KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS HASANUDDIN
FAKULTAS KEDOKTERAN
Jl. Perintis Kemerdekaan Km. 10 Makassar 90245 Telp.(0411)586010, 586297

SURAT KETERANGAN JURNAL

Yang bertanda tangan dibawah ini menerangkan bahwa :

Nama	: Sri Rahayu
No Pokok	: P0200314417
Program Pendidikan	: Doktor (S3)
Program Studi	: Ilmu Kedokteran
Judul Jurnal	:

Analyis of p53 Gene mRNA Ekspression and Capcase-3 Levels as Pre-Cervical Cancer Animals Model in Wistar Rat with *Diethylstilbestrol* Induced

Naskah tersebut telah terindeks SCOPUS, IOP Ebooks, IOP Publishing Series Part Environmental, society, science and technologi, xxxdoi:10.1088/1755-315/170/5/052001

Demikian surat keterangan ini dibuat untuk dipergunakan sebagaimana mestinya.

Makassar , 27 November 2018
an. Dekan
Wakil Dekan Bidang Akademik dan Pengembangan,

Dr. dr. Irfan Idris, M.Kes
NIP.19671103 199802 1 001

KOMUNITAS KOLABORASI PUBLIKASI INDONESIA
Journal of Physics: Conference Series
IOP Publishing
Scopus

WMA-WESTECH 2018

Workshop on Multidisciplinary and Its Applications
Part Environment, Society Science and Technology
Jalan Sumatera No. 1-2, Bukit Indah, Lhokseumawe, Aceh, Indonesia
Telp/Fax: +62 645 8450348, Hp. +62-852 5596 2536 / +62-812 6326 393

Saturday, 11 November2018

Dear Mr/Mrs.
S. Rahayu

On behalf of the committee, we are pleased to inform that your manuscript is **ACCEPTED** to be presented/workshop at the WMA-WESTECH 2018, scheduled on 8 December 2018.

Number : **Medan-WMA-WESTECH-016**
Title : **ANALYSIS OF P53 GENE mRNA EXPRESSION AND CASPASE-3 LEVELS AS PRE-CERVICAL CANCER ANIMALS MODEL IN WISTAR RAT WITH DIETHYLSTILBESTROL-INDUCED**
Author(s) : **S. Rahayu**

Here are some important things we would like you to do in relation to the paper acceptance:

1. Please kindly complete the payment of:
   IDR 1.800.000,- ***(due date 31st November 2018)***
   The payment should be transferred to the following bank account:
   Bank BNI
   0476328621
   an. Robbi Rahim
2. Confirm your payment through our email on: robbirahim@ko2pi.org by sending the scan of the receipt of payment, your name, and title of the paper,
   with **Subject**: **Fee-WMA-WESTECH-016**
3. All accepted papers will be published in **IOP Earth and Environmental Science** and will be submitted for indexation by **Scopus**.
4. Process reviewed will check the templates and plagiarism. We suggest you to use Professional Translation Services **not using *Google Translate*** and using Mendeley, Zotero or EndNote to manage your citation and references.

We thank you a lot for your participation and again congratulate for your achievement.
We are looking forward to seeing you in Medan on 8 December 2018

Best Regards,

KOMUNITAS KOLABORASI PUBLIKASI INDONESIA

Robbi Rahim
WMA-WESTECH Chairman

IOP. Series: Part Environmental,society,Science and technologi. xxx doi:10.1088/1755-315/170/5/052001

Analysis of p53 gene mRNA expression and caspase-3 levels as pre-cervical cancer animals model in wistar rat with *diethylstilbestrol*-induced

S.Rahayu[1], Rosdiana Natzird[2], Muh Nasrum Massie[2], Syahrul Rauf[2], , Mochammad Hatta[2], Nurpuji Astuti A.Taslim[2] Muh.Husni Cangara[2], Dondin Sujuti,[3]Ilhamjaya Patellongi[2], Khairuddin Djawad,[2]

[1]Faculty of Health Science, State University of Singaperbangsa Karawang 41361 Indonesia.

[2]Faculty of Medicine, Hasanudin university, Makassar 90245 Indonesia

[3]Primate Research Center (PSSP) Bogor Agricultural University, 16680 west Java,Indonesia

*Corresponding author e-mail: sri.rahayu@staff.unsika.ac.id,(m):+6281212831000

The use of hormones as one of the therapies is increasingly prevalent at this time, there are many uses obtained by hormone therapy such as preventing miscarriage in pregnant women or as a contraceptive. It was reported that the contraceptive hormone was the most used method by women compared to other contraceptive methods. Diethylstilbestrol (DES) is one of the synthetic estrogen hormones that is useful as hormone replacement therapy. However, the use of DES can also trigger abnormal cell growth that will develop into cervical cancer. This study was conducted to analyze the effect of DES on the p53 gene expression and caspase-3 levels. The method of this study was a post-test design in vivo experiment in animal model which was induced with doses of DES 1500 μgram / BB at 3 days of age after birth, then observed at 35 days. Thus, it was concluded that DES has given to 3-day-old models animals and evaluate at the 35-days-old effect on decreasing p53 gene expression and caspase-3 levels.

