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

Preprint · March 2019

DOI: 10.13140/RG.2.2.16419.50721

6 authors, including:

Sri Rahayu
Universitas Singaperbangsa Karawang
20 PUBLICATIONS **12** CITATIONS
SEE PROFILE

Rosdiana Natzir
Universitas Hasanuddin
80 PUBLICATIONS **116** CITATIONS
SEE PROFILE

Muhammad Nasrum
Universitas Hasanuddin
96 PUBLICATIONS **344** CITATIONS
SEE PROFILE

Syahrul Rauf
6 PUBLICATIONS **0** CITATIONS
SEE PROFILE

ISSN 0975-2366
https://doi.org/10.31838/ijpr/2019.11.01.007

Research Article

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

S.RAHAYU[1]*, ROSDIANA NATZIRD[2], MUH NASRUM MASSIE[3], SYAHRUL RAUFF[4], MOCHAMMAD HATTA[5], MUH HUSNI CANGARA[6].

[1]Faculty of Health Science, State University of SingaperbangsaKarawang 41361 Indonesia.
Email: a.sri.rahayu@staff.unsika.ac.id,(m):+6281212831000b.
[2,5]Department of Biochemistry, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia, Email:rosdiananatzir@yahoo.com, Email: hattaram@indosat.net.id
[3]Molecular Biology and Immunology Laboratory for Infections Diseases, Faculty of Medicine, Hasanuddin University, Makassar 90245. Indonesia Email:nasrumm2000@yahoo.com,
[4] 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:syahrulrauf@yahoo.com,[6]Email:drhusnihusni1977@gmail.com.
Received: 10.10.18, Revised: 09.12.18, Accepted: 19.01.19

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. Ocimumhas 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 CIN3Stadium, 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 withp-value > 0.05.

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

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. [1]–[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 1-8cineol compounds, arigin, anetol compounds, flavonoids, boron, stigma sterol, eugenol, beta-carotene, magnesium, tryptophan, and volatile. Ocimumhas an anti-cancer effect through the caspase-3 induction which stimulates PARF to release cytochrome-c[10]. Ocimumhas antioxidant activity, protects cell damage, and acts as a *scavenger highly reactive free radicals*.Ocimummetabolites, namely; alkaloids, polyphenols, triterpene flavonoids. These metabolites cause apoptosis by modulating p53, Bcl-2, and caspase.[11].Ocimumleaves 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 Ocimumleaves 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]. Ocimumleaves 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].Ocimumleaves 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 andAdministration of Ocimum

The type of Ocimum was used namely OcimumCitriodorum 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 OcimumCitriodorumpowder and the second stage was making OcimumCitriodorumherbal 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 wasformed 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.

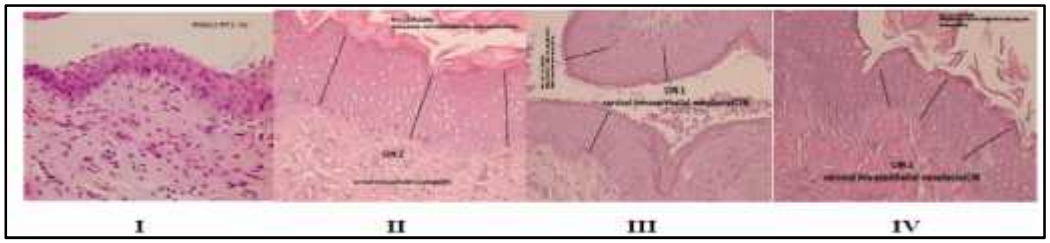

Figure 1. CIN Description in the treatment and control groups

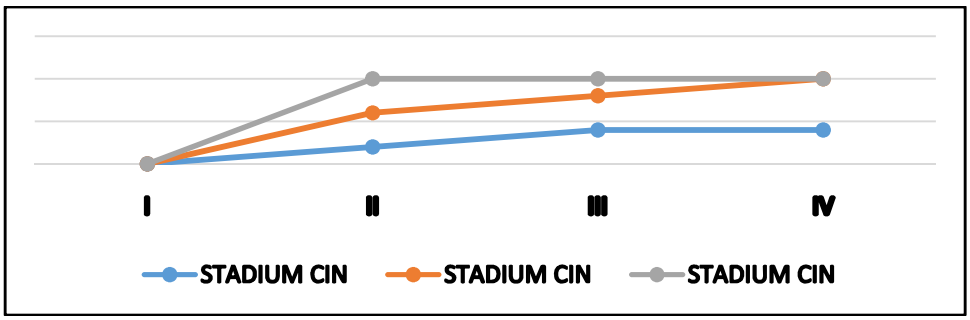

Figure 2. CIN Frequency Distribution in 5 Wistar Rat groups in Percent (%)

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 CIN3stadium. 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]

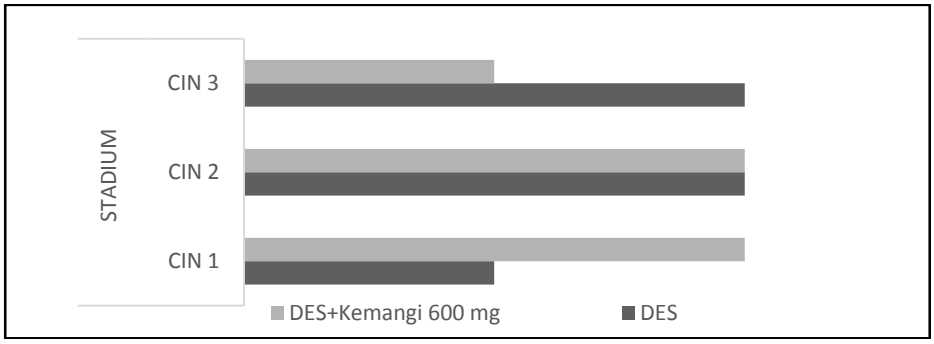

Figure 3. CINStadium, the Rat got DES and DES with Ocimum600 mg/kgBB

The *Chi-square* test results showed, there was no significant difference in the mean group which was given DES and Ocimumextract, 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 wasdifficult to observe the effect of Ocimumon non-homogeneous CINstadium.It recommends, in the future research to see the effect of Ocimumon pre-cancer, the group of rat must be at the same CINstadium. However, the results of the study illustrate that the group was given DES and Ocimumextract doses of 600mg/KgBB, the frequency of CIN3 stadium was lower than CIN1 and 2.

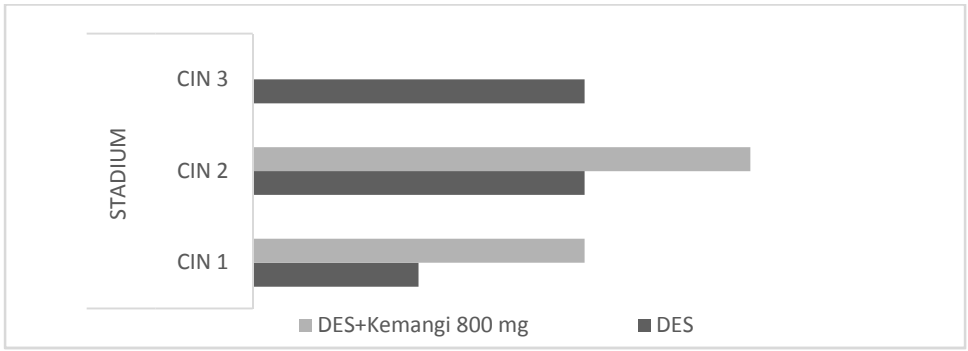

Figure 4. CIN Stadium, the Rat got DES and DES with Ocimum800 mg/kgBB

Chi-square statistic results were obtained p> 0.05, which means, there was no significant difference the mean of groups were given DES and Ocimumextract 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 Ocimumon non-homogeneous CINstadium.Giving Ocimumleaves 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 Ocimumextract, it was not found CIN3medium.

Conclusion

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 ofOcimumextract dose of 600mg/Kg BB and a dose of

800mg/KgBBon 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 whereOcimumhad 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.

    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.