# Analysis of P53 Gene Mrna Expression and Caspase-3 Levels As PreCervical Cancer Animals Model in Wistar Rat With DiethylstilbestrolInduced

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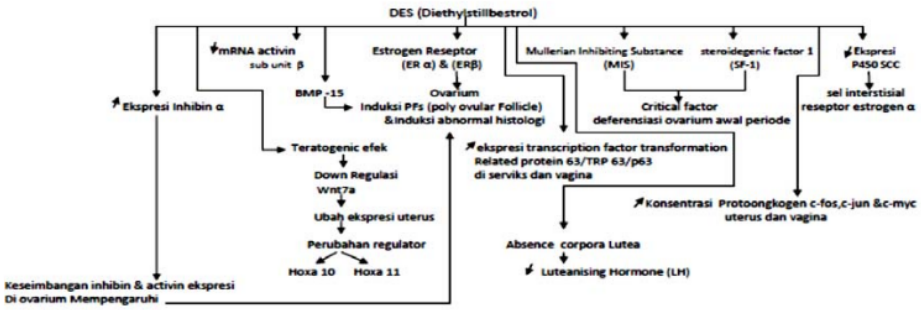
**Abstract.** The use of hormones as one of the therapies is increasingly prevalent at this time, there are many uses obtained by hormone therapy such as preventing miscarriage in pregnant women or as a contraceptive. It was reported that the contraceptive hormone was the most used method by women compared 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. P53 is a cancer suppressor gene and caspase 3 is a gene that plays a vital role in the process of apoptosis. 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. The results showed that there was a significant decrease in p53 gene expression and caspase-3 between DES-induced animals models compared with DES-uninduced with p-value = <0.05. 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.

## 1. Introduction

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] 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]

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.

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 overexpression of p53 protein. *Nonsense mutation*, insertion, and deletion in p53 are also found [16].

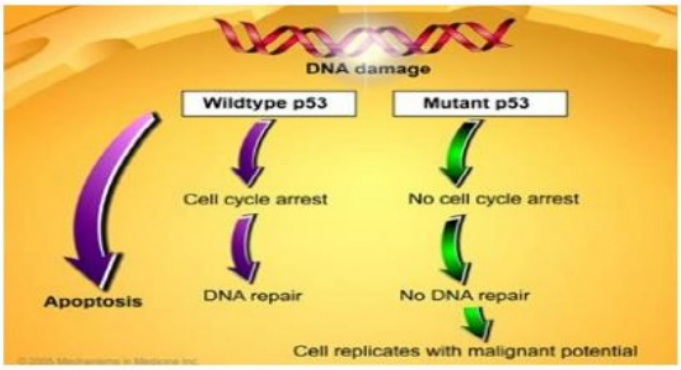



**Figure 2.** Normal and abnormal mechanism of p53 [17]

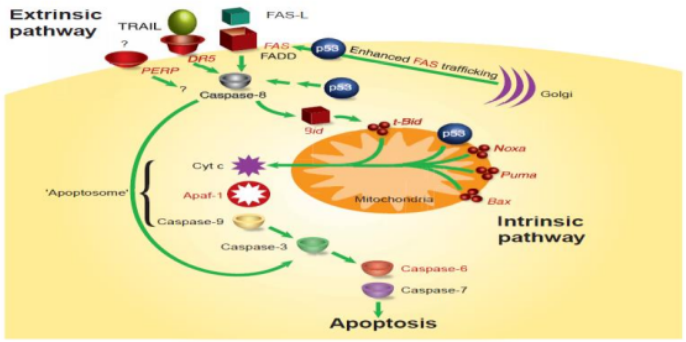



**Figure 3.** Model of p53 mediated apoptosis [18].

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]

## 2. Method, Time, And Research Site

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.

## 3. 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.

## 4. Results And Discussions

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.

a. 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.

b. 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.

## 5. Conclusion

a. Diethylstilbestrol 1500 µ gram was given at 3 days old can significantly reduce p53 gene expression at 35 days of age.
b. Diethylstilbestrol 1500 µ gram was given at 3 days old can significantly reduce caspase 3-levels at 35 days of age.
c. Good expertise is needed during DES injection on Wistar rats at 3 days of age because it is possible the rats are eaten by their mother after injection.
d. The results of this study will be used for further research entitle the effect of Ocimum Citriodorum on animal model pre-cervical cancer induced with diethylstilbestrol

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