI: 10.3923/jpt.2017.57.75
 22. Young WC, Boring JL & Landau R., 1941, The vaginal smear picture, sexual receptivity and time of ovulation in the albino rat. *Anat. Rec.*, 80 : 37-45.
22
 23. Schwartz NB, Acute effects of ovariectomy on pituitary LH, uterine weight, and vaginal cornification. *Am. J. Physiol.* 1964. 107: 1251-1259.
 24. Patsoula E. et al. 2001. Expression of mRNA for the LH and FSH receptors in mouse oocytes and preimplantation embryos *Reproduction* (2001) 121, 455-461
 25. Julian A, Gingold, Joseph A, Lee, Michael, Whitehouse, Jorge Rodriguez-Purata, Benjamin Sandler, Lawrence Grunfeld, Tanmoy Mukherjee and Alan B Copperman. Maximum basal FSH predicts reproductive outcome better than cycle-specific basal FSH levels: waiting for a "better" month conveys limited retrieval benefits. *Reproductive Biology and Endocrinology* 13:91, 2015
 26. Brown P and McNeilly AS
Transcriptional regulation of pituitary gonadotrophin subunit genes. University of Edinburgh, Edinburgh EH3 9EW, UK: *Reviews of Reproduction*. 1999 . 4, 117-124
 27. Thackray VG., Fox Tales: Regulation of Gonadotropin Gene Expression by Forkhead Transcription Factors. *Mol Cell Endocrinol*. 2014 March 25; 385

- (0): ³⁵ 62-70. (doi: 10.1016 / ¹⁷ j.mce.2013.09.034.)
28. ⁸ Sinha, A. and AR Rao. Induction of shape abnormality and unscheduled DNA synthesis by arecoline in the germ cells of mice. 1985. *Mutat. Res.* 158: 189-192.
 29. Chou WW, Guh JY, Tsai JF, Hwang CC, Chiou SJ, Chuang LY. Arecoline-induced phosphorylated p53 and p21WAF1 protein expression is dependent on ATM / ATR and phosphatidylinositol-3-kinase in clone-9 cells. *Journal of Cellular Biochemistry* 2009; 107 (3): 408-417
 30. ⁶ ByunSJ , Kim HS, Jeon SM, Park YB and MS Choi: Supplementation of Areca catechu L. extract alters triglyceride absorption and cholesterol metabolism in rats. *Ann NutrMetab.* 2001; 45 (6): 279-84.
 31. ² YB Park, SM Jeon, SJ Byun, HS Kim and MS Choi: Abnormal intestinal cholesterol absorption was reduced by supplementation of Areca catechu L. extract in mice. *Life Sci.* 2002 Mar 8; 70 (16): 1849-59.
 32. ⁶ B Chempakam: Hypoglycemic activity of arecoline in betel nut Areca catechu L., *Ind. J of Exp. Biol;* 1993, 31 (5), 474-475.
 33. ⁶ M SenthilAmudhan, and V Hazeena Begum: Protective effect of Areca catechu extract on ethanol-induced gastric mucosa of lesions in online *Pharmacology rat* 2008; 1: 97-106
 34. David R Garris, Douglas L Coleman and Carl R Morgan. 1985. Age- and Diabetes-related Changes in Tissue Glucose Uptake and Estradiol Accumulation in the C57BL / KsJMouse . *Diabetes* 1985 Jan; 34 (1): 47-52.
<https://doi.org/10.2337/diab.34.1.47>
 35. Callum LIVINGSTONE, Mary COLLISON. Sex steroids and insulin resistance . *Clinical Science* Feb 01, 2002, 102 (2) 151-166; DOI: 10.1042 / cs1020151
 36. George A. BurghenJames R. GivensAbbas E. Kitabchi Correlation of Hyperandrogenism with Hyperinsulinism in Polycystic Ovarian Disease *Endocrinology & Metabolism*, Volume 50, Issue 1, 1 January 1980, Pages 113-116,
<https://doi.org/10.1210/jcem-50-1-113>
 37. ³ Ashleigh C. McLean, Nicolas Valenzuela, Stephen Fai, Steffany AL Bennett1, Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse

- Estrous Cycle Staging Identification, 67
(389): 1 - 6, 2012
38. Marcondes, FK, FJ Bianchi, & AP Tanno. Determination of the estrous cycle phase of rats: some helpful considerations. *Journal Brazilian Archives of Biology and Technology*. 2002.4A: 600-614.
 39. Martins RR, Pereira NML, Silva TMA. Liquid-base cytology: a new method for oestral cycle study in Wistar's rats. *Acta Cirúrgica Brasileira / Sociedade Brasileira de Desenvolvimento Pesquisa em Cirurgia*. 20 (1): 78-81, 2005 [PubMed: 16186975]
 40. Montes GS, Luque EH. Effects of ovarian steroids on vaginal smears in the rat. *Acta Anatomica*. 133: 192-19, 1988. [PubMed: 3227778]
 41. Shatha H.Ali, Ali M.A.Al-Nuaimi, Bushra J. Al-Musawi. Serum irisin and leptin levels in obese and non-obese women with polycystic ovary syndrome with reference to glucose homeostasis. *Int J Pharm Pharm Sci*, Vol 8, Issue 10, 276-283, 2016

