Smoke Effects of Disturbances Folliculogenesis (Mda, Gnrh, Hsp70, Apoptosis, and Follicles) in Ovarian on Mice Balb / C

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Abstract

One of the triggers for the disruption of the follicle maturation process along with the oocyte core is the presence of reactive oxygen species (ROS) can affect oxidative stress caused by substances in cigarette smoke. Female passive smokers experience menstrual disorders in the reproductive system, less fetal weight and other reproductive problems. The reality in the community, smoking habits are still difficult to stop, even though health workers and the media have often been socializing about the dangers of cigarette smoke.

The aim of study was to explain the effect of cigarette smoke on damage to theca cells in follicles in the ovaries in balb / c mice exposed to cigarette smoke. The subjects used in this study were 20 mice (Mus Musculus) Balbb / c females aged around 8-10 weeks with a bodyweight of 25-30 grams . he results showed the test statistic Mann Whithney smoke increased MDA (Malondeldehide) levels (P= .000), decreased GnRH (Gonadothrophin Relesing Hormone) levels (P= .000) and increased the theca cell apoptosis index (P= .000), and the results of the Independent T-statistic test results Tests showed that exposure to cigarette smoke decreased HSP (Heat Shock Protein) 70 expression (P= .002) and decreased the number of follicles in the ovary (P= .004).

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Introduction

Infertility is a problem for women and men throughout the world. The prevalence of causes of infertility in women according to WHO among them is ovulation disorders 33%. Disruption of the process of ovulation is caused by disorders of the hypothalamus-pituitary, ovary, and endometrium, resulting in disruption of the follicular maturation process and impaired implantation. One of the triggers for oocyte maturation process disruption is the presence of ROS which can affect the occurrence of oxidative stress, one of which is caused by substances contained in cigarette smoke. Cigarette smoke contains 4800 kinds of dangerous chemical compounds and one of them is free radicals[1,22].

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Hot steam in cigarette smoke can enter the respiratory tract, then react with NOX to NADPH oxidase and O2 O (superoxide). There are no product standards that for controlling the levels of dosing, contaminants, toxins, and carcinogens in the liquids used in ecigarettes as well as the aerosols they produce[14,23].

Help with SOD, the superoxide radical (O2-) is catalyzed into hydrogen peroxide (H2O2) and oxygen (O2). H2O2 oxidants are systemic that can affect all cells in the body including the female reproductive organs, namely the ovaries[2,24,25]. H2O2 will enter the blood vessels and react with the lipid membrane, then lipid membrane peroxidation will occur, causing MDA levels in the blood to increase. MDA levels are oxidative stress biomarkers if MDA levels increase indicates that oxidative stress occurs in cells[3]. The role of GnRH in folliculogenesis is the Gonadotropin-releasing hormone (GnRH) hormone produced by the hypothalamus, part of your large brain. Its function is to stimulate the body to make and release FSH and LH hormones. Hormone follicle-stimulating hormone

(FSH) itself, produced in the pituitary gland in the brain. This hormone functions to make the eggs in the egg sacs (ovaries) mature and ