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Dari: "Mochamad Lazuardi" <ardiunair@gmail.com>
Kepada: "editor@univmed.org" <editor@univmed.org>
Cc: "Prof Mochammad Lazuardi" <lazuardi@fkh.unair.ac.id>, "bambang_h@fk.unair.ac.id" <bambang_h@fk.unair.ac.id>, "Affaveti" <ardiunair@yahoo.co.uk>
Terkirim: Min, 23 Feb 2014 pada 19:55
Judul: Re: Vancouver

Yth. Prof Adi, bersama ini saya kirimkan kembali revisi dari editor Univmed (lihat attachment).

Selanjutnya untuk re-view dari Prof. Eni dan Prof mae apa sudah ada hasilnya ?

Hormat Lazuarfi

Pada 18 Februari 2014 22.24, Mochamad Lazuardi <ardiunair@gmail.com> menulis:
Terima kasih akan saya revisi kembali

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Pada 16 Februari 2014 18.32, <editor@univmed.org> menulis:

Pada guidelines for authors (advice for authors) di http://www.univmed.org jelas Universa Medicina menggunakan sistem Vancouver. Silahkan dibaca kembali, misalkan 6 authors baru et al.nama jurnal disingkat, nomor halaman yang sama tidak diulang. dll. Upayakan total references sekitar 20 an dan mutakhir (10 tahun twerakhir) dari jurnal dan bukan buku, terima kasih.
AMA mirip tetapi ada sedikit perbedaan.

Salam,

Adi

On 16.02.2014 09:04, Mochamad Lazuardi wrote:
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[1].

Salam,

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Area lampiran

Mochamad Lazuardi 21 Jul 2021 18.40 (2 hari yang lalu)

---------- Forwarded message --------- Dari: Mochamad Lazuardi <lazuardi@fkh.unair.ac.id> Date: Rab, 21 Jul 2021 pukul 18.38 Subject: Fwd: Re: Vancouver To: Bay

Mochamad Lazuardi 21 Jul 2021 18.42 (2 hari yang lalu)

kepada **Affaveti**, Bayu, saya

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Kiriman pertama

Determinationof FSHProgesterone and influenced to oestrouscycles on ratsat orally Extract *Benalu Duku*

Running text:
FSH Progesterone levels and influenced to oestrous cycle on rats post orally *Benalu duku*

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ABSTRACT

BACKGROUND

Cases in human infertilities in Indonesia were known tends to increase at about 2-5 % each year's since of early 2000. The other side knew that many tropical plants in Indonesia had potential sources to make a new anti-infertility compounds (i.e*Benalu duku* or *Dendrophthoepetandra*L. Miq., grewth on *Lanciumdemesticum*). The objective of this research was to identify performance of crude extract methanol *Benalu duku* leaves on rat to induce oestrous cycle and influence their FSH progesterone perform.

METHODS

Fourteen *RattusNorvegicusWistar strain* rats were divided into two groups (namely the treatment group and the control group) and arranged to oestrous perform via pheromone synchronizing method. The rats at treatment groups were given crude extract 100 mg/kg body weight^{-1}*(s.d.d*) during the 4 days, via intra muscularly. The control groups were given 0.25 ml phosphate buffer saline intramuscularly (*s.d.d)* during the 4 days. To determine of FSH Progesterone levels, the whole blood samples were prepared by Evidence Investigators Analyzer Method.

RESULTS

The result showed that FSH at treatment groups were obtained 09.28 ±06.72 mIU/ml (lowest two times than control groups at 24.80 ±16.35 mIU/ml, $p<0.05$). Progesterone hormones at treatment groups were obtained 33.55±13.96 nmol/L (more twice than the control groups at 18.47±06.47 nmol/L, $p<0.05$). This research was conclusion that the crude methanol extract of *Benalu duku* leaves was better used to be stimulate of Progesterone production up to more level than usual level in rats, but not recommended yet to using the other fertility hormone.

Key words: Herbal medicine**,**Synchronize oestrous, Evidence Investigator Method, Cubic Cell, Vaginal Smear.

ABSTRAK

LATAR BELAKANG

Kasusketidaksuburanpadamanusia di Indonesia cenderungnaik 2-5 % setiaptahunsejaktahun 2000. Disisi lain diketahuibanyaktumbuhantropisdi Indonesia yang berpotensisebagaisumberkomponenbaru anti infertilitas (contohBenalu duku atau*Dendrophthoepetandra*L. *Miq*., tumbuh di *Lanciumdemesticum*). Tujuanpenelitianiniadalahmengidentifikasikankinerjaekstrakkasarmetanoldaun Benalu duku padatikusterhadapinduksisiklusoestrousdanpengaruhnyaterhadapkinerja FSH danProgesteron.

METODE

Sebanyakempatbelastikus (*RattusNorvegicusWistar)* strain dibagiduakelompok (kelompokperlakuandankelompokkontrol) dandiaturmenjadioestrousmelaluimetodesinkronisasiferomon. Kelompokperlakuanselanjutnyadiberiekstrakkasar 100 mg/kg beratbadan(satu kali sehari)selama 4 harimelalui intramuskular. Kelompokkontroldiberi 0,25 ml garamfosfat buffer secara intra muskular (satu kali sehari) selama 4 hari. Untukmenetapkanderajat FSH danProgesteron,sampeldarahdiprosesmenggunakanmetodeanalisa Evidence Investigator

HASIL

Hasilmenunjukkanbahwa FSH padakelompokperlakuan 09,28 ± 06,72 mIU/ml (lebihrendahdarikelompokkontrolpada 24,80 ± 16,35 mIU/ml, ($p<0,05$). HormonProgesteronpadakelompokperlakuandidapat 33,55 ± 13,96 nmol/L (dua kali lebihtinggidarikelompokkontrolpada 18,47 ± 06,47 nmol/L, $p<0,05$). PenelitianinidapatdisimpulkanbahwaekstrakkasarmetanoldaunBenalu dukubaikdigunakanuntukmendorongproduksiprogesteronsampaidengan di ataskadarlazimpadatukis. Dalampenelitianinimasihbelumdianjurkanuntukmenggunakan hormone fertilitaslainnyasebagaipenghambatataupeningkat hormone pada tikus.

Kata kunci: Tanamanobat, penyeragamanbirahi, Metoda Evidence Investigator, selkubus, ulas vaginal

INTRODUCTION

Follicle stimulating hormone (FSH) and progesteroneis part of fertility hormone in the human female and important for control cycle of menstruation period. The fertility hormones can not only test for pregnancy in woman but are also very useful in the diagnosis of other conditions such as the onset of menopause and gonadal dysfunction. In men, fertility hormones can be accurate indicators of condition such as liver cirrhosis and testicular cancer.[1]The fertility hormones can be produced from herbal medicine as follows; *Jatropha*

curcas producing testosterone[2], *Dioscorea macrostachya* producing *diosgenine*(intermediate product of cortisone)and anti-estrogenic activity.[3]

Benalu duku or*Dendrophthoepetandra (L.)Miq* grew on *Lansiumdomestic* was known herbal medicine since 1990 and still explored as a local and systemic anticancer agent.[4] As a parasite trees, *Benalu duku* was known have a beneficial compound as follows; anti myeloma cell and antibiotics.[5,6] Some researcher reported that Benalu duku have a some beneficial compounds (i.e. essential amino acid and alkaloid, flavonoid, polyphenol, terpenoide, free steroid) as referred to Lazuardi*et al*.[4] The last the other report described that Family of *Dendrophthoe spec*., were probably have an immune hormone prevention substances for protected internal free radical caused external stimulant substances.[7]But other species of *Dendrophthoe* familly, esspecially *Petandra L. Miq* grew in *Lansium domesticum*(*Benalu duku*) for treated cycle menstruation dissorders wasnot reported yet.

By research background as described at above, we were tried to explore the effect of FSH and Progesterone levels as a part of steroid hormones after giving crude extract *Benalu duku* leaves on healthy adult female rat. The objectives of these research was to obtained influence of crude extract methanol compound of *Benalu duku* leaves on fertility hormone level in female rat subject during the oestrous period.

METHODS

Research design

This study was true experiment design with posttest only control group model by animal model as a subject research. This research was use indoor clinical trial laboratory on Veterinary Pharmacy Subdivision Department of Basic Science, Faculty of Veterinary Medicine Airlangga University. The study was conducted from July to October 2013.

Sample size

The sample size were calculate according to Rumke's tablewith assumption 100% successful after giving crude extract methanol *Benalu duku* leaves but 40% failed without consumption crude extract methanol Benalu duku leaves.[4,8]

Herbal medicine

Benalu duku leaves (*Dendrophthoepetandra L., Miq* growth in *Lanciumdomesticum*). was collected from natural habitats, Palembang District, South of Sumatera, and checked for authenticate by Mrs. Yayah, Research Center For Biology - Cibinong Science Center (CSC), JL. Raya Jakarta - Bogor Km.46 Cibinong 16911 Bogor –Indonesia, Phone: +62-21-87907604/87907636 Fax: +62-21-87907612, e-mail: biologi@mail.lipi.go.id. The fresh materials of *Benalu duku* leaves were separated shade dried and powdered using the electric homogenizer. The powdered samples at 450 g were extracted with 2 L of methanol pro analysis grade for 72 h by using rotating percolation method. The crude extract methanol of *Benalu duku* leaves were dried by warm dried method with nitrogen gas as a stimulate gas to reduced methanol levels. The crude extract of methanol were prepared to injection dose perform as follows; free from pyrogenic agents, sterile perform and stable at iso-tonic, iso-ionic, iso-hydricperform on ranging pH 7.2-7.5. The last prepare of crude extract methanol *Benalu duku* leaves were filtrated at 0.20 µm and keep on disposable peek sterile vials at 4 ^{0}C.

Experimental animals

Fourteen healthy adult female rats at ranging 3-4 month years old *(Rattusnorwegicuswistar strain)* were obtained fromRachmadPriyadi DVM at, Trosobo, Sidoarjo. The rats were grouping in two groups design as control group and treatment groups. The two groups were marking as follows; $K_3$0; K_3KA; K_3KI; K_3KK; K_4KI; K_4KK (control groups)andP_3KA; P_3KI; P_3KK; $P_3$0; $P_4$0; P_4KA; P_4KI (treatment groups).The mean of $K_3$0 is number code of sample i.e K_3 = Third cages, 0 = no ear marker. The K_3KA as a K_3

is a third cage and KA = ear marker in right side, KI = ear marker in left side and KK ear marker in right and left. Code of P_3 and P_4 at treatment groups are code of cages (third cage and fouth cage) and KA, KI, KK code of ear marker.

Synchronized oestrous

The all rats were synchronizing oestrouscycle by whitten effect or pheromone effect techniqueduring the two period cycle's oestrous at ranging 10 days as follows; fifteen female rats at two groups were cages on two level cages. The upper level cages were filled by male rats and the other level cages (bottom level) filled female rats.[8,9]The rats were examined by Giemsa staining method at magnified 1000x to find the cubic cells and cornification cells from vaginal smear test during the research period.[10]

Clinical trials

Research trials starting after all of the rats were oestrous as referred to Bambang and Lazuardi.[8]The treatment groups were treated with crude extract methanol *Benalu duku* leaves diluted on aqua pro injection (b/v) at dosing 100 mg/kg body weight during the fourdays (*semel in die*) by intramuscular injection. Control groups were giving aqua pro injection by intramuscular injection at dosing 1 ml during the four days(*semel in die*). The end treated periods, the rats were sacrified and whole blood samples collected at ranging 1.5 to 2 ml by cardiac suction after.The plasma samples were separated by centrifugation at 8000 g for 15 minutes and were stored at 2-8^{0}C until ready to analyzing concentrationsof the FSH and Progesterone.

Principle of determined FSH Progesterone

The principle of analysis levels of FSH and Progesterone are used to perform simultaneous quantitative detection of multiple analytes from a single subject sample. The core technology is the Randox Biochip, a (9 mm^2) solid substrate containing an array of discrete test region of immobilized antibodies specific to different fertility markers. A

chemiluminescent immunoassay is employed. The light signal generated from each of the test regions on the biochip is detected using a CCD camera and state-of-the-art digital imaging technology. The light signal generate from each of the test regions on the biochip is detected using digital imaging technology and compared to that from a store calibration curve. From this the concentration is calculated.[11]

Quantitative analysis of FSH Progesterone

The amount of FSH and progesterone was measured by Evidence Investigator^{TM}using the method described previously by Randox Corp (in triplo process). Briefly, One package of fertility hormone array (FERTILITY) Cat No. EV3610 were ready to use. Cat No. EV 3610 wasconsisting of six compounds i.e. (i) fertility dilution assay, (ii) fertility conjugate, (iii) fertility biochip, (iv) fertility calibrate, (v) luminace or PX, (vi) buffer washer (concentration). Pipette 150 µl of assay diluent into the appropriate biochip wells as required. Pipette 75 µl of calibrator/sample/ control into appropriate biochip wells. Gently tap all edges of the handling try to mix reagents. Secure the handling tray to the base plate of the thermoshaker. Incubate for 30 minutes at +37 ^{0}C and 350 rpm. Following incubation, remove the handling tray containing carriers from the thermoshaker. Pipette 75 µl of conjugate into the appropriate biochip wells as required. Gently tap all edges of the handling tray to mix reagents. Secure the handling tray to the base plate of the thermoshaker. Incubate for 30 minutes at +37 ^{0}C and 350 rpm. Following incubation, remove the handling tray containing carriers from the thermoshaker. Discharge reagents to waste using a sharp flicking action of the handling tray. Immediately carry out 2 quick wash cycles. Using wash bottle with diluted wash buffer (refer to kit insert for dilution), add approximately 350 µl wash buffer to each well, gently tap all edges of the handling tray to release any reagents trappedbelow the biochip, and flick to waste with a sharp action. Take care not to overfill wells during wash in order to reduce potential for well-to-well contamination. Carry out a

further 4 wash cycles; for each cycle gently tap all edges of the handling tray for approximately 10 to 15 seconds then leave the biochips to soak in wash buffer for 2 minutes. After final wash, fill wells with wash buffer and leave to soak until directly prior to imaging (no carrier should be left to soak for longer 30 minutes). Imaging process was individually sampled. Those awaiting imaging should be protected from light. Remove the first carrier to be imaged from the handling tray. Directly before addition of signal, remove wash buffer using a sharp, flicking action and tap the carrier onto lint free tissue to remove any residual wash buffer. Add 250 µl of working signal reagent to each well and cover to protect the light. Place the carrier into Evidence Investigator machine after exactly 2 minutes (±10 seconds). Use of a timer is recommended to ensure imaging occurs at the correct time. Capture of images will be automatically initiated as defined by dedicated software. All blood samples (treatment groups and control groups) were measured at triplo process by code as follows; n1, n2,n3.

Data analysis

Data calibrator was analysis by linearity program method. Analysis validation method were use reference by Evidence Investigator operator manual.Data samples were analysis by minitab statistic software 17.0 with two sample independent student t test at 95% significant levels.

Ethical clearance

The rats were handling by principles use for animal experiments under control Commission Animal Ethic Clearance from Faculty of Veterinary Medicine, Airlangga University and requirement standard handling for animal experiment from unit ethic animal experimental of Indonesia Veterinary Pharmacy and Pharmacology Association (www.affaveti.org).

RESULTS

Research result appeared that synchronize oestrous perform all rats were obtained at about 10 days. The view of cubic cell from vaginal smears test by Giemsa staining method were appeared at Figure 1and Figure2 (1000x magnify).

Calibration curve of FSH (mIU/ml) by serial consentrations 00.00; 05.65; 28.30; 44.62 were appeared linear with average squared difference of the error in the actual to the predicted values of the date (S) = 5.27620 at level correlation (R-Sq) = 95.7% and level adjective correlation orR-Sq (adj) = 93.5%. Calibration curve of Progesterone (nmol/L) in serials 000.00; 001.74; 048.22; 232.02 were obtained less than liner by average squared difference of the error in the actual to the predicted values of the date (S) = 66.0114, level correlation (R-Sq) = 76.0% and level adjective correlation or R-Sq(adj) = 64.0%. Sensitivity test fordetermined the mean low concentrations of FSH (22.49 mIU/ml and 43.70 mIU/ml) and the mean high concentrations of FSH (37.4966mIU/ml and 72.86 mIU/ml) at 36th replicates were obtained at ranging coeficient variation 10.01% to 16.68%.The sensitivty test of Progesterone at mean low concentrations (30.86 nmol/L and 138.68 nmol/L) and mean high concentration (51.42 nmol/L and 231.16 nmol/L) were obtained at ranging of CV 10.00% to 16.67%. The result research to determine of FSH Progesterone level on two groups treatment and control groupsby triplo measurement were described Table 1 at bellow.

DISCUSSION

Oestrus syncrhronyze technique using whittten effect method was appeared succesfully, result research were obtained cubic cell and cornification cell up to 10 days available all female rats at the same cycle oestrus. Figure 1 and Figure 2 is clear evidence that the findings of cubic cells and cornification cell to be an achievement condition of oestrus. Figure 1 appeared that cubic cells from vaginal smear test were pink color and grouping. Figure 2 appeared that cornification cells from vaginal smear test were blue color and separated. The time line to obtained synchronized oestrus by whitten effect method were avarage at 8 to 10

days after using the method.Our method to obtain the estrous cycle was more fastest than "Bruce effect" method as described other researcher mice.[12,13]Result research at Tabel 1 appeared that influence of analites (crude extract methanol *Benalu duku* leaves) to FSH Progesterone hormone were caused contradiction performing each other hormones.

FSH level in the treartment groups were significantly lowest third times than FSH in control groups ($p>0.05$). But Progesterone levels of the treartment groups were appeared highest twicelevels than Progesterone in control groups ($p<0.05$). These fact was indicated that analites have some unknown substances with two action pharmacodynamic as a (a) FSH suppression product and (b) Progesterone stimulant product. That "unknown substances from crude extract of *Benalu duku* leaves" were identifyed as a suppresed FSH like effect and stimulant Progesterone like effect. Some other substances of suppresed FSH like effect were known as follows; human seminal plasma compouns with 92-amino acid polypeptide andalpha-inhibin-92 (alpha-IB-92).[14]Synthetic Progesterone like effect had been known early of year 2000 as follows; Dydrogesterone, 17- α- Hydroxyprogesterone carproate, Medroxy progesterone acetat, Megestrol acetat.

The FSH suppression phenomenon was analog using concept of "receptor down regulation of Gonadothrophine Releasing Hormon (GnRH)".As down-regulation occurs, production of gonadotrophins by the pituitary i.e follicle-stimulating hormone (FSH) ceases, effectively shutting down control hormones for cyclic ovarian function in the female. This effect is well known and as early as 1989 had been suggested as a potential estrous suppression hormone for the bitch.[15]Analytes from crude extract of methanol were probably made actions to occupy GnRH receptors at the pituitary and after a short period of stimulation cause the cells to reduce or stop the synthesis of the receptor protein, making the cells insensitive to GnRH.[15]The Progesterone will be increasing after the anterior pituitary

give them “a calling signal” with impact to the corpus luteum for inhibit the development of Graafian folicles in the ovary. The relationship beetwen the inhibit of FSH inhibition and increased of Progesteronewere not directly but other fertility hormon influence i.e., Luteinizing hormone (LH), Estrogen hormone may be have a role important to make closely of their relationship.[16]

Hormones are generally administered to patient for one of three purposes. First, when an patients fail to produce sufficient quantities of hormones, therapy is directed at correcting the deficiency. Second, when no deficiency exists, hormones are used to obtain a desired effect. For example, synthetic progesterone, used as a birth control agent, may be administered to normal bitches.Third, when the products of fertility hormones more ecsessive then usual, therapy is directtly to the target with treatment via other antagonist hormones to inhibit mechanismtarget for dismiss the excessive product.Result reseach probably indicate that analytes are stimulating to corpus luteum for production of Progesterone more twice than usual and of course will be make a feedback mechanism to inhibit FSH product via coding of GnRH to inhibit FSH as referred to analogy of Konishi *et al*.[17]Our study may be not clearly explain, how is the mechanism of analytes to make action causing low level of FSH and encourage excessive product of Progesterone after giving crude methanol extract of *Benalu duku* leaves. But may be by advance explore of Progesterone like effect of crude extract methanol *Benalu duku* leaves can be find the mechanism action of that analytes.As an incompletely of our study we are not study enzyme expression from encode sub units proteins of base-paired microsomal DNA as “a messenger control” to inhibit FSH and stimulate Progesterone that possibilities can be answer mechanism action of analytes.

In woman with fertility cases, the unique phenomena of crude extract methanol *Benalu duku* leaves to inhibit FSH and stimulate Progesterone can be use to treat pregnant woman with low Progesterone levels condition esspecially in early pregnancy with miscarriage risk.

In early woman pregnancy, abdominal pain and vaginal bleeding may be signs of a miscarriage, but by consumption of crude extract methanol of *Benalu duku* leaves probably will be help to make better the Progesteronelevel. Some natural herbal or plants had been produce as a stimulate of Progesterone hormone in ealy of pregnant as follows; oils in yam and soy plants, *Curcuma comosa* esspecially diaryl-heptanoid compounds, wastewater plants (WWTPs) of Beijing, China., alkaloid leaves of *Digitalis lanata* as known 5-β-cardenolide.[17,18,19]As explained by other researcher i.e.,Gupta and Kachhawa that some alkaloids of *Dendrophthoe* family was usefully to threat cases steroid disorder in animal experimental.[20]Other researchers even mentionedthat *Demdrophthoe* species was potential as components for muscle relaxation.[21] But for future direction, by next research explore of alkaloid *Benalu duku* leaves as a parts of *Dendrophthoe* familly, probably we are find a new compounds to treatment the cases of low level Progesterone concentration at early of pregnant or as a maintenance uterus condition during the non-fertile.

CONCLUSSION AND RECOMMENDED

The crude extract methanol of *Benalu duku* leaves was potential for using stimulate agent to produce the Progesterone hormone on healthy adult female rats. The crude extract methanol of *Benalu duku* leaves was suitable for suppressor agents to produce FSH hormone on healthy adult female rats. Result research was recommend to explored the compound of crude extract methanol *Benalu duku* leaves with effect stimulate Progesterone hormone on next research advances. Other fertility hormone i.e., Prolactine, Testosterone, Oestrogen, Lutinize hormone were not recommended yet to use treated other cases of steroide cases in healthy female rats.

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REFERENCES

1. ShalenderBhasin, Glenn R. Cunningham, Frances J. Hayes, Alvin M. Matsumoto, Peter J. Snyder, et al. The Endocrine Society's Clinical Guidelines: Testosterone Therapy in Adult Men with Androgen Deficiency Syndromes: An Endocrine Society Clinical Practice Guideline. J ClinEndocrinolMetab 2010; 95(6): 2536–59.

2. Onyeka CA1, Aligwekwe AU1, Olawuyi TS, Nwakanma AA, Kalu EC, Oyeyemi AW. Antifertility Effects of Ethanolic Root Bark Extract of *Chrysophyllumalbidum* in Male Albino Rats. Int J Appl Res Nat Prod 2012; 5 (1): 12-17.

3. Pathak AK, Mallurwar VR, Kondalkar AK, Soni S. A review of plants with anti-fertility activity. Nig J Nat Prod Med 2005;9: 4-10.

4. Lazuardi M, Nuraini F, Ratna SM, Roostantia I. Screening of *benalu duku* for antiproliferation of myeloma. In :Puji S, et al. Editors. Strategies for the control prevention of zoonotic diseases. Surabaya: Airlangga University Press. 2010. p 65-68.

5. Lazuardi M. The overview of *Benalu duku* (*Dendrophthoe spec.,*) as a parasites herbal medicine with potential for treatment Trypanosomiasis. In: Endang E, et al., Editors. Abstract and Programs PertemuanIlmiahTahunanNasional I PerhimpunanFarmasiKedokteran Indonesia (PEFARDI) 7-8 Mei 2011. Bandung: Bag. FarmasiKedokteranUniversitasMaranatha. 2011. p 15-20.

6. Zaimah I. Skriningfitokimiaekstrak herbal *Benalu duku* (*Dendrophthoepetandra L. Miq.*,)danujiaktivasiterhadap larva udang (*Artemiasalina leach*) denganmetode BSLT (Brine Shrimp Lethality Test) [Skripsi]. FakultasSainsdanTeknologi (FST): UniversitasJenderalSoedirman Solo; 2011.

7. Nina A, Taufik F, Akhmad D. Bioactivities Evaluation of Indonesian Mistletoes (*Dendrophthoepentandra (L.) Miq*.) Leaves Extracts. J Appl Pharm Sci 02 (01); 2012: 24-27.

8. Bambang H and Lazuardi M. Assessment of luteinizing testosterone prolactin estrogen hormone after giving crude methanol extract of *Benalu duku* leaves on healthy adult female at. In: Lazuardi and Rinidar. Editors. Proceeding 2nd MusyawarahNasional Indonesia Veterinary Pharmacy and Pharmacology Association 21-22 of September 2013. Surabaya: Airlangga University Press. 2014. (In press).

9. Anne Fawcett. Guidelines for the housing of mice in scientific institutions. NSW: Animal Welfare Unit, NSW Department of Primary Industries, Locked Bag 21, Orange NSW. April 2012. p 112-114.

10. Saadat P, Latiffah AL, Sabariah AR, Mohammad AD, Hanachi P. Assessing estrogenic activity of Nigella sativa in ovariectomized rats using vaginal cornification assay. African J PharmPharmacol 2011; 5(2): 137-142.

11. RandoxLaboratorium Ltd. Fertility Hormone Array (FERT) [Brochure]. 55 Diamond Road. Crumlin, County Antrim UK: (Revised) May 20th 2013: 1-16.

12. Izuchukwu SO, Chike FO, Chinaza CO. The effect of increasing number of strange male mice on bruce effect. Veterinarskhi Arhiv 2012; 82 (1): 103-114.

13. Cornelius PCP. Study of effect of sweet (*Ocimum basillicum Linn*) essential oil of various dosages on estrus cycle of sparque dawley rats. J Sains Vet 2012; 30 (2): 87-96.

14. Yu WH, McCann SM, Li CH. Synthetic human seminal alpha-inhibin-92 selectively suppresses follicle-stimulating hormone release in vivo. Proc Natl Acad Sci USA 1988; 85(1): 289–292.

15. Briggs Joice. Alliance for contraceptic in cat and dog.USA: ACC& D Press; 2013. p 13-14.

16. Otto JR, Freeman MJ, Malau-Aduli BS, Nichols PD, Lane PA, Malau-Aduli EO.Reproduction and Fertility Parameters of Dairy Cows Supplemented with Omega-3 Fatty Acid-rich Canola Oil. Ann Res & Rev in Biol 2014; 4(10): 1611-1636.

17. Konishi S1, Brindle E, Guyton A, O'Connor KA. Salivary concentration of progesterone and cortisol significantly differs across individuals after correcting for blood hormone values.Am J Phys Anthropol 2012;149 (2):231-41.

18. Chang H1, Wan Y, Wu S, Fan Z, Hu J. Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: comparison to estrogens. Water Res 2011;45(2):732-40.

19. Ernst M1, de Padua RM, Herl V, Müller-Uri F, Kreis W.Expression of 3beta-HSD and P5betaR, genes respectively coding for Delta5-3beta-hydroxysteroid dehydrogenase and progesterone 5beta-reductase, in leaves and cell cultures of *Digitalis lanata* EHRH.Planta Med. 2010; 76(9):923-7.

20. Gupta RS1, Kachhawa JB.Evaluation of contraceptive activity of methanol extract of *Dendrophthoe falcata* stem in male albino rats.J Ethnopharmacol 2007; 30;112(1):215-8.

21. Pooja S, Irchhaiya R, Bhawna S, Gayatri S, Santosh K. Anticonvulsant and muscle relaxant activity of the ethanolic extract of stems of *Dendrophthoe falcata (Linn. F*.) in mice. Indian J Pharmacol 2011; 43(6): 710–713.

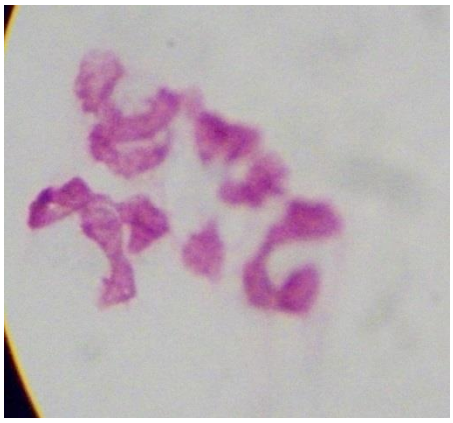

Figure 1. Cubic cell from vaginal smear test (Giemsa staining, 1000x)

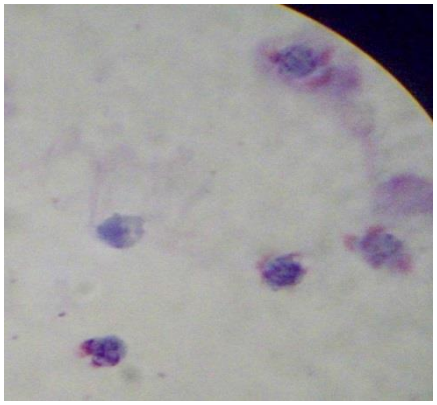

Figure 2. Cornification cells from vaginal smear test (Giemsa staining, 1000x)

Table 1. Research result of FSH Progesterone on treatment groups and control groups.

Sample code, body weight (g)	Treatment groups							
	FSH (mIU/ml)				Progesterone (nmol/L)			
	(n1)	(n2)	(n3)	Mean ± SD	(n1)	(n2)	(n3)	Mean ± SD
P_3KA (166 g)	00.67	49.14	01.12	16.98^{a}±27.85	71.88	11.88	54.35	46.04^{c}±30.85
P_3KI (120 g)	00.38	01.93	11.91	04.74^{a}±06.26	82.27	15.53	17.88	38.56^{c}±37.87
P_3KK (167 g)	01.68	15.74	26.19	14.54^{a}±12.30	37.37	0.99	10.75	16.30^{c}±18.86
$P_3$0 (177 g)	25.91	06.39	-	16.15^{a}±13.80	17.95	6.12	-	12.03^{c}±08.36
$P_4$0 (196 g)	02.28	01.81	-	02.04^{a}±00.33	38.47	32.85	-	35.66^{c}±03.97
P_4KA (132 g)	16.68	01.87	-	09.27^{a}±10.47	-	38.77	-	38.77^{c}±00.00
P_4KI (111 g)	02.45	00.45	00.90	01.27^{a}±01.05	18.29	63.18	61.08	47.52^{c}±25.33
Mean ± SD				09.28±06.72	Mean ± SD			33.55±13.96
Sample code, body weight (g)	Control groups							
	FSH (mIU/ml)				Progesterone (nmol/L)			
	(n1)	(n2)	(n3)	Means ± SD	(n1)	(n2)	(n3)	Means ± SD
$K_3$0 (153 g)	10.80	-	-	10.80^{b}±00.00	48.54	-	8.88	28.67^{d}±28.10
K_3KA (198 g)	07.05	25.13	13.54	15.24^{b}±9.16	5.32	17.13	17.91	13.45^{d}±05.76
K_3KI (148 g)	22.68	01.69	28.29	17.55^{b}±14.02	11.01	26.38	8.88	15.40^{d}±09.58
K_3KK (147 g)	09.42	00.42	07.37	05.74^{b}±04.72	22.33	53.08	6.68	27.36^{d}±23.61
K_4KA (170)	27.59	-	42.69	35.14^{b}±10.68	5.83	-	18.22	12.02^{d}±08.76
K_4KI (159 g)	38.71	45.76	-	42.23^{b}±04.98	-	-	-	-
K_4KK (165 g)	89.86	30.61	20.29	46.92^{b}±37.54	-	7.48	26.34	16.91^{d}±13.34
Mean ± SD				24.80±16.35	Mean ± SD			18.47±06.47

Notes:

- Blood samples lysis. Code column n1,n2,n3 is *triplo*measurement.

P_3, P_4 and K_3, K_4at treatment and control sample code is third and fourth cages.

0,KA,KI,KK at treatment and control samples code is no ear marker, right ear marker, left ear marker, right and left ear marker of the rats.

Superscript a vs., b at same column was different at $p < 0.05$ by independent sample t test

Superscript c vs., d at same column was different at $p < 0.05$ by independent sample t test

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Determinationof FSHProgesterone and influenced to oestrouscycles on ratsat orally Extract *Benalu Duku*

Running text:
FSH Progesterone levels and influenced to oestrous cycle on rats post orally *Benalu duku*

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ABSTRACT

BACKGROUND

Cases in human infertilities in Indonesia were known tends to increase at about 2-5 % each year's since of early 2000. The other side knew that many tropical plants in Indonesia had potential sources to make a new anti-infertility compounds (i.e*Benalu duku* or *Dendrophthoepetandra*L. Miq., grewth on *Lanciumdemesticum*). The objective of this research was to identify performance of crude extract methanol *Benalu duku* leaves on rat to induce oestrous cycle and influence their FSH progesterone perform.

METHODS

Fourteen *RattusNorvegicusWistar strain* rats were divided into two groups (namely the treatment group and the control group) and arranged to oestrous perform via pheromone synchronizing method. The rats at treatment groups were given crude extract 100 mg/kg body weight^{-1}*(s.d.d*) during the 4 days, via intra muscularly. The control groups were given

0.25 ml phosphate buffer saline intramuscularly (*s.d.d)* during the 4 days. To determine of FSH Progesterone levels, the whole blood samples were prepared by Evidence Investigators Analyzer Method.

RESULTS

The result showed that FSH at treatment groups were obtained 09.28 ±06.72 mIU/ml (lowest two times than control groups at 24.80 ±16.35 mIU/ml, $p<0.05$). Progesterone hormones at treatment groups were obtained 33.55±13.96 nmol/L (more twice than the control groups at 18.47±06.47 nmol/L, $p<0.05$). This research was conclusion that the crude methanol extract of *Benalu duku* leaves was better used to be stimulate of Progesterone production up to more level than usual level in rats, but not recommended yet to using the other fertility hormone.

Key words: Herbal medicine**,**Synchronize oestrous, Evidence Investigator Method, Cubic Cell, Vaginal Smear.

ABSTRAK

LATAR BELAKANG

Kasusketidaksuburanpadamanusia di Indonesia cenderungnaik 2-5 % setiaptahunsejaktahun 2000. Disisi lain diketahuibanyaktumbuhantropisdi Indonesia yang berpotensisebagaisumberkomponenbaru anti infertilitas (contohBenalu duku atau*Dendrophthoepetandra*L. *Miq*., tumbuh di *Lanciumdemesticum*). Tujuanpenelitianiniadalahmengidentifikasikankinerjaekstrakkasarmetanoldaun Benalu duku padatikusterhadapinduksisiklusoestrousdanpengaruhnyaterhadapkinerja FSH danProgesteron.

METODE

Sebanyakempatbelastikus (*RattusNorvegicusWistar)* strain dibagiduakelompok (kelompokperlakuandankelompokkontrol) dandiaturmenjadioestrousmelaluimetodesinkronisasiferomon. Kelompokperlakuanselanjutnyadiberiekstrakkasar 100 mg/kg beratbadan(satu kali sehari)selama 4 harimelalui intramuskular. Kelompokkontroldiberi 0,25 ml garamfosfat buffer secara intra muskular (satu kali sehari) selama 4 hari. Untukmenetapkanderajat FSH danProgesteron,sampeldarahdiprosesmenggunakanmetodeanalisa Evidence Investigator

HASIL

Hasilmenunjukkanbahwa FSH padakelompokperlakuan 09,28 ± 06,72 mIU/ml (lebihrendahdarikelompokkontrolpada 24,80 ± 16,35 mIU/ml, ($p<0,05$). HormonProgesteronpadakelompokperlakuandidapat 33,55 ± 13,96 nmol/L (dua kali lebihtinggidarikelompokkontrolpada 18,47 ± 06,47 nmol/L, $p<0,05$). Penelitianinidapatdisimpulkanbahwaekstrakkasarmetanoldaun Benalu dukubaikdigunakanuntukmendorongproduksiprogesteronsampaidengan di ataskadarlazimpadatukis. Dalampenelitianinimasihbelumdianjurkanuntukmenggunakan hormone fertilitaslainnyasebagaipenghambatataupeningkat hormone pada tikus.

Kata kunci: Tanamanobat, penyeragamanbirahi, Metoda Evidence Investigator, selkubus, ulas vaginal

INTRODUCTION

Follicle stimulating hormone (FSH) and progesteroneis part of fertility hormone in the human female and important for control cycle of menstruation period. The fertility hormones can not only test for pregnancy in woman but are also very useful in the diagnosis of other conditions such as the onset of menopause and gonadal dysfunction. In men, fertility hormones can be accurate indicators of condition such as liver cirrhosis and testicular cancer.[1]The fertility hormones can be produced from herbal medicine as follows; *Jatropha curcas* producing testosterone[2], *Dioscorea macrostachya* producing *diosgenine*(intermediate product of cortisone)and anti-estrogenic activity.[3]

Benalu duku or*Dendrophthoepetandra (L.)Miq* grew on *Lansiumdomestic* was known herbal medicine since 1990 and still explored as a local and systemic anticancer agent.[4] As a parasite trees, *Benalu duku* was known have a beneficial compound as follows; anti myeloma cell and antibiotics.[5,6] Some researcher reported that Benalu duku have a some beneficial compounds (i.e. essential amino acid and alkaloid, flavonoid, polyphenol, terpenoide, free steroid) as referred to Lazuardi*et al*.[4] The last the other report described that Family of *Dendrophthoe spec*., were probably have an immune hormone prevention substances for protected internal free radical caused external stimulant substances.[7]But other species of *Dendrophthoe* familly, esspecially *Petandra L. Miq* grew in *Lansium domesticum*(*Benalu duku*) for treated cycle menstruation dissorders wasnot reported yet.

By research background as described at above, we were tried to explore the effect of FSH and Progesterone levels as a part of steroid hormones after giving crude extract *Benalu duku* leaves on healthy adult female rat. The objectives of these research was to obtained influence of crude extract methanol compound of *Benalu duku* leaves on fertility hormone level in female rat subject during the oestrous period.

METHODS

Research design

This study was true experiment design with posttest only control group model by animal model as a subject research. This research was use indoor clinical trial laboratory on Veterinary Pharmacy Subdivision Department of Basic Science, Faculty of Veterinary Medicine Airlangga University. The study was conducted from July to October 2013.

Sample size

The sample size were calculate according to Rumke's tablewith assumption 100% successful after giving crude extract methanol *Benalu duku* leaves but 40% failed without consumption crude extract methanol Benalu duku leaves.[4,8]

Herbal medicine

Benalu duku leaves (*Dendrophthoepetandra L., Miq* growth in *Lanciumdomesticum*). was collected from natural habitats, Palembang District, South of Sumatera, and checked for authenticate by Mrs. Yayah, Research Center For Biology - Cibinong Science Center (CSC), JL. Raya Jakarta - Bogor Km.46 Cibinong 16911 Bogor –Indonesia, Phone: +62-21-87907604/87907636 Fax: +62-21-87907612, e-mail: biologi@mail.lipi.go.id. The fresh materials of *Benalu duku* leaves were separated shade dried and powdered using the electric homogenizer. The powdered samples at 450 g were extracted with 2 L of methanol pro analysis grade for 72 h by using rotating percolation method. The crude extract methanol of *Benalu duku* leaves were dried by warm dried method with nitrogen gas as a stimulate gas to reduced methanol levels. The crude extract of methanol were prepared to injection dose perform as follows; free from pyrogenic agents, sterile perform and stable at iso-tonic, iso-ionic, iso-hydricperform on ranging pH 7.2-7.5. The last prepare of crude extract methanol *Benalu duku* leaves were filtrated at 0.20 µm and keep on disposable peek sterile vials at 4 ^{0}C.

Experimental animals

Fourteen healthy adult female rats at ranging 3-4 month years old *(Rattusnorwegicuswistar strain)* were obtained fromRachmadPriyadi DVM at, Trosobo, Sidoarjo. The rats were grouping in two groups design as control group and treatment groups. The two groups were marking as follows; $K_3$0; K_3KA; K_3KI; K_3KK; K_4KI; K_4KK (control groups)andP_3KA; P_3KI; P_3KK; $P_3$0; $P_4$0; P_4KA; P_4KI (treatment groups).The mean of $K_3$0 is number code of sample i.e K_3 = Third cages, 0 = no ear marker. The K_3KA as a K_3 is a third cage and KA = ear marker in right side, KI = ear marker in left side and KK ear marker in right and left. Code of P_3 and P_4 at treatment groups are code of cages (third cage and fouth cage) and KA, KI, KK code of ear marker.

Synchronized oestrous

The all rats were synchronizing oestrouscycle by whitten effect or pheromone effect techniqueduring the two period cycle's oestrous at ranging 10 days as follows; fifteen female rats at two groups were cages on two level cages. The upper level cages were filled by male rats and the other level cages (bottom level) filled female rats.[8,9]The rats were examined by Giemsa staining method at magnified 1000x to find the cubic cells and cornification cells from vaginal smear test during the research period.[10]

Clinical trials

Research trials starting after all of the rats were oestrous as referred to Bambang and Lazuardi.[8]The treatment groups were treated with crude extract methanol *Benalu duku* leaves diluted on aqua pro injection (b/v) at dosing 100 mg/kg body weight during the fourdays (*semel in die*) by intramuscular injection. Control groups were giving aqua pro injection by intramuscular injection at dosing 1 ml during the four days(*semel in die*). The end treated periods, the rats were sacrified and whole blood samples collected at ranging 1.5 to 2 ml by cardiac suction after.The plasma samples were separated by centrifugation at 8000 g for 15

minutes and were stored at 2-8^{0}C until ready to analyzing concentrationsof the FSH and Progesterone.

Principle of determined FSH Progesterone

The principle of analysis levels of FSH and Progesterone are used to perform simultaneous quantitative detection of multiple analytes from a single subject sample. The core technology is the Randox Biochip, a (9 mm^2) solid substrate containing an array of discrete test region of immobilized antibodies specific to different fertility markers. A chemiluminescent immunoassay is employed. The light signal generated from each of the test regions on the biochip is detected using a CCD camera and state-of-the-art digital imaging technology. The light signal generate from each of the test regions on the biochip is detected using digital imaging technology and compared to that from a store calibration curve. From this the concentration is calculated.[11]

Quantitative analysis of FSH Progesterone

The amount of FSH and progesterone was measured by Evidence Investigator^{TM}using the method described previously by Randox Corp (in triplo process). Briefly, One package of fertility hormone array (FERTILITY) Cat No. EV3610 were ready to use. Cat No. EV 3610 wasconsisting of six compounds i.e. (i) fertility dilution assay, (ii) fertility conjugate, (iii) fertility biochip, (iv) fertility calibrate, (v) luminace or PX, (vi) buffer washer (concentration). Pipette 150 µl of assay diluent into the appropriate biochip wells as required. Pipette 75 µl of calibrator/sample/ control into appropriate biochip wells. Gently tap all edges of the handling try to mix reagents. Secure the handling tray to the base plate of the thermoshaker. Incubate for 30 minutes at +37 ^{0}C and 350 rpm. Following incubation, remove the handling tray containing carriers from the thermoshaker. Pipette 75 µl of conjugate into the appropriate biochip wells as required. Gently tap all edges of the handling tray to mix reagents. Secure the handling tray to the base plate of the thermoshaker. Incubate

for 30 minutes at +37 ^{0}C and 350 rpm. Following incubation, remove the handling tray containing carriers from the thermoshaker. Discharge reagents to waste using a sharp flicking action of the handling tray. Immediately carry out 2 quick wash cycles. Using wash bottle with diluted wash buffer (refer to kit insert for dilution), add approximately 350 µl wash buffer to each well, gently tap all edges of the handling tray to release any reagents trappedbelow the biochip, and flick to waste with a sharp action. Take care not to overfill wells during wash in order to reduce potential for well-to-well contamination. Carry out a further 4 wash cycles; for each cycle gently tap all edges of the handling tray for approximately 10 to 15 seconds then leave the biochips to soak in wash buffer for 2 minutes. After final wash, fill wells with wash buffer and leave to soak until directly prior to imaging (no carrier should be left to soak for longer 30 minutes). Imaging process was individually sampled. Those awaiting imaging should be protected from light. Remove the first carrier to be imaged from the handling tray. Directly before addition of signal, remove wash buffer using a sharp, flicking action and tap the carrier onto lint free tissue to remove any residual wash buffer. Add 250 µl of working signal reagent to each well and cover to protect the light. Place the carrier into Evidence Investigator machine after exactly 2 minutes (±10 seconds). Use of a timer is recommended to ensure imaging occurs at the correct time. Capture of images will be automatically initiated as defined by dedicated software. All blood samples (treatment groups and control groups) were measured at triplo process by code as follows; n1, n2,n3.

Data analysis

Data calibrator was analysis by linearity program method. Analysis validation method were use reference by Evidence Investigator operator manual.Data samples were analysis by minitab statistic software 17.0 with two sample independent student t test at 95% significant levels.

Ethical clearance

The rats were handling by principles use for animal experiments under control Commission Animal Ethic Clearance from Faculty of Veterinary Medicine, Airlangga University and requirement standard handling for animal experiment from unit ethic animal experimental of Indonesia Veterinary Pharmacy and Pharmacology Association (www.affaveti.org).

RESULTS

Research result appeared that synchronize oestrous perform all rats were obtained at about 10 days. The view of cubic cell from vaginal smears test by Giemsa staining method were appeared at Figure 1and Figure2 (1000x magnify).

Calibration curve of FSH (mIU/ml) by serial consentrations 00.00; 05.65; 28.30; 44.62 were appeared linear with average squared difference of the error in the actual to the predicted values of the date (S) = 5.27620 at level correlation (R-Sq) = 95.7% and level adjective correlation orR-Sq (adj) = 93.5%. Calibration curve of Progesterone (nmol/L) in serials 000.00; 001.74; 048.22; 232.02 were obtained less than liner by average squared difference of the error in the actual to the predicted values of the date (S) = 66.0114, level correlation (R-Sq) = 76.0% and level adjective correlation or R-Sq(adj) = 64.0%. Sensitivity test fordetermined the mean low concentrations of FSH (22.49 mIU/ml and 43.70 mIU/ml) and the mean high concentrations of FSH (37.4966mIU/ml and 72.86 mIU/ml) at 36th replicates were obtained at ranging coeficient variation 10.01% to 16.68%.The sensitivty test of Progesterone at mean low concentrations (30.86 nmol/L and 138.68 nmol/L) and mean high concentration (51.42 nmol/L and 231.16 nmol/L) were obtained at ranging of CV 10.00% to 16.67%. The result research to determine of FSH Progesterone level on two groups treatment and control groupsby triplo measurement were described Table 1 at bellow.

DISCUSSION

Oestrus syncrhronyze technique using whittten effect method was appeared succesfully, result research were obtained cubic cell and cornification cell up to 10 days available all female rats at the same cycle oestrus. Figure 1 and Figure 2 is clear evidence that the findings of cubic cells and cornification cell to be an achievement condition of oestrus. Figure 1 appeared that cubic cells from vaginal smear test were pink color and grouping. Figure 2 appeared that cornification cells from vaginal smear test were blue color and separated. The time line to obtained synchronized oestrus by whitten effect method were avarage at 8 to 10 days after using the method.Our method to obtain the estrous cycle was more fastest than "Bruce effect" method as described other researcher mice.[12,13]Result research at Tabel 1 appeared that influence of analites (crude extract methanol *Benalu duku* leaves) to FSH Progesterone hormone were caused contradiction performing each other hormones.

FSH level in the treartment groups were significantly lowest third times than FSH in control groups ($p>0.05$). But Progesterone levels of the treartment groups were appeared highest twicelevels than Progesterone in control groups ($p<0.05$). These fact was indicated that analites have some unknown substances with two action pharmacodynamic as a (a) FSH suppression product and (b) Progesterone stimulant product. That "unknown substances from crude extract of *Benalu duku* leaves" were identifyed as a suppresed FSH like effect and stimulant Progesterone like effect. Some other substances of suppresed FSH like effect were known as follows; human seminal plasma compouns with 92-amino acid polypeptide andalpha-inhibin-92 (alpha-IB-92).[14]Synthetic Progesterone like effect had been known early of year 2000 as follows; Dydrogesterone, 17- α- Hydroxyprogesterone carproate, Medroxy progesterone acetat, Megestrol acetat.

The FSH suppression phenomenon was analog using concept of "receptor down regulation of Gonadothrophine Releasing Hormon (GnRH)".As down-regulation occurs,

production of gonadotrophins by the pituitary i.e follicle-stimulating hormone (FSH) ceases, effectively shutting down control hormones for cyclic ovarian function in the female. This effect is well known and as early as 1989 had been suggested as a potential estrous suppression hormone for the bitch.[15]Analytes from crude extract of methanol were probably made actions to occupy GnRH receptors at the pituitary and after a short period of stimulation cause the cells to reduce or stop the synthesis of the receptor protein, making the cells insensitive to GnRH.[15]The Progesterone will be increasing after the anterior pituitary give them “a calling signal” with impact to the corpus luteum for inhibit the development of Graafian folicles in the ovary. The relationship beetwen the inhibit of FSH inhibition and increased of Progesteronewere not directly but other fertility hormon influence i.e., Luteinizing hormone (LH), Estrogen hormone may be have a role important to make closely of their relationship.[16]

Hormones are generally administered to patient for one of three purposes. First, when an patients fail to produce sufficient quantities of hormones, therapy is directed at correcting the deficiency. Second, when no deficiency exists, hormones are used to obtain a desired effect. For example, synthetic progesterone, used as a birth control agent, may be administered to normal bitches.Third, when the products of fertility hormones more ecsessive then usual, therapy is directtly to the target with treatment via other antagonist hormones to inhibit mechanismtarget for dismiss the excessive product.Result reseach probably indicate that analytes are stimulating to corpus luteum for production of Progesterone more twice than usual and of course will be make a feedback mechanism to inhibit FSH product via coding of GnRH to inhibit FSH as referred to analogy of Konishi *et al*.[17]Our study may be not clearly explain, how is the mechanism of analytes to make action causing low level of FSH and encourage excessive product of Progesterone after giving crude methanol extract of *Benalu duku* leaves. But may be by advance explore of Progesterone like effect of crude extract

methanol *Benalu duku* leaves can be find the mechanism action of that analytes.As an incompletely of our study we are not study enzyme expression from encode sub units proteins of base-paired microsomal DNA as “a messenger control” to inhibit FSH and stimulate Progesterone that possibilities can be answer mechanism action of analytes.

In woman with fertility cases, the unique phenomena of crude extract methanol *Benalu duku* leaves to inhibit FSH and stimulate Progesterone can be use to treat pregnant woman with low Progesterone levels condition esspecially in early pregnancy with miscarriage risk. In early woman pregnancy, abdominal pain and vaginal bleeding may be signs of a miscarriage, but by consumption of crude extract methanol of *Benalu duku* leaves probably will be help to make better the Progesteronelevel. Some natural herbal or plants had been produce as a stimulate of Progesterone hormone in ealy of pregnant as follows; oils in yam and soy plants, *Curcuma comosa* esspecially diaryl-heptanoid compounds, wastewater plants (WWTPs) of Beijing, China., alkaloid leaves of *Digitalis lanata* as known 5-β-cardenolide.[17,18,19]As explained by other researcher i.e.,Gupta and Kachhawa that some alkaloids of *Dendrophthoe* family was usefully to threat cases steroid disorder in animal experimental.[20]Other researchers even mentionedthat *Demdrophthoe* species was potential as components for muscle relaxation.[21] But for future direction, by next research explore of alkaloid *Benalu duku* leaves as a parts of *Dendrophthoe* familly, probably we are find a new compounds to treatment the cases of low level Progesterone concentration at early of pregnant or as a maintenance uterus condition during the non-fertile.

CONCLUSSION AND RECOMMENDED

The crude extract methanol of *Benalu duku* leaves was potential for using stimulate agent to produce the Progesterone hormone on healthy adult female rats. The crude extract methanol of *Benalu duku* leaves was suitable for suppressor agents to produce FSH hormone on healthy adult female rats. Result research was recommend to explored the compound of

crude extract methanol *Benalu duku* leaves with effect stimulate Progesterone hormone on next research advances. Other fertility hormone i.e., Prolactine, Testosterone, Oestrogen, Lutinize hormone were not recommended yet to use treated other cases of steroide cases in healthy female rats.

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REFERENCES

1. ShalenderBhasin, Glenn R. Cunningham, Frances J. Hayes, Alvin M. Matsumoto, Peter J. Snyder, et al. The Endocrine Society's Clinical Guidelines: Testosterone Therapy in Adult Men with Androgen Deficiency Syndromes: An Endocrine Society Clinical Practice Guideline. J ClinEndocrinolMetab 2010; 95(6): 2536–59.

2. Onyeka CA1, Aligwekwe AU1, Olawuyi TS, Nwakanma AA, Kalu EC, Oyeyemi AW. Antifertility Effects of Ethanolic Root Bark Extract of *Chrysophyllumalbidum* in Male Albino Rats. Int J Appl Res Nat Prod 2012; 5 (1): 12-17.

3. Pathak AK, Mallurwar VR, Kondalkar AK, Soni S. A review of plants with anti-fertility activity. Nig J Nat Prod Med 2005;9: 4-10.

4. Lazuardi M, Nuraini F, Ratna SM, Roostantia I. Screening of *benalu duku* for antiproliferation of myeloma. In :Puji S, et al. Editors. Strategies for the control prevention of zoonotic diseases. Surabaya: Airlangga University Press. 2010. p 65-68.

5. Lazuardi M. The overview of *Benalu duku* (*Dendrophthoe spec.,*) as a parasites herbal medicine with potential for treatment Trypanosomiasis. In: Endang E, et al., Editors.

Abstract and Programs PertemuanIlmiahTahunanNasional I PerhimpunanFarmasiKedokteran Indonesia (PEFARDI) 7-8 Mei 2011. Bandung: Bag. FarmasiKedokteranUniversitasMaranatha. 2011. p 15-20.

6. Zaimah I. Skriningfitokimiaekstrak herbal *Benalu duku* (*Dendrophthoepetandra L. Miq.*,)danujiaktivasiterhadap larva udang (*Artemiasalina leach*) denganmetode BSLT (Brine Shrimp Lethality Test) [Skripsi]. FakultasSainsdanTeknologi (FST): UniversitasJenderalSoedirman Solo; 2011.

7. Nina A, Taufik F, Akhmad D. Bioactivities Evaluation of Indonesian Mistletoes (*Dendrophthoepentandra (L.) Miq*.) Leaves Extracts. J Appl Pharm Sci 02 (01); 2012: 24-27.

8. Bambang H and Lazuardi M. Assessment of luteinizing testosterone prolactin estrogen hormone after giving crude methanol extract of *Benalu duku* leaves on healthy adult female at. In: Lazuardi and Rinidar. Editors. Proceeding 2nd MusyawarahNasional Indonesia Veterinary Pharmacy and Pharmacology Association 21-22 of September 2013. Surabaya: Airlangga University Press. 2014. (In press).

9. Anne Fawcett. Guidelines for the housing of mice in scientific institutions. NSW: Animal Welfare Unit, NSW Department of Primary Industries, Locked Bag 21, Orange NSW. April 2012. p 112-114.

10. Saadat P, Latiffah AL, Sabariah AR, Mohammad AD, Hanachi P. Assessing estrogenic activity of Nigella sativa in ovariectomized rats using vaginal cornification assay. African J PharmPharmacol 2011; 5(2): 137-142.

11. RandoxLaboratorium Ltd. Fertility Hormone Array (FERT) [Brochure]. 55 Diamond Road. Crumlin, County Antrim UK: (Revised) May 20th 2013: 1-16.

12. Izuchukwu SO, Chike FO, Chinaza CO. The effect of increasing number of strange male mice on bruce effect. Veterinarskhi Arhiv 2012; 82 (1): 103-114.

13. Cornelius PCP. Study of effect of sweet (*Ocimum basillicum Linn*) essential oil of various dosages on estrus cycle of sparque dawley rats. J Sains Vet 2012; 30 (2): 87-96.

14. Yu WH, McCann SM, Li CH. Synthetic human seminal alpha-inhibin-92 selectively suppresses follicle-stimulating hormone release in vivo. Proc Natl Acad Sci USA 1988; 85(1): 289–292.

15. Briggs Joice. Alliance for contraceptic in cat and dog.USA: ACC& D Press; 2013. p 13-14.

16. Otto JR, Freeman MJ, Malau-Aduli BS, Nichols PD, Lane PA, Malau-Aduli EO.Reproduction and Fertility Parameters of Dairy Cows Supplemented with Omega-3 Fatty Acid-rich Canola Oil. Ann Res & Rev in Biol 2014; 4(10): 1611-1636.

17. Konishi S1, Brindle E, Guyton A, O'Connor KA. Salivary concentration of progesterone and cortisol significantly differs across individuals after correcting for blood hormone values.Am J Phys Anthropol 2012;149 (2):231-41.

18. Chang H1, Wan Y, Wu S, Fan Z, Hu J. Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: comparison to estrogens. Water Res 2011;45(2):732-40.

19. Ernst M1, de Padua RM, Herl V, Müller-Uri F, Kreis W.Expression of 3beta-HSD and P5betaR, genes respectively coding for Delta5-3beta-hydroxysteroid dehydrogenase and progesterone 5beta-reductase, in leaves and cell cultures of *Digitalis lanata* EHRH.Planta Med. 2010; 76(9):923-7.

20. Gupta RS1, Kachhawa JB.Evaluation of contraceptive activity of methanol extract of *Dendrophthoe falcata* stem in male albino rats.J Ethnopharmacol 2007; 30;112(1):215-8.