ORIGINALITY REPORT

60%

SIMILARITY INDEX

55%

INTERNET SOURCES

51%

PUBLICATIONS

17%

STUDENT PAPERS

PRIMARY SOURCES

1	T Rina Aritonang, R Natzir, A Wardihan Sinrang, M Nasrum Massi, M Hatta, Kamelia. "The Effect of Administration of Extract from Areca Nut Seeds (Areca Catechu L) on the Estradiol and Estrus Cycle Balb/C Female Rats", Journal of Physics: Conference Series, 2020 Publication	14%
2	www.researchgate.net Internet Source	13%
3	www.gssrr.org Internet Source	7%
4	www.ncbi.nlm.nih.gov Internet Source	3%
5	innovareacademics.in Internet Source	2%
6	ijpsr.com Internet Source	2%
7	Yin-Ju Chen. "Head and neck cancer in the betel quid chewing area: recent advances in	1%

molecular carcinogenesis", Cancer Science,

08/2008

Publication

8	indonesianjpharm.farmasi.ugm.ac.id Internet Source	1 %
9	clinical-and-molecular-endocrinology.imedpub.com Internet Source	1 %
10	www.innovareacademics.in Internet Source	1 %
11	www.pubmedcentral.nih.gov Internet Source	1 %
12	gssrr.org Internet Source	1 %
13	repositorio.unicamp.br Internet Source	1 %
14	ijpt.iums.ac.ir Internet Source	1 %
15	journals.lww.com Internet Source	1 %
16	rbej.biomedcentral.com Internet Source	1 %
17	shodhgangotri.inflibnet.ac.in Internet Source	1 %

www.forsyth.org

18	Internet Source	1 %
19	academic.oup.com Internet Source	1 %
20	en.wikipedia.org Internet Source	1 %
21	f1000research.com Internet Source	1 %
22	onlinelibrary.wiley.com Internet Source	1 %
23	ajpendo.physiology.org Internet Source	<1 %
24	core.ac.uk Internet Source	<1 %
25	Moslem Heydari pur, Mohammad Mehdi Bahri, Ebrahim Sheykhi. "Evaluation of Burn Injuries and Epidemiological Factors in Patients Hospitalized in Shohada Mehrab Hospital in Yazd-Iran 2019", Journal of Community Health Research, 2021 Publication	<1 %
26	Nataša Nestorović. "Somatostatin-14 influences pituitary-ovarian axis in peripubertal rats", Histochemistry and Cell Biology, 10/2008 Publication	<1 %

27	profiles.ucsd.edu Internet Source	<1 %
28	Submitted to International School Yangon Student Paper	<1 %
29	iopscience.iop.org Internet Source	<1 %
30	mail.scialert.net Internet Source	<1 %
31	www.scielo.br Internet Source	<1 %
32	Ajesh Kumar A, S.S. Syed Abuthahir, Hassan Y. Aboul-Enein. "Phytochemical extraction and comparative analysis of antioxidant activities of Areca catechu L. nut extracts", Pharmacia, 2022 Publication	<1 %
33	E Patsoula. "Expression of mRNA for the LH and FSH receptors in mouse oocytes and preimplantation embryos", Reproduction, 03/01/2001 Publication	<1 %
34	coek.info Internet Source	<1 %
35	www.frontiersin.org Internet Source	<1 %

36

Submitted to Ateneo de Manila University

Student Paper

<1 %

37

verizonaonlinepublishing.com

Internet Source

<1 %

38

Abdullah Ansari, Tarique Mahmood,
Paramdeep Bagga, Farogh Ahsan, Arshiya
Shamim, Shoaib Ahmad, Mohammad Shariq,
Saba Parveen. " : A phytopharmacological
legwork ", Food Frontiers, 2021

Publication

<1 %

39

Linlin Wu, Qian Lei, Zirong Gao, Wei Zhang.
"Research Progress on Phenotypic
Classification of Acute Respiratory Distress
Syndrome: A Narrative Review", International
Journal of General Medicine, 2022

Publication

<1 %

40

iajps.com

Internet Source

<1 %

41

ir.unilag.edu.ng

Internet Source

<1 %

42

journal.unair.ac.id

Internet Source

<1 %

43

journals.sagepub.com

Internet Source

<1 %

44

orbi.uliege.be

Internet Source

<1 %

45

qspace.library.queensu.ca

Internet Source

<1 %

46

www.ajol.info

Internet Source

<1 %

47

www.nature.com

Internet Source

<1 %

48

Saha, I.. "Arecoline augments cellular proliferation in the prostate gland of male Wistar rats", *Toxicology and Applied Pharmacology*, 20110901

Publication

<1 %

49

Giri, Sarbani, Jeffrey R. Idle, Chi Chen, T. Mark Zabriskie, Kristopher W. Krausz, and Frank J. Gonzalez. "A Metabolomic Approach to the Metabolism of the Areca Nut Alkaloids Arecoline and Arecaidine in the Mouse", *Chemical Research in Toxicology*, 2006.

Publication

<1 %

50

Kadi, Adnan, Mohamed Attwa, and A.F.M. Rahman. "A preliminary study of arecoline and guvacoline presence in the saliva of a "betel-quist" chewer using liquid-chromatography ion trap mass spectrometry", *European Journal of Mass Spectrometry*, 2013.

Publication

<1 %

Exclude quotes Off

Exclude matches Off

Exclude bibliography Off

7

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12