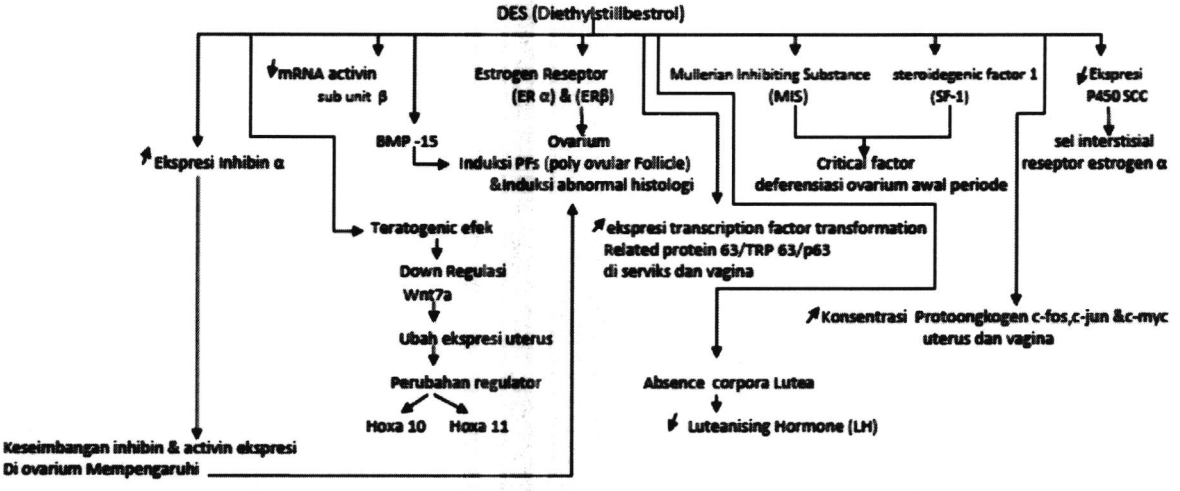
Key Words: p53, caspase-3, diethylstilbestrol, in vivo

1. Introduction

The use of hormones as one of the therapies is increasingly prevalent at this time, there are many uses obtained by hormone therapy such as preventing miscarriage in pregnant women or as a contraceptive. It was reported that the contraceptive hormone was the most used method by women compared to other contraceptive methods. Diethylstilbestrol (DES) is one of the synthetic estrogen hormones that is useful as hormone replacement therapy. However, the use of DES can also trigger abnormal cell growth that will develop into cervical cancer. In the female reproductive tract, DES can trigger cell growth (proliferation) which can develop into cancer cells [1], [2]

2. The role of DES effect

DES will also interfere the genes that regulate uterine development such as *wingless int* (wnt7a, wnt5a) and *homeobox* A (Hoxa10, Hoxa11). DES will interfere the function of tumor suppressor protein p63 gene (TRP 63/p63) which can cause abnormal cell growth, and affect cancer in the cervix and vagina [3] The use of DES with certain doses can be beneficial to the female reproductive system. However, DES also has a negative impact on the development of ovarian follicles if it used continuously with high doses and can cause infertility in women. According to [4]. DES induces *caspase-dependent apoptosis* in the human T-ALL cell line (Jurkat cells). Giving DES will trigger apoptosis at caspase, but it needs further research. On the contrary, according to [5] there is no relationship between caspase-3 and the administration of DES. Some literature explains that DES has a negative effect on the reproductive system as shown below:



Sumber: Hajek, ra. *et al.*,2014, Laronda, *et al.*, 2014, Kirigayo akiko.*et al.*,2009, Wehua, *et al.*,2003,Jefferson,*et al.*,2002,Newbold 1990,Kurita *et al.*,2001,Forsberg 1972

Figure 1. Effects of DES on the female reproductive system [6]

2.1. Neonatal exposure of DES

Neonatal exposure to estrogenic chemicals causes irreversible complex damage in the hypothalamic-pituitary-gonadal axis and reproductive system of women. Some lesions are noted after maturation as a delayed adverse effect. Uterine anomalies were detected at 1,500 mg/kg. These results show that neonatal exposure on DES which uses estrogenic in vivo activity induces a detrimental effect on the female rat in a dose-dependent manner.[7]. Damage to the hypothalamic-pituitary-gonad axis is the most worrying problem with perinatal exposure to chemicals that have estrogenic activity because the changes are caused during the development period can last throughout life. The effects of exposure may be

qualitatively different from those experienced in adulthood of rodents and humans [7]. Despite the differences in neonatal effects, it is known as the ovarian response to the rat vagina but it is not in rat. [8] (Katsuda et al., 2002; Takasugi et al., 1976) or adenomyosis induction by tamoxifen in rat [7] but uterine cancer in rat (Carthlew et al. 2000) There are a lot of experimental data on perinatal exposure to estrogen using rodents consider tend to be relevant to humans [6]. Perinatal exposure to estrogen or estrogenic compounds during the critical period disrupts the function of the hypothalamus, which results in lower production of gonadotropin (FSH and LH) [9] This disorder causes many complex abnormalities in the hypothalamic-pituitary-gonadal axis and genital tract, and a direct effect of estrogen on the genital system is also added. In general, this study was conducted to determine the effect of DES on the GEN p53 expression and caspase-3 levels.

2.2. Protein 53 /P53

The p53 protein was first identified in 1979 as a transformation related protein and protein that accumulated in the cancer nucleus and it was strongly linked to the simian virus 40 (SV40) T antigen. However, ten years later, the researchers found that the protein is a mutation from the initial form of p53 / wild-type p53 (wt p53). And the oncogenic nature of p53 is actually the result of the mutation of p53 [10]. The p53 gene in humans is located on the short arm of chromosome 17, stretching along 20 kb of DNA, consisting of 11 exons, and expressed on almost all body tissues. When DNA damage occurs, the expression of p53 in cells increases. This condition causes cell growth to stop in the G1 phase to allow DNA *repair genes* to repair DNA before the cycle continues to S phase to DNA synthesis, or in the G2/M phase before mitosis occurs. [11] P53 is a tumor suppressor protein that can prevent cancer. The ability of p53 to eliminate excess, damage, or infected cells through apoptosis. P53 also plays a role in regulating cell proliferation in multicellular organisms. P53 is activated by external and internal stress signals will cause *nuclear accumulation* in the active form, p53 prevents DNA damage or neoplastic transformation potential. P53 contributes to cell processes such as differentiation, DNA repair, and angiogenesis. Almost 50% of cancers in humans due to the mutation of the p53 gene.[12][5]. In normal conditions, p53 lives short (short-lived protein). p53 inhibitors namely Mdm2 (Hdm2 in humans) are largely responsible for maintaining the p53 balance. Mdm2 inhibits transcription, increases degradation through proteasome. P53 drastically increases when cells are exposed to stress, DNA damage, oncogenes, hypoxia and lack of nucleotides.

Generally, p53 levels are influenced by signal stress, cell type, and stress exposure time. p53 activates the target gene through a canonical sequence bond, p53 induces gene 3 (PIG3) as a sign of increased *reactive* oxygen species and apoptotic induction. PIG3 is stimulated p53 through a microsatellite sequence in an *untranslated* region, for example, a gene that encodes pro-apoptotic phosphate PAC1, p53-induced bonding is a palindrome *binding site.*[13]. The *protein* product of this gene. p53 is one of the most important molecules in biology. The various roles of p53 related to cancer are constantly being investigated. The functions of p53 have been known include regulation of the cell cycle, cell aging, apoptosis, repair of DNA damage caused by genotoxic agents, angiogenesis and regulation of oxidative stress. With a very broad relevance of functions, p53 has a controlling position that is responsible for various processes related to cancer. furthermore, the large number of interaction partners, it is not surprising that deviations in p53 are very often found in cancer [14]. Therefore, the p53 protein as a guardian of the genome is an important inhibitor of tumor development, it explains why this gene has become the most frequently mutated in human cancer. [14]. In general, apoptosis is characterized by shrinking cell size, blebbing on the membrane, chromatin condensation, and core fragmentation.[15]. There is a close relationship between missense mutation and

2.3. Caspase 3

Apoptosis is intermediated by a proteases family called caspase, which is activated through proteolysis from its inactive precursor form (zymogen). Caspase is an *endoprotease* which has the active

side of *Cys (C)* and it splits at terminal C on Asp residues, therefore it is known as Caspases (Cys containing Asp specific protease). Now, 13 members of the caspases family have been found in humans. Some members of the caspase family involved in apoptosis are divided into 2 groups. The first group consists of caspase 8, 9, 10 which contains a long prodomain at terminal N, its function as an initiator in the process of cell death. The second group consists of caspase 3, 6, 7 which contains a short prodomain, they have a function as an effector, dividing various dead substrate which ultimately leads to morphological and biochemical changes in apoptotic cells. Caspase is inactive until one of the caspases is activated by a signal, then a series of the next caspase activation reactions occur through a proteolytic process.[19]

3. Results And Discussions

This research was an *in vivo true experiment control group design.* It was conducted started from January 2017 until May 2018, animal model injection was done at Murine Farm Malang and Biochemistry laboratory of Brawijaya Malang University. Furthermore, the biomolecular examination was carried out in the Biomolecular and Immunology laboratory of the Faculty of Medicine, Hasanuddin University, Makassar. This study was conducted on Wistar rats from 4 mothers were injected with DES at 3 days of age. DES has dissolved with corn oil at a single dose of 1500 μ gram/kgBB. At 21 days of age during the weaning period with their mothers, the rats were separated between males and females, then 10 female rats were divided into 2 groups in 5. K0 was a group of rats that was not injected with DES, meanwhile, the K1 group was the one injected with the doses of DES 1500 μg at 3 days of age. At the age of 35 days, P53 gene mRNA expression and caspase-3 levels were examined. Materials And Examination Procedures : The drug used was DES obtained from *SIGMA-ALDRICH.Co.3050 Sprunce Street, ST Louis M @, 63103, USA 314-7715763.* which contains >99% of synthetic estrogen and Corn oil as DES solvents. Measurement of P53 gene mRNA expression used Real-Time PCR (RT-PCR) [20], the DNA extract activity used the boom protocol. The primary used was P53 human, a primer of *GAPDH Forward: 5'-grc cac taa agg gca tcc-3g 'reverse: 5'-cca agg tag cca tga gat cc-3, base pair 189, accesion no. XM-001067852.1* and *P53 forward: 5'-ccgc or not, gg-3 ', reverse: 5'-tt gcc ggg acg tag ac-3, base pair 125, accesion no. NM-030989.3* with one-step technology RT PCR / one-step RT PCR (Macrogen, Korea). Examination of Caspase-3 levels response was done with *rat caspase-3/caspase-3 Elisa kit* no *catalog no LS-F4138 SANDWICH,* it was done based on protocol standards on the kit.

After examination results, it found that there was a difference of P53 gene mRNA expression between K0 and K1 (12.61 ± 0.29 vs. 6.80 ± 0.48). Likewise, the serum Caspase-3 levels which was given by DES in a single dose of 1500 mcg/BB at 3 days of age and observed after 35 days of age, there was a mean difference in caspase-3 levels between K0 and K1 (4.42 ± 0.53 vs 3.27 ± 0.56). Those differences showed that the value after the DES induction where P53 gene mRNA expression and the serum Caspase-3 levels became lower; or in other words DES induction decreases P53 gene mRNA expression and serum Caspase-3 levels. Evidently, there was an effect of DES induction, thus the result of this study could strengthen the previous research that has been.

3.1. DES effect on P53 gene mRNA expression

The results of the analysis summary in table 2 showed that there was an effect after DES induction with differences (5.81 ± 0.26), from (12.61 ± 0.29^{a}) to (6.80 ± 0.48^{b}). Statistically, the results of the analysis summary in table 2, there was a significant difference ($p < 0.05$) in the group was given DES compared to the group without DES. It means, DES was given at 3 days of ages after birth can reduce the p53 gene expression at 35 days of age.

3.2. Effect of DES on Caspase-3 Levels

From the results of the analysis showed a difference between the group that was not DES-induced and after DES induction, namely: (1.14 ± 0.35) from (4.42 ± 0.53^{a}) to (3.27 ± 0.56^{b}). Statistically, there was a

significant difference ($p < 0.05$) in the group without DES *(best line)* compared to those were given DES. It means that DES was given at 3 days of age after birth can significantly reduce caspase-3 levels at 35 days of age.

4. Conclusion

Diethylstilbestrol 1500 μ gram was given at 3 days old can significantly reduce p53 gene expression at 35 days of age. Diethylstilbestrol 1500 μ gram was given at 3 days old can significantly reduce caspase 3-levels at 35 days of age.

Acknowledgments

This work was financially supported by The project of Prof . Dr.rer.nat. Marianti Manggau, Apt. and implementation of this research was carried out after training about managing animals of research in Primate Research Center (PSSP) Bogor Agricultural University. with the support of funding from the PSSP Bogor.

References

[1] M. Behbahani, "Evaluation of In Vitro Anticancer Activity of Ocimum Basilicum , Alhagi Maurorum , Calendula Officinalis and Their Parasite Cuscuta Campestris," *PLoS One*, vol. 9, no. 12, pp. 1–13, 2014.

[2] M. M. Laronda, K. Unno, L. M. Butler, and T. Kurita, "The development of cervical and vaginal adenosis as a result of diethylstilbestrol exposure in utero," *elsivier*, vol. 84, no. 3, p. 253260, 2012.

[3] and T. K. Monica M.Laronda, Kenji Unno, Lindsey M Butler, "The Development of Cervical and Vaginal Adenosis as a Result of Diethylstilbestrol Exposure In Utero," *NIH Public Access*, vol. 84, no. 3, pp. 252–260, 2013.

[4] el all Sungwook Chun1, "The Neutrophil-Lymphocyte Ratio Predicts Recurrence of Cervical Intraepithelial Neoplasia," *J. Cancer*, vol. 8, no. 12, 2017.

[5] D. P. Lane, C. F. Cheok, and S. Lain, "P53-Based Cancer Therapy.," *Cold Spring Harb. Perspect. Biol.*, vol. 2, no. 9, pp. 1–24, 2010.

[6] H. R. Troisi R, Hatch EE, Palmer JR, Titus L, Robboy SJ, Strohsnitter WC, Herbst AL, Adam E, Hyer M, "Prenatal diethylstilbestrol exposure and high- grade squamous cell neoplasia of the lower genital tract .," *PubMed*, vol. 215, no. 3, 2016.

[7] M. Yoshida, M. Takahashi, K. Inoue, S. Hayashi, A. Maekawa, and A. Nishikawa, "Delayed Adverse Effects of Neonatal Exposure to Diethylstilbestrol and Their Dose Dependency in Female Rats," *Toxicol. Pathol.*, vol. 39, no. 5, pp. 823–834, Aug. 2011.

[8] T. Ariyoshi, M. Arakaki, K. Ideguchi, Y. Ishizuka, and H. Ide, "Studies on the metabolism of d-limonene (p-mentha-1,8-diene): III. Effects of d-limonene on the lipids and drug-metabolizing enzymes in rat livers," *Xenobiotica*, vol. 5, no. 1, pp. 33–38, 1975.

[9] M. Yoshida, M. Takahashi, K. Inoue, S. Hayashi, A. Maekawa, and A. Nishikawa, "Delayed adverse effects of neonatal exposure to diethylstilbestrol and their dose dependency in female rats," *Toxicol. Pathol.*, vol. 39, no. 5, pp. 823–834, 2011.

[10] L. L. John D. Jacobson, MD, "Cervical Intraephitelial Neoplasia," *medlineplus*, vol. 136, no. 14

january. US National Library of Medicine, 2018.

[11] B. Divya, A. N. U, and S. Honnappa, "Comparative study of P53 expression between inflammatory and mild dysplasia of cervical epithelium," *Indian J. Obstet. Gynecol. Res.*, vol. 4, no. 4, pp. 356–358, 2017.

[12] K. S. Korach, M. Metzler, and J. A. McLachlan, "Estrogenic activity in vivo and in vitro of some diethylstilbestrol metabolites and analogs," *Proc. Natl. Acad. Sci.*, vol. 75, no. 1, pp. 468–471, 1978.

[13] J.-F. Lesgards, N. Baldovini, N. Vidal, and S. Pietri, "Anticancer Activities of Essential Oils Constituents and Synergy with Conventional Therapies: A Review," *Phyther. Res.*, vol. 28, no. 10, pp. 1423–1446, Oct. 2014.

[14] t. g. n. chandragiram, "Ekspresi Protein 53 (p53) Berhubungan Positif Dengan Derajat Differensiasi Sel Pada Kanker Ovarium Epitelial," Universitas Udayana Denpasar, 2014.

[15] C. H. Buckley, E. B. Butler, and H. Fox, "Review article Cervical intraepithelial neoplasia," *J. Clin. Pathol.*, vol. 35, no. 20 july 1981, pp. 1–13, 1982.

[16] H. S. Zeeshan Javed, Mukhtar Ullah*, Hafiz Ahsan Ashfaq, Afzaal Hussain Shah, Muhammad Shahzad, Muhammad Bilal, Aleena Sumrin, Hamid Bashir, Muhammad Hassan Siddiqi, "Role of MicroRNA in Endometrial Carcinoma," *Int. Q. J. Biol. Sci. Aritical*, vol. 4, no. 1, pp. 8–13, 2016.

[17] G. Gracy, K. Sadhna, J. Jacqueline, and K. Deepika, "Highlights Of P53 Mutation And It ' s Role In Cervical Cancer Metastasis," *Int. J. Biol. Med. Res.*, vol. 3, no. 1, pp. 3772–3779, 2014.

[18] S. W. Lowe and A. W. Lin, "Apoptosis in cancer," *Carcinogenesis*, vol. 21, no. 3, pp. 485–495, 2000.

[19] S. Niazi, M. Purohit, and J. H. Niazi, "Role of p53 circuitry in tumorigenesis: A brief review," *Eur. J. Med. Chem.*, vol. 158, pp. 7–24, 2018.

[20] R. R. Newbold, E. Padilla-banks, W. N. Jefferson, T. Park, and N. Carolina, "Adverse Effects of the Model Environmental Estrogen Diethylstilbestrol Are Transmitted to Subsequent Generations," *Endocr. Soc.*, vol. 147, no. December, pp. 11–17, 2016.

Research Article

Effect of Ocimum on Cervical Intraepithelial Neoplasia (CIN) Pre Cervical Cancer

S.RAHAYU*, ROSDIANA NATZIRD, MUH NASRUM MASSIE, SYAHRUL RAUF, MOCHAMMAD HATTA, MUH HUSNI CANGARA.

a Faculty of Health Science, State University of Singaperbangsa Karawang 41361Indonesia. b,e Department of Biochemistry, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia, c Molecular Biology and Immunology Laboratory for Infections Diseases, Faculty of Medicine, Hasanuddin University, Makassar 90245. Indonesia d Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia. Department of anatomical pathology faculty of medicine Hasanudin University Makassar 90245, Indonesia

Email:a.sri.rahayu@staff.unsika.ac.id,(m):+628121283 1000b.Email:rosdiananatzir@yahoo.com,c.Email:nasrumm2000@yahoo.com,d.Email:syahrulrauf@yahoo.com,e.Email:hattaram@indosat.net.id,f.Email:drhusnihusni1977@gmail.com.

Received: xxx, Revised: xxx, Accepted: xxx

Abstract

Diethylstilbestrol is a synthetic estrogen hormone which is useful as hormone replacement therapy, but it also can trigger the abnormal cells growth that will develop into cervical cancer. Cervical cancer is associated with invasion and metastasis potential where the gold standard is a histopathological examination. [1][2] Histopathological examination is a method of seeing *Cervical Intraepithelial Neoplasia* growth. There is no therapy needed at the CIN1 medium, 80% can return to normal. CIN II & III can develop into cancer cells (true cervical cancers precursors). CIN or pre-cancer therapy is more effective and efficient, and also low cost before it develops into malignant cancer cells. Accurate diagnosis and prediction of malignancy are important issues in clinical management, including biomarker identification for CIN diagnosis. CIN screening is an effective method to identify the presence of cancer earlier to decrease the mortality. CIN that has been treated can regrow/recur by some trigger factors such as age, menopause, and surgery. Ocimum has an anti-cancer effect through the caspase-3 induction which stimulates PARF to release cytochrome-c. PARF induces apoptosis in cancer cells through ROS increase. Ocimum has antioxidant activity, protects cell damage, and acts as a *scavenger highly reactive free radicals*. The results of the study group who received 100% diethylstilbestrol had Cervical Intraepithelial Neoplasm (CIN). 40% at CIN2 and CIN3 Stadium, and 20% at CIN1. Chi-square test results showed that there was a significant effect between the administration of *diethylstilbestrol* and the occurrence of CIN in p-value <0.05. Then, there was no significant effect between the administration of Ocimum extract and CIN stadium with p-value > 0.05.

Keyword: Pre, Cervical Cancer, Diethylstilbestrol, CIN, Ocimum, Perinatal, Wistar Rat, In vivo

Introduction

Based on the WHO report in 2015, it was found more than 528,000 new cervical cancer cases. About 80-90% are in developing countries. Cervical cancer is in second-ranking in all cancer cases in the world.[1]. In 2012, almost 266,000 women in the world died of cervical cancer. The number of people with cervical cancer is predicted to increase 1-5 times in 2030. [2]-[3] Cervical cancer is 98% related to the *Human Papilloma Virus* (HPV). HPV infection does not develop into cervical cancer as a whole because endogenous and exogenous factors are indicated to affect the process of cervical cancer[1], [4]. Some risk factors for cervical cancer are (1) socio-demographic; age, socioeconomic status, (2) factors of sexual activity; the age of first sexual intercourse. There is no therapy needed at the CIN1 medium, 80% from this cases can return to be normal. [5]. Women with CIN II & III need therapy such as a laser, *cryotherapy, LEEP* (*Loop Electrosurgical Excision Procedure*), and laser conization. CIN II and III cases can develop into true cervical cancers precursors within 12 months.[5]

Almost 330,000 CIN cases have been found in Europe, 50% of them are CIN2 and CIN3. 1.5 per 1000 women in developing countries were found diagnosed with CIN2 and CIN3, the highest incidence was 25 to 29 years. [6]. CIN does not cause specific symptoms. Accurate diagnosis and prediction of malignancy are important issues in clinical management, including the identification of biomarkers for CIN diagnosis.[5]

CIN *screening* is an effective method to identify the presence of cancer earlier to decrease the mortality. CIN that has been treated can regrow/recur by some trigger factors such as age, menopause, and surgery.[7].

Pre-cancer therapy is more effective and efficient, and also low cost before it develops into malignant cancer cells.[8][9]. Ocimum contains a number of important compounds such as 1-8 cineol compounds, arigin, anetol compounds, flavonoids, boron, stigma sterol, eugenol, beta-carotene, magnesium, tryptophan, and volatile. Ocimum has an anti-cancer effect through the caspase-3 induction which stimulates PARF to release cytochrome-c[10]. Ocimum has antioxidant activity, protects cell damage, and acts as a *scavenger highly reactive free radicals*. Ocimum metabolites, namely; alkaloids, polyphenols, triterpene flavonoids. These metabolites cause apoptosis by modulating p53, Bcl-2, and caspase. [11].

Ocimum leaves contain a highly active substance eugenol, by giving eugenol 100 mg/kg BB with a frequency of 3 times a week in mice induced *N-methyl-N'-nitro-N-nitrosoguanidine* (MNNG) gastric cancer for 26 weeks, causing tumor cell apoptosis and it does not cause apoptosis in the control group.[12]. Rosmarinic acid in Ocimum leaves inhibits the proliferation of tumor necrosis factor-a, inhibits the G0 – G1 and G1 – S phases.[13]. The content of terpene in Ocimum (β-element) increases H1 (Histon1) which will inhibit transcription. Histone is a protective protein of DNA structure. [14]. Research question. [15]. Ocimum leaves have an anti-cancer effect through induction of apoptosis in *cell line* (HeLa) cervical cancer. The effect of its anti-cancer is by decreasing Bcl-2 expression and increasing Bax, cytochrome-c, and caspase-3 expression.[16]. Ocimum leaves have an anti-cancer effect through induction of apoptosis in cell line (HeLa) cervical cancer. The effect of its anti-cancer is by decreasing Bcl-2 expression and increasing Bax, cytochrome-c, and caspase-3 expression.

The results of various studies above showed that Ocimum exposure causes disruption in abnormal cell growth which will activate the proapoptotic signal. Based on the explanation above, an *in vivo* study was conducted on the effect of Ocimum leaves extract in CIN stadium of pre-cervical cancer mice models after induction of diethylstilbestrol (DES)[17].

Materials and Methods

Solution Preparation and Administration of DES

DES was obtained *from SIGMA-ALDRICH.Co.3050 Sprunce Street, ST Louis M @, 63103, USA 314-7715763,* which contains >99% synthetic estrogen content. Preparation of DES 1500 dose µgram / kg BB to see the presence of CIN was done by dissolving DES in a solution of corn oil and it was given a single dose subcutaneously in 3-day-old Wistar rats, 10 grams in weight, 0.01 ml.

Extract Preparation and Administration of Ocimum

The type of Ocimum was used namely Ocimum Citriodorum which is widely available in Indonesia (Java). The extract uses Ocimum leaves from BALITRO, Bogor, West Java. The procedure of making extracts consists of 2 stages. The first stage was making Ocimum Citriodorum powder and the second stage was making Ocimum Citriodorum herbal extract by maceration with variations in the composition of ethanol and water. [18]. Then the Ocimum extract was given in a sonde to Wistar rats according to the dosage. For untreated mice, the NaCMC solution was given as a placebo.

Histopathology Examination

The cervical tissue was fixed by a frozen section method by soaking the tissue with an ethanol solution to remove liquid from the tissue, followed by a solution of toluene or xylene, then with paraffin. The final result was formed paraffin block, where it was then sliced thinly with a special microtome knife. The thin slices were then placed on a glass object, fixed further with the same solution as the chemical fixation method. The next step was painting. The tissue was painted with special dyes. Finally, after the tissue was fixed and colored, tissue in the glass object can be read under a microscope and epithelial cells can be seen in the cervical tissue.

Observation Time

Rats were divided into 4 groups, each group was consisted of 5 rats. Group 1 was the negative control group, only given a placebo in the form of 1% NaCMC. Group II was given DES at a dose of 1500µgram / kgBB without Ocimum, group III and IV was given a dose of 1500 µgram / kgBB with Ocimum extract, each dose of 600 mg / kgBB and 800 mg / kgBB, the four groups then euthanized at 49 days or 14 days after administration (given starting at 35 days), the cervical epithelium was examined in stadium CI observation for each group, the results were compared to controls.

Data Analysis

This research was an experimental research which was conducted *in vivo.*

Data analysis in this research used chi-square test.

Results and Discussions

The results of CIN description study on the cervical epithelium group given by DES and the group that received DES with Ocimum can be seen in the figure below.

Figure 1. CIN Description in the treatment and control groups

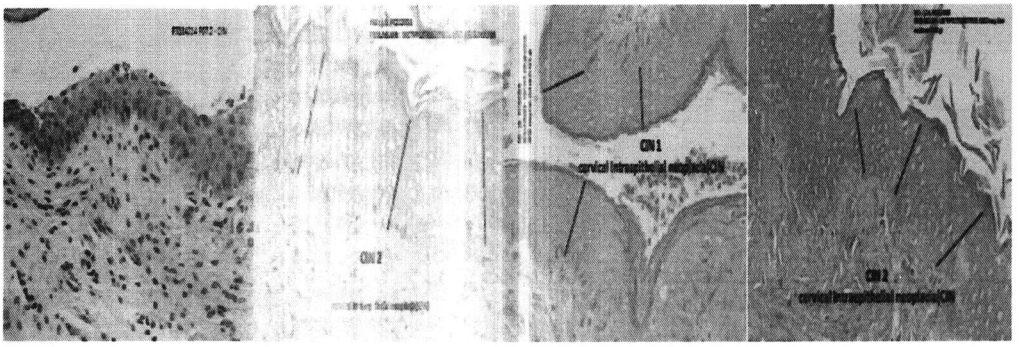
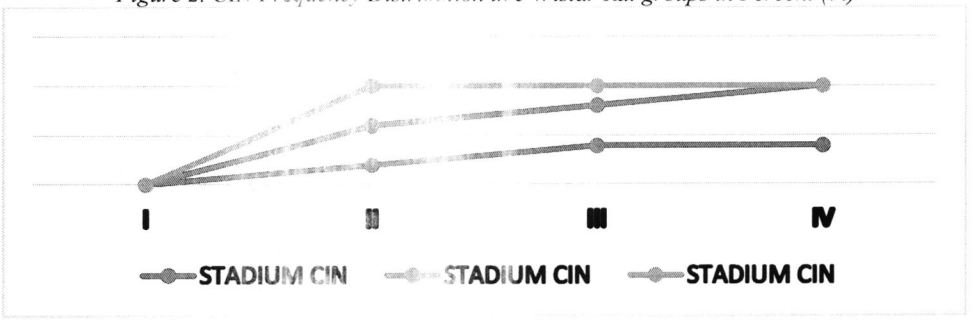


Figure 2. CIN Frequency Distribution in 5 Wistar Rat groups in Percent (%)



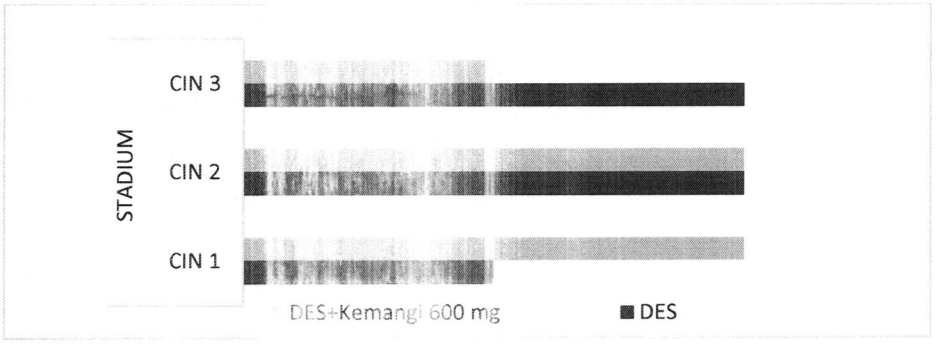
Group I, all epithelial cells grew normally. Meanwhile, group II, III, and IV cervical epithelial cells had abnormal growth (dysplasia). CIN was found in group I, II and III. CIN Stadium was seen in group IV, it was not found CIN3 stadium. This group IV received Ocimum extract at a dose of 800 mg/KgBB. The result of the *chi-square* test showed that there was a significant effect between the mean of the control group (only given DES) and the occurrence of CIN with p-value = <0.01.

The results showed that administration of DES 100% caused cervical epithelial dysplasia (CIN). [19] and stimulates the formation of the abnormal cell. DES works to interfere neuroendocrine, inhibits the release of the hormone gonadotrophin-release hormone (GnRH). DES will reduce the level of *kiss peptin*1, mRNA will reduce GnRH stimulation in neurons stimulated by *kiss peptin*. *Kiss Peptin* is a regulator or regulates the production of GnRH. *Kiss peptin*, also regulates ovulation, estrus cycles, sex differentiation, and affects puberty. [20].

DES causes proliferation of vaginal epithelium through estrogen receptors. [21] Estrogen receptors are in the nucleus, estrogen bonds, and estrogen receptors increase transcription in the target gene. Activation of these target genes will increase the tissue response, followed by the increasing of protooncogene including mRNA *c-fos, c-jun,* and *c-myc.* In the vaginal epithelium, the increasing of protooncogene *c-fos, c-jun* is almost 4-fold. [22]

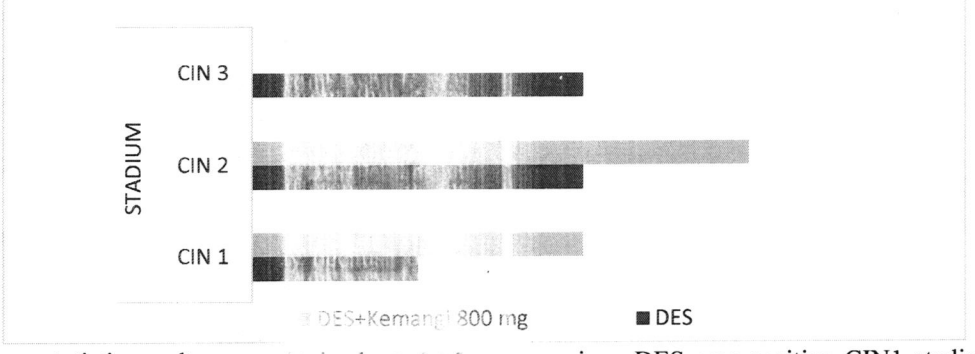
DES or diethylstilbestrol is a synthesis of estrogen, has the chemical formula is $C_{18}H_{20}O_2$. DES is used to prevent miscarriage or premature labor. In 1946-1971, around 2 to 4 million individuals were exposed to DES during their pregnancy.[13] Women exposed to DES during pregnancy causes a disorder called DES *daughters.* DES *daughters* show reproductive tract abnormalities in the form of vaginal and cervical adenoid followed by the development of columnar epithelium in the cervix and vagina. DES *daughter* has a risk of developing *cervical clear cell-adenoma.* Adenosis cervical vaginal is a precursor of adenocarcinoma. [5]

Figure 3. CIN Stadium, the Rat got DES and DES with Ocimum 600 mg/kgBB



The *Chi-square* test results showed, there was no significant difference in the mean group which was given DES and Ocimum extract, with a dose of 600mg/KgBB compared to the control group with the p-value = >0.05. There was no significant difference in the statistical test results because the number of samples was relatively small (5 rats) or possibly because of the CIN stadium was not homogeneous/different, thus it was difficult to observe the effect of Ocimum on non-homogeneous CIN stadium. It recommends, in the future research to see the effect of Ocimum on pre-cancer, the group of rat must be at the same CIN stadium. However, the results of the study illustrate that the group was given DES and Ocimum extract doses of 600 mg/KgBB, the frequency of CIN3 stadium was lower than CIN1 and 2.

Figure 4. CIN Stadium, the Rat got DES and DES with Ocimum 800 mg/kgBB



Chi-square statistic results were obtained p > 0.05, which means, there was no significant difference the mean of groups were given DES and Ocimum extract before and after administration of 800mg/KgBB with CIN medium with p-value = > 0.05. (0.08). There was no significant difference in the statistical test results it possible because of the lack of doses, thus the future research, it should increase the doses. The number of samples was relatively small (5 rats) or possibly because of the CIN stadium was not homogeneous/different, thus it was difficult to observe the effect of Ocimum on non-homogeneous CIN stadium. Giving Ocimum leaves extract doses of 800 mg/Kg BB in CIN, all rat groups were given DES was positive CIN1 stadium, 2, and 3 but DES group with Ocimum extract, it was not found CIN3 medium.

Conclusions

The results of this study, there was a significant effect between administration of diethylstilbestrol with CIN ($p < 0.05$) and there was no significant correlation between the effects of Ocimum extract dose of 600mg/Kg BB and a dose of 800mg/KgBB on CIN with $p > 0.05$. This condition may be caused by the number of small samples or inappropriate dose given to inhibit the growth of abnormal cells in the cervix. However, there was no CIN3 found in the 800mg dose group where Ocimum had an anti-cancer effect.

References

[1] P. Tsikouras *et al.*, "Cervical cancer: Screening, diagnosis and staging," *Journal of B.U.ON.*, vol. 21, no. 2. pp. 320–325, 2016.

[2] L. A. Torre, F. Bray, R. L. Siegel, J. Ferlay, J. Lortet-tieulent, and A. Jemal, "Global Cancer Statistics, 2012," *CA a cancer J. Clin.*, vol. 65, no. 2, pp. 87–108, 2015.

[3] N. Kumar, "Women's Health & Gynecology Cervical Cancer; a Nightmare for Womanhood: Review of Recent Advances," *Womens Heal. Gynecol.*, vol. 2, no. 2, pp. 30–34, 2012.

[4] GracyG, SadhnaK, JacquelineJ, and DeepikaK, "Highlights Of P53 Mutation And It's Role In Cervical Cancer Metastasis," *Int. J. Biol. Med. Res. Int J Biol Med Res. Int J Biol Med Res*, vol. 5, no. 1, pp. 3772–3779, 2014.

[5] A. Mitildzans, A. Arechvo, D. Rezeberga, and S. Isajevs, "Expression of p63, p53 and ki-67 in patients with cervical intraepithelial neoplasia," *Turkish J. Pathol.*, vol. 33, pp. 9–16, 2016.

[6] S. Vegunta, J. A. Files, and M. N. W. Do, "Screening Women at High Risk for Cervical," *Mayo Clin. Proc.*, vol. 92, no. 8, pp. 1272–1277, 2017.

[7] H.-B. K. Sungwook Chun1, Kyusik Shin, Ki Hyung Kim, Heung Yeol Kim, Wankyu Eo, Ji Young Lee, Jeong Namkung, Sang Hoon Kwon8, Suk Bong Koh, "The Neutrophil-Lymphocyte Ratio Predicts Recurrence of Cervical Intraepithelial Neoplasia," *J. Cancer*, vol. 8, no. 12, 2017.

[8] B. Divya, A. N. U, and S. Honnappa, "Comparative study of P53 expression between inflammatory and mild dysplasia of cervical epithelium," *Indian J. Obstet. Gynecol. Res.*, vol. 4, no. 4, pp. 356–358, 2017.

[9] S. Vegunta, J. A. Files, and M. N. Wasson, "Screening Women at High Risk for Cervical Cancer: Special Groups of Women Who Require More Frequent Screening," *Mayo Clin. Proc.*, vol. 92, no. 8, pp. 1272–1277, 2017.

[10] M. U. K. Harsimran Singh*1, Mishu Sharma1, Jagdeep Kaur1, Pms Bedi1, "Diverse Role of Ocimum Sanctum : A Magic Remedy of Nature," *Indo Am. J. Pharm. Res.*, vol. 11, no. 3, 2018.

[11] M. Behbahani, "Evaluation of In Vitro Anticancer Activity of Ocimum Basilicum , Alhagi Maurorum , Calendula Officinalis and Their Parasite Cuscuta Campestris," *PLoS One*, vol. 9, no. 12, pp. 1–13, 2014.

[12] N. Singh, P. Verma, B. R. Pandey, and M. Bhalla, "Review Article Therapeutic Potential of Ocimum sanctum in Prevention and Treatment of Cancer and Exposure to Radiation : An Overview," *Int. J. Pharm. Sci. Drug Res. 2012;*, vol. 4, no. 2, pp. 97–104, 2012.

[13] J. Manosroi, P. Dhumtanom, and A. Manosroi, "Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines," *elsevier*, vol. 235, no. 8, pp. 114–120, 2006.

[14] J.-F. Lesgards, N. Baldovini, N. Vidal, and S. Pietri, "Anticancer Activities of Essential Oils Constituents and Synergy with Conventional Therapies: A Review," *Phyther. Res.*, vol. 28, no. 10, pp. 1423–1446, Oct. 2014.

[15] A. K. Jha, M. Jha, and J. Kaur, "Ethanolic extracts of Ocimum sanctum, Azadirachta indica and Withania somnifera cause apoptosis in SiHa cells," *Res. J. Pharm. Biol. Chem. Sci.*, vol. 3, no. 2, pp. 557–562, 2012.

[16] M. A. Webb, S. I. N. Ekunwe, B. Herbert, and G. Begonia, "The Mechanisms By Which Fractions Of Ocimum gratissimum (Og) Leaf Extracts Inhibit The Proliferation Of Prostate Cancer," in *Poster Session A [Students]*, 2016, p. 200.

[17] S. Rahayu *et al.*, "Ocimum Basilicum as Alternative Natural Cancer Care," *Int. J. Sci. Basic Appl. Res.*, vol. 34, no. 3, pp. 302–308, 2017.

[18] A. B. S. Lestari, L. U. Susanti, and Y. Dwiatmaka, "Optimasi Pelarut Etanol-Air dalam Proses Ekstraksi Herba Pegagan (Centella Asiatica (L.) Urban) pada Suhu Terukur," *Bionatura J. Ilmu-ilmu Hayati dan Fis.*, vol. 14, no. 2, pp. 87–93, 2012.

[19] B. Joseph, "Ocimum Sanctum Linn . (Holy Basil): Pharmacology Behind Its Anti- Cancerous Effect," *Int. J. Pharma Bio Sci.*, vol. 4, no. 2, pp. 556–575, 2013.

[20] M. Yoshida, M. Takahashi, K. Inoue, S. Hayashi, A. Maekawa, and A. Nishikawa, "Delayed Adverse Effects of Neonatal Exposure to Diethylstilbestrol and Their Dose Dependency in Female Rats," *Toxicol. Pathol.*, vol. 39, no. 5, pp. 823–834, Aug. 2011.

[21] S. G. Silverberg, "Problems in the differential diagnosis of endometrial hyperplasia and carcinoma," *Mod. Pathol.*, vol. 13, no. 3, pp. 309–327, 2000.

[22] O. E. Rivera, J. Varayoud, H. A. Rodríguez, M. Muñoz-de-Toro, and E. H. Luque, "Neonatal exposure to bisphenol A or diethylstilbestrol alters the ovarian follicular dynamics in the lamb," *Reprod. Toxicol.*, 2011.