21. Pooja S, Irchhaiya R, Bhawna S, Gayatri S, Santosh K. Anticonvulsant and muscle relaxant activity of the ethanolic extract of stems of *Dendrophthoe falcata (Linn. F*.) in mice. Indian J Pharmacol 2011; 43(6): 710–713.

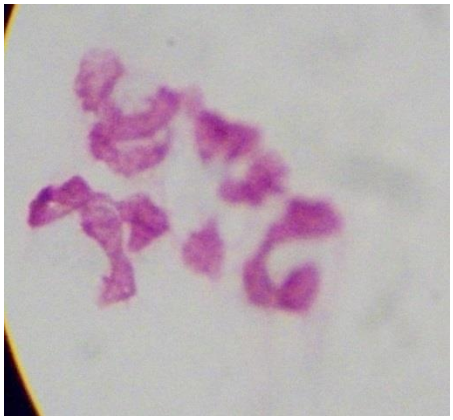

Figure 1. Cubic cell from vaginal smear test (Giemsa staining, 1000x)

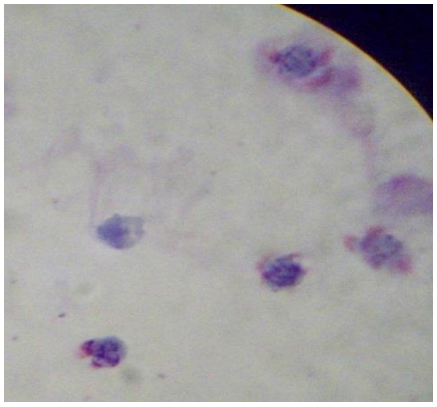

Figure 2. Cornification cells from vaginal smear test (Giemsa staining, 1000x)

Table 1. Research result of FSH Progesterone on treatment groups and control groups.

Sample code, body weight (g)	Treatment groups							
	FSH (mIU/ml)				Progesterone (nmol/L)			
	(n1)	(n2)	(n3)	Mean ± SD	(n1)	(n2)	(n3)	Mean ± SD
P_3KA (166 g)	00.67	49.14	01.12	16.98^{a}±27.85	71.88	11.88	54.35	46.04^{c}±30.85
P_3KI (120 g)	00.38	01.93	11.91	04.74^{a}±06.26	82.27	15.53	17.88	38.56^{c}±37.87
P_3KK (167 g)	01.68	15.74	26.19	14.54^{a}±12.30	37.37	0.99	10.75	16.30^{c}±18.86
P_30 (177 g)	25.91	06.39	-	16.15^{a}±13.80	17.95	6.12	-	12.03^{c}±08.36
P_40 (196 g)	02.28	01.81	-	02.04^{a}±00.33	38.47	32.85	-	35.66^{c}±03.97
P_4KA (132 g)	16.68	01.87	-	09.27^{a}±10.47	-	38.77	-	38.77^{c}±00.00
P_4KI (111 g)	02.45	00.45	00.90	01.27^{a}±01.05	18.29	63.18	61.08	47.52^{c}±25.33
Mean ± SD				09.28±06.72	Mean ± SD			33.55±13.96
Sample code, body weight (g)	Control groups							
	FSH (mIU/ml)				Progesterone (nmol/L)			
	(n1)	(n2)	(n3)	Means ± SD	(n1)	(n2)	(n3)	Means ± SD
K_30 (153 g)	10.80	-	-	10.80^{b}±00.00	48.54	-	8.88	28.67^{d}±28.10
K_3KA (198 g)	07.05	25.13	13.54	15.24^{b}±9.16	5.32	17.13	17.91	13.45^{d}±05.76
K_3KI (148 g)	22.68	01.69	28.29	17.55^{b}±14.02	11.01	26.38	8.88	15.40^{d}±09.58
K_3KK (147 g)	09.42	00.42	07.37	05.74^{b}±04.72	22.33	53.08	6.68	27.36^{d}±23.61
K_4KA (170)	27.59	-	42.69	35.14^{b}±10.68	5.83	-	18.22	12.02^{d}±08.76
K_4KI (159 g)	38.71	45.76	-	42.23^{b}±04.98	-	-	-	-

K_4KK (165 g)	89.86	30.61	20.29	46.92^{b}±37.54	-	7.48	26.34	16.91^{d}±13.34
Mean ± SD				24.80±16.35	Mean ± SD			18.47±06.47

Notes:
- Blood samples lysis. Code column n1,n2,n3 is *triplo*measurement.
P_3, P_4 and K_3, K_4at treatment and control sample code is third and fourth cages.
0,KA,KI,KK at treatment and control samples code is no ear marker, right ear marker, left ear marker, right and left ear marker of the rats.
Superscript a vs., b at same column was different at p <0.05 by independent sample t test
Superscript c vs., d at same column was different at p <0.05 by independent sample t test

Revisi-2 Terakhir

Determinationof FSHProgesterone and influenced to oestrouscycles on ratsat orally Extract *Benalu Duku*

Running text:
FSH Progesterone levels and influenced to oestrous cycle on rats post orally *Benalu duku*

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ABSTRACT

BACKGROUND

Cases in human infertilities in Indonesia were known tends to increase at about 2-5 % each year's since of early 2000. The other side knew that many tropical plants in Indonesia had potential sources to make a new anti-infertility compounds (i.e*Benalu duku* or *Dendrophthoepetandra*L. Miq., grewth on *Lanciumdemesticum*). The objective of this research was to identify performance of crude extract methanol *Benalu duku* leaves on rat to induce oestrous cycle and influence their FSH progesterone perform.

METHODS

Fourteen *RattusNorvegicusWistar strain* rats were divided into two groups (namely the treatment group and the control group) and arranged to oestrous perform via pheromone synchronizing method. The rats at treatment groups were given crude extract 100 mg/kg

body weight^{-1}*(s.d.d*) during the 4 days, via intra muscularly. The control groups were given 0.25 ml phosphate buffer saline intramuscularly (*s.d.d)* during the 4 days. To determine of FSH Progesterone levels, the whole blood samples were prepared by Evidence Investigators Analyzer Method.

RESULTS

The result showed that FSH at treatment groups were obtained 09.28 ±06.72 mIU/ml (lowest two times than control groups at 24.80 ±16.35 mIU/ml, $p<0.05$). Progesterone hormones at treatment groups were obtained 33.55±13.96 nmol/L (more twice than the control groups at 18.47±06.47 nmol/L, $p<0.05$). This research was conclusion that the crude methanol extract of *Benalu duku* leaves was better used to be stimulate of Progesterone production up to more level than usual level in rats, but not recommended yet to using the other fertility hormone.

Key words: Herbal medicine**,**Synchronize oestrous, Evidence Investigator Method, Cubic Cell, Vaginal Smear.

ABSTRAK

LATAR BELAKANG

Kasusketidaksuburanpadamanusia di Indonesia cenderungnaik 2-5 % setiaptahunsejaktahun 2000. Disisi lain diketahuibanyaktumbuhantropisdi Indonesia yang berpotensisebagaisumberkomponenbaru anti infertilitas (contohBenalu duku atau*DendrophthoepetandraL. Miq*., tumbuh di *Lanciumdemesticum*). Tujuanpenelitianiniadalahmengidentifikasikankinerjaekstrakkasarmetanoldaun Benalu duku padatikusterhadapinduksisiklusoestrousdanpengaruhnyaterhadapkinerja FSH danProgesteron.

METODE

Sebanyakempatbelastikus (*RattusNorvegicusWistar)* strain dibagiduakelompok (kelompokperlakuandankelompokkontrol) dandiaturmenjadioestrousmelaluimetodesinkronisasiferomon. Kelompokperlakuanselanjutnyadiberiekstrakkasar 100 mg/kg beratbadan(satu kali sehari)selama 4 harimelalui intramuskular. Kelompokkontroldiberi 0,25 ml garamfosfat buffer secara intra muskular (satu kali sehari) selama 4 hari. Untukmenetapkanderajat FSH danProgesteron,sampeldarahdiprosesmenggunakanmetodeanalisa Evidence Investigator

HASIL

Hasilmenunjukkanbahwa FSH padakelompokperlakuan 09,28 ± 06,72 mIU/ml (lebihrendahdarikelompokkontrolpada 24,80 ± 16,35 mIU/ml, ($p<0,05$). HormonProgesteronpadakelompokperlakuandidapat 33,55 ± 13,96 nmol/L (dua kali lebihtinggidarikelompokkontrolpada 18,47 ± 06,47 nmol/L, $p<0,05$). PenelitianinidapatdisimpulkanbahwaekstrakkasarmetanoldaunBenalu dukubaikdigunakanuntukmendorongproduksiprogesteronsampaidengan di ataskadarlazimpadatukis. Dalampenelitianinimasihbelumdianjurkanuntukmenggunakan hormone fertilitaslainnyasebagaipenghambatataupeningkat hormone pada tikus.

Kata kunci: Tanamanobat, penyeragamanbirahi, Metoda Evidence Investigator, selkubus, ulas vaginal

INTRODUCTION

Follicle stimulating hormone (FSH) and progesteroneis part of fertility hormone in the human female and important for control cycle of menstruation period. The fertility hormones can not only test for pregnancy in woman but are also very useful in the diagnosis of other conditions such as the onset of menopause and gonadal dysfunction. In men, fertility hormones can be accurate indicators of condition such as liver cirrhosis and testicular cancer.[1]The fertility hormones can be produced from herbal medicine as follows; *Jatropha curcas* producing testosterone[2], *Dioscorea macrostachya* producing *diosgenine*(intermediate product of cortisone)and anti-estrogenic activity.[3]

Benalu duku or*Dendrophthoepetandra (L.)Miq* grew on *Lansiumdomestic* was known herbal medicine since 1990 and still explored as a local and systemic anticancer agent.[4] As a parasite trees, *Benalu duku* was known have a beneficial compound as follows; anti myeloma cell and antibiotics.[5,6] Some researcher reported that Benalu duku have a some beneficial compounds (i.e. essential amino acid and alkaloid, flavonoid, polyphenol, terpenoide, free steroid) as referred to Lazuardi*et al*.[4] The last the other report described that Family of *Dendrophthoe spec*., were probably have an immune hormone prevention substances for protected internal free radical caused external stimulant substances.[7]But other species of *Dendrophthoe* familly, esspecially *Petandra L. Miq* grew in *Lansium domesticum*(*Benalu duku*) for treated cycle menstruation dissorders wasnot reported yet.

By research background as described at above, we were tried to explore the effect of FSH and Progesterone levels as a part of steroid hormones after giving crude extract *Benalu duku* leaves on healthy adult female rat. The objectives of these research was to obtained influence of crude extract methanol compound of *Benalu duku* leaves on fertility hormone level in female rat subject during the oestrous period.

METHODS

Research design

This study was true experiment design with posttest only control group model by animal model as a subject research. This research was use indoor clinical trial laboratory on Veterinary Pharmacy Subdivision Department of Basic Science, Faculty of Veterinary Medicine Airlangga University. The study was conducted from July to October 2013.

Sample size

The sample size were calculate according to Rumke's tablewith assumption 100% successful after giving crude extract methanol *Benalu duku* leaves but 40% failed without consumption crude extract methanol Benalu duku leaves.[4,8]

Herbal medicine

Benalu duku leaves (*Dendrophthoepetandra L., Miq* growth in *Lanciumdomesticum*). was collected from natural habitats, Palembang District, South of Sumatera, and checked for authenticate by Mrs. Yayah, Research Center For Biology - Cibinong Science Center (CSC), JL. Raya Jakarta - Bogor Km.46 Cibinong 16911 Bogor –Indonesia, Phone: +62-21-87907604/87907636 Fax: +62-21-87907612, e-mail: biologi@mail.lipi.go.id. The fresh materials of *Benalu duku* leaves were separated shade dried and powdered using the electric homogenizer. The powdered samples at 450 g were extracted with 2 L of methanol pro analysis grade for 72 h by using rotating percolation method. The crude extract methanol of *Benalu duku* leaves were dried by warm dried method with nitrogen gas as a stimulate gas to reduced methanol levels. The crude extract of methanol were prepared to injection dose perform as follows; free from pyrogenic agents, sterile perform and stable at iso-tonic, iso-ionic, iso-hydricperform on ranging pH 7.2-7.5. The last prepare of crude extract methanol *Benalu duku* leaves were filtrated at 0.20 µm and keep on disposable peek sterile vials at 4 ^{0}C.

Experimental animals

Fourteen healthy adult female rats at ranging 3-4 month years old *(Rattusnorwegicuswistar strain)* were obtained fromRachmadPriyadi DVM at, Trosobo, Sidoarjo. The rats were grouping in two groups design as control group and treatment groups. The two groups were marking as follows; K_30; K_3KA; K_3KI; K_3KK; K_4KI; K_4KK (control groups)andP_3KA; P_3KI; P_3KK; P_30; P_40; P_4KA; P_4KI (treatment groups).The mean of K_30 is number code of sample i.e K_3 = Third cages, 0 = no ear marker. The K_3KA as a K_3 is a third cage and KA = ear marker in right side, KI = ear marker in left side and KK ear marker in right and left. Code of P_3 and P_4 at treatment groups are code of cages (third cage and fouth cage) and KA, KI, KK code of ear marker.

Synchronized oestrous

The all rats were synchronizing oestrouscycle by whitten effect or pheromone effect techniqueduring the two period cycle's oestrous at ranging 10 days as follows; fifteen female rats at two groups were cages on two level cages. The upper level cages were filled by male rats and the other level cages (bottom level) filled female rats.[8,9]The rats were examined by Giemsa staining method at magnified 1000x to find the cubic cells and cornification cells from vaginal smear test during the research period.[10]

Clinical trials

Research trials starting after all of the rats were oestrous as referred to Bambang and Lazuardi.[8]The treatment groups were treated with crude extract methanol *Benalu duku* leaves diluted on aqua pro injection (b/v) at dosing 100 mg/kg body weight during the fourdays (*semel in die*) by intramuscular injection. Control groups were giving aqua pro injection by intramuscular injection at dosing 1 ml during the four days(*semel in die*). The end treated periods, the rats were sacrified and whole blood samples collected at ranging 1.5 to 2 ml by cardiac suction after.The plasma samples were separated by centrifugation at 8000 g for 15

minutes and were stored at 2-8^{0}C until ready to analyzing concentrationsof the FSH and Progesterone.

Principle of determined FSH Progesterone

The principle of analysis levels of FSH and Progesterone are used to perform simultaneous quantitative detection of multiple analytes from a single subject sample. The core technology is the Randox Biochip, a (9 mm^2) solid substrate containing an array of discrete test region of immobilized antibodies specific to different fertility markers. A chemiluminescent immunoassay is employed. The light signal generated from each of the test regions on the biochip is detected using a CCD camera and state-of-the-art digital imaging technology. The light signal generate from each of the test regions on the biochip is detected using digital imaging technology and compared to that from a store calibration curve. From this the concentration is calculated.[11]

Quantitative analysis of FSH Progesterone

The amount of FSH and progesterone was measured by Evidence Investigator^{TM}using the method described previously by Randox Corp (in triplo process). Briefly, One package of fertility hormone array (FERTILITY) Cat No. EV3610 were ready to use. Cat No. EV 3610 wasconsisting of six compounds i.e. (i) fertility dilution assay, (ii) fertility conjugate, (iii) fertility biochip, (iv) fertility calibrate, (v) luminace or PX, (vi) buffer washer (concentration). Pipette 150 µl of assay diluent into the appropriate biochip wells as required. Pipette 75 µl of calibrator/sample/ control into appropriate biochip wells. Gently tap all edges of the handling try to mix reagents. Secure the handling tray to the base plate of the thermoshaker. Incubate for 30 minutes at +37 ^{0}C and 350 rpm. Following incubation, remove the handling tray containing carriers from the thermoshaker. Pipette 75 µl of conjugate into the appropriate biochip wells as required. Gently tap all edges of the handling tray to mix reagents. Secure the handling tray to the base plate of the thermoshaker. Incubate

for 30 minutes at +37 ^{0}C and 350 rpm. Following incubation, remove the handling tray containing carriers from the thermoshaker. Discharge reagents to waste using a sharp flicking action of the handling tray. Immediately carry out 2 quick wash cycles. Using wash bottle with diluted wash buffer (refer to kit insert for dilution), add approximately 350 µl wash buffer to each well, gently tap all edges of the handling tray to release any reagents trappedbelow the biochip, and flick to waste with a sharp action. Take care not to overfill wells during wash in order to reduce potential for well-to-well contamination. Carry out a further 4 wash cycles; for each cycle gently tap all edges of the handling tray for approximately 10 to 15 seconds then leave the biochips to soak in wash buffer for 2 minutes. After final wash, fill wells with wash buffer and leave to soak until directly prior to imaging (no carrier should be left to soak for longer 30 minutes). Imaging process was individually sampled. Those awaiting imaging should be protected from light. Remove the first carrier to be imaged from the handling tray. Directly before addition of signal, remove wash buffer using a sharp, flicking action and tap the carrier onto lint free tissue to remove any residual wash buffer. Add 250 µl of working signal reagent to each well and cover to protect the light. Place the carrier into Evidence Investigator machine after exactly 2 minutes (±10 seconds). Use of a timer is recommended to ensure imaging occurs at the correct time. Capture of images will be automatically initiated as defined by dedicated software. All blood samples (treatment groups and control groups) were measured at triplo process by code as follows; n1, n2,n3.

Data analysis

Data calibrator was analysis by linearity program method. Analysis validation method were use reference by Evidence Investigator operator manual.Data samples were analysis by minitab statistic software 17.0 with two sample independent student t test at 95% significant levels.

Ethical clearance

The rats were handling by principles use for animal experiments under control Commission Animal Ethic Clearance from Faculty of Veterinary Medicine, Airlangga University and requirement standard handling for animal experiment from unit ethic animal experimental of Indonesia Veterinary Pharmacy and Pharmacology Association (www.affaveti.org).

RESULTS

Research result appeared that synchronize oestrous perform all rats were obtained at about 10 days. The view of cubic cell from vaginal smears test by Giemsa staining method were appeared at Figure 1and Figure2 (1000x magnify).

Calibration curve of FSH (mIU/ml) by serial consentrations 00.00; 05.65; 28.30; 44.62 were appeared linear with average squared difference of the error in the actual to the predicted values of the date (S) = 5.27620 at level correlation (R-Sq) = 95.7% and level adjective correlation orR-Sq (adj) = 93.5%. Calibration curve of Progesterone (nmol/L) in serials 000.00; 001.74; 048.22; 232.02 were obtained less than liner by average squared difference of the error in the actual to the predicted values of the date (S) = 66.0114, level correlation (R-Sq) = 76.0% and level adjective correlation or R-Sq(adj) = 64.0%. Sensitivity test fordetermined the mean low concentrations of FSH (22.49 mIU/ml and 43.70 mIU/ml) and the mean high concentrations of FSH (37.4966mIU/ml and 72.86 mIU/ml) at 36th replicates were obtained at ranging coeficient variation 10.01% to 16.68%.The sensitivty test of Progesterone at mean low concentrations (30.86 nmol/L and 138.68 nmol/L) and mean high concentration (51.42 nmol/L and 231.16 nmol/L) were obtained at ranging of CV 10.00% to 16.67%. The result research to determine of FSH Progesterone level on two groups treatment and control groupsby triplo measurement were described Table 1 at bellow.

DISCUSSION

Oestrus syncrhronyze technique using whittten effect method was appeared succesfully, result research were obtained cubic cell and cornification cell up to 10 days available all female rats at the same cycle oestrus. Figure 1 and Figure 2 is clear evidence that the findings of cubic cells and cornification cell to be an achievement condition of oestrus. Figure 1 appeared that cubic cells from vaginal smear test were pink color and grouping. Figure 2 appeared that cornification cells from vaginal smear test were blue color and separated. The time line to obtained synchronized oestrus by whitten effect method were avarage at 8 to 10 days after using the method.Our method to obtain the estrous cycle was more fastest than "Bruce effect" method as described other researcher mice.[12,13]Result research at Tabel 1 appeared that influence of analites (crude extract methanol *Benalu duku* leaves) to FSH Progesterone hormone were caused contradiction performing each other hormones.

FSH level in the treartment groups were significantly lowest third times than FSH in control groups ($p>0.05$). But Progesterone levels of the treartment groups were appeared highest twicelevels than Progesterone in control groups ($p<0.05$). These fact was indicated that analites have some unknown substances with two action pharmacodynamic as a (a) FSH suppression product and (b) Progesterone stimulant product. That "unknown substances from crude extract of *Benalu duku* leaves" were identifyed as a suppresed FSH like effect and stimulant Progesterone like effect. Some other substances of suppresed FSH like effect were known as follows; human seminal plasma compouns with 92-amino acid polypeptide andalpha-inhibin-92 (alpha-IB-92).[14]Synthetic Progesterone like effect had been known early of year 2000 as follows; Dydrogesterone, 17- α- Hydroxyprogesterone carproate, Medroxy progesterone acetat, Megestrol acetat.

The FSH suppression phenomenon was analog using concept of "receptor down regulation of Gonadothrophine Releasing Hormon (GnRH)".As down-regulation occurs,

production of gonadotrophins by the pituitary i.e follicle-stimulating hormone (FSH) ceases, effectively shutting down control hormones for cyclic ovarian function in the female. This effect is well known and as early as 1989 had been suggested as a potential estrous suppression hormone for the bitch.[15]Analytes from crude extract of methanol were probably made actions to occupy GnRH receptors at the pituitary and after a short period of stimulation cause the cells to reduce or stop the synthesis of the receptor protein, making the cells insensitive to GnRH.[15]The Progesterone will be increasing after the anterior pituitary give them “a calling signal” with impact to the corpus luteum for inhibit the development of Graafian folicles in the ovary. The relationship beetwen the inhibit of FSH inhibition and increased of Progesteronewere not directly but other fertility hormon influence i.e., Luteinizing hormone (LH), Estrogen hormone may be have a role important to make closely of their relationship.[16]

Hormones are generally administered to patient for one of three purposes. First, when an patients fail to produce sufficient quantities of hormones, therapy is directed at correcting the deficiency. Second, when no deficiency exists, hormones are used to obtain a desired effect. For example, synthetic progesterone, used as a birth control agent, may be administered to normal bitches.Third, when the products of fertility hormones more ecsessive then usual, therapy is directtly to the target with treatment via other antagonist hormones to inhibit mechanismtarget for dismiss the excessive product.Result reseach probably indicate that analytes are stimulating to corpus luteum for production of Progesterone more twice than usual and of course will be make a feedback mechanism to inhibit FSH product via coding of GnRH to inhibit FSH as referred to analogy of Konishi *et al*.[17]Our study may be not clearly explain, how is the mechanism of analytes to make action causing low level of FSH and encourage excessive product of Progesterone after giving crude methanol extract of *Benalu duku* leaves. But may be by advance explore of Progesterone like effect of crude extract

methanol *Benalu duku* leaves can be find the mechanism action of that analytes.As an incompletely of our study we are not study enzyme expression from encode sub units proteins of base-paired microsomal DNA as “a messenger control” to inhibit FSH and stimulate Progesterone that possibilities can be answer mechanism action of analytes.

In woman with fertility cases, the unique phenomena of crude extract methanol *Benalu duku* leaves to inhibit FSH and stimulate Progesterone can be use to treat pregnant woman with low Progesterone levels condition esspecially in early pregnancy with miscarriage risk. In early woman pregnancy, abdominal pain and vaginal bleeding may be signs of a miscarriage, but by consumption of crude extract methanol of *Benalu duku* leaves probably will be help to make better the Progesteronelevel. Some natural herbal or plants had been produce as a stimulate of Progesterone hormone in ealy of pregnant as follows; oils in yam and soy plants, *Curcuma comosa* esspecially diaryl-heptanoid compounds, wastewater plants (WWTPs) of Beijing, China., alkaloid leaves of *Digitalis lanata* as known 5-β-cardenolide.[17,18,19]As explained by other researcher i.e.,Gupta and Kachhawa that some alkaloids of *Dendrophthoe* family was usefully to threat cases steroid disorder in animal experimental.[20]Other researchers even mentionedthat *Demdrophthoe* species was potential as components for muscle relaxation.[21] But for future direction, by next research explore of alkaloid *Benalu duku* leaves as a parts of *Dendrophthoe* familly, probably we are find a new compounds to treatment the cases of low level Progesterone concentration at early of pregnant or as a maintenance uterus condition during the non-fertile.

CONCLUSSION AND RECOMMENDED

The crude extract methanol of *Benalu duku* leaves was potential for using stimulate agent to produce the Progesterone hormone on healthy adult female rats. The crude extract methanol of *Benalu duku* leaves was suitable for suppressor agents to produce FSH hormone on healthy adult female rats. Result research was recommend to explored the compound of

crude extract methanol *Benalu duku* leaves with effect stimulate Progesterone hormone on next research advances. Other fertility hormone i.e., Prolactine, Testosterone, Oestrogen, Lutinize hormone were not recommended yet to use treated other cases of steroide cases in healthy female rats.

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REFERENCES

1. ShalenderBhasin, Glenn R. Cunningham, Frances J. Hayes, Alvin M. Matsumoto, Peter J. Snyder, et al. The Endocrine Society's Clinical Guidelines: Testosterone Therapy in Adult Men with Androgen Deficiency Syndromes: An Endocrine Society Clinical Practice Guideline. J ClinEndocrinolMetab 2010; 95(6): 2536–59.

2. Onyeka CA1, Aligwekwe AU1, Olawuyi TS, Nwakanma AA, Kalu EC, Oyeyemi AW. Antifertility Effects of Ethanolic Root Bark Extract of *Chrysophyllumalbidum* in Male Albino Rats. Int J Appl Res Nat Prod 2012; 5 (1): 12-17.

3. Pathak AK, Mallurwar VR, Kondalkar AK, Soni S. A review of plants with anti-fertility activity. Nig J Nat Prod Med 2005;9: 4-10.

4. Lazuardi M, Nuraini F, Ratna SM, Roostantia I. Screening of *benalu duku* for antiproliferation of myeloma. In :Puji S, et al. Editors. Strategies for the control prevention of zoonotic diseases. Surabaya: Airlangga University Press. 2010. p 65-68.

5. Lazuardi M. The overview of *Benalu duku* (*Dendrophthoe spec.,*) as a parasites herbal medicine with potential for treatment Trypanosomiasis. In: Endang E, et al., Editors.

Abstract and Programs PertemuanIlmiahTahunanNasional I PerhimpunanFarmasiKedokteran Indonesia (PEFARDI) 7-8 Mei 2011. Bandung: Bag. FarmasiKedokteranUniversitasMaranatha. 2011. p 15-20.

6. Zaimah I. Skriningfitokimiaekstrak herbal *Benalu duku* (*Dendrophthoepetandra L. Miq.*,)danujiaktivasiterhadap larva udang (*Artemiasalina leach*) denganmetode BSLT (Brine Shrimp Lethality Test) [Skripsi]. FakultasSainsdanTeknologi (FST): UniversitasJenderalSoedirman Solo; 2011.

7. Nina A, Taufik F, Akhmad D. Bioactivities Evaluation of Indonesian Mistletoes (*Dendrophthoepentandra (L.) Miq*.) Leaves Extracts. J Appl Pharm Sci 02 (01); 2012: 24-27.

8. Bambang H and Lazuardi M. Assessment of luteinizing testosterone prolactin estrogen hormone after giving crude methanol extract of *Benalu duku* leaves on healthy adult female at. In: Lazuardi and Rinidar. Editors. Proceeding 2nd MusyawarahNasional Indonesia Veterinary Pharmacy and Pharmacology Association 21-22 of September 2013. Surabaya: Airlangga University Press. 2014. (In press).

9. Anne Fawcett. Guidelines for the housing of mice in scientific institutions. NSW: Animal Welfare Unit, NSW Department of Primary Industries, Locked Bag 21, Orange NSW. April 2012. p 112-114.

10. Saadat P, Latiffah AL, Sabariah AR, Mohammad AD, Hanachi P. Assessing estrogenic activity of Nigella sativa in ovariectomized rats using vaginal cornification assay. African J PharmPharmacol 2011; 5(2): 137-142.

11. RandoxLaboratorium Ltd. Fertility Hormone Array (FERT) [Brochure]. 55 Diamond Road. Crumlin, County Antrim UK: (Revised) May 20th 2013: 1-16.

12. Izuchukwu SO, Chike FO, Chinaza CO. The effect of increasing number of strange male mice on bruce effect. Veterinarskhi Arhiv 2012; 82 (1): 103-114.

13. Cornelius PCP. Study of effect of sweet (*Ocimum basillicum Linn*) essential oil of various dosages on estrus cycle of sparque dawley rats. J Sains Vet 2012; 30 (2): 87-96.

14. Yu WH, McCann SM, Li CH. Synthetic human seminal alpha-inhibin-92 selectively suppresses follicle-stimulating hormone release in vivo. Proc Natl Acad Sci USA 1988; 85(1): 289–292.

15. Briggs Joice. Alliance for contraceptic in cat and dog.USA: ACC& D Press; 2013. p 13-14.

16. Otto JR, Freeman MJ, Malau-Aduli BS, Nichols PD, Lane PA, Malau-Aduli EO.Reproduction and Fertility Parameters of Dairy Cows Supplemented with Omega-3 Fatty Acid-rich Canola Oil. Ann Res & Rev in Biol 2014; 4(10): 1611-1636.

17. Konishi S1, Brindle E, Guyton A, O'Connor KA. Salivary concentration of progesterone and cortisol significantly differs across individuals after correcting for blood hormone values.Am J Phys Anthropol 2012;149 (2):231-41.

18. Chang H1, Wan Y, Wu S, Fan Z, Hu J. Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: comparison to estrogens. Water Res 2011;45(2):732-40.

19. Ernst M1, de Padua RM, Herl V, Müller-Uri F, Kreis W.Expression of 3beta-HSD and P5betaR, genes respectively coding for Delta5-3beta-hydroxysteroid dehydrogenase and progesterone 5beta-reductase, in leaves and cell cultures of *Digitalis lanata* EHRH.Planta Med. 2010; 76(9):923-7.

20. Gupta RS1, Kachhawa JB.Evaluation of contraceptive activity of methanol extract of *Dendrophthoe falcata* stem in male albino rats.J Ethnopharmacol 2007; 30;112(1):215-8.

21. Pooja S, Irchhaiya R, Bhawna S, Gayatri S, Santosh K. Anticonvulsant and muscle relaxant activity of the ethanolic extract of stems of *Dendrophthoe falcata (Linn. F*.) in mice. Indian J Pharmacol 2011; 43(6): 710–713.

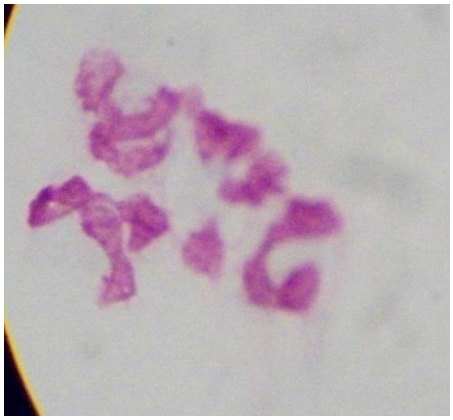

Figure 1. Cubic cell from vaginal smear test (Giemsa staining, 1000x)

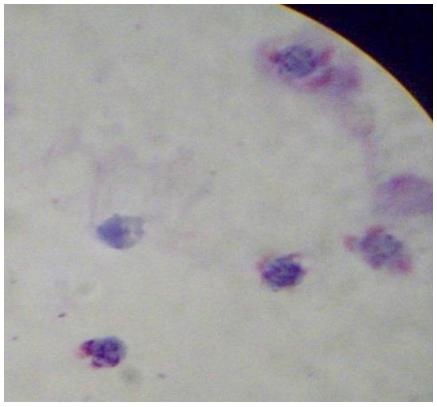

Figure 2. Cornification cells from vaginal smear test (Giemsa staining, 1000x)

Table 1. Research result of FSH Progesterone on treatment groups and control groups.

Sample code, body weight (g)	Treatment groups							
	FSH (mIU/ml)				Progesterone (nmol/L)			
	(n1)	(n2)	(n3)	Mean ± SD	(n1)	(n2)	(n3)	Mean ± SD
P_3KA (166 g)	00.67	49.14	01.12	16.98^{a}±27.85	71.88	11.88	54.35	46.04^{c}±30.85
P_3KI (120 g)	00.38	01.93	11.91	04.74^{a}±06.26	82.27	15.53	17.88	38.56^{c}±37.87
P_3KK (167 g)	01.68	15.74	26.19	14.54^{a}±12.30	37.37	0.99	10.75	16.30^{c}±18.86
P_30 (177 g)	25.91	06.39	-	16.15^{a}±13.80	17.95	6.12	-	12.03^{c}±08.36
P_40 (196 g)	02.28	01.81	-	02.04^{a}±00.33	38.47	32.85	-	35.66^{c}±03.97
P_4KA (132 g)	16.68	01.87	-	09.27^{a}±10.47	-	38.77	-	38.77^{c}±00.00
P_4KI (111 g)	02.45	00.45	00.90	01.27^{a}±01.05	18.29	63.18	61.08	47.52^{c}±25.33
Mean ± SD				09.28±06.72	Mean ± SD			33.55±13.96
Sample code, body weight (g)	Control groups							
	FSH (mIU/ml)				Progesterone (nmol/L)			
	(n1)	(n2)	(n3)	Means ± SD	(n1)	(n2)	(n3)	Means ± SD
K_30 (153 g)	10.80	-	-	10.80^{b}±00.00	48.54	-	8.88	28.67^{d}±28.10
K_3KA (198 g)	07.05	25.13	13.54	15.24^{b}±9.16	5.32	17.13	17.91	13.45^{d}±05.76
K_3KI (148 g)	22.68	01.69	28.29	17.55^{b}±14.02	11.01	26.38	8.88	15.40^{d}±09.58
K_3KK (147 g)	09.42	00.42	07.37	05.74^{b}±04.72	22.33	53.08	6.68	27.36^{d}±23.61
K_4KA (170)	27.59	-	42.69	35.14^{b}±10.68	5.83	-	18.22	12.02^{d}±08.76
K_4KI (159 g)	38.71	45.76	-	42.23^{b}±04.98	-	-	-	-

K_4KK (165 g)	89.86	30.61	20.29	46.92^{b}±37.54	-	7.48	26.34	16.91^{d}±13.34
Mean ± SD				24.80±16.35	Mean ± SD			18.47±06.47

Notes:

- Blood samples lysis. Code column n1,n2,n3 is *triplo*measurement.

P_3, P_4 and K_3, K_4at treatment and control sample code is third and fourth cages.

0,KA,KI,KK at treatment and control samples code is no ear marker, right ear marker, left ear marker, right and left ear marker of the rats.

Superscript a vs., b at same column was different at $p < 0.05$ by independent sample t test

Superscript c vs., d at same column was different at $p < 0.05$ by independent sample t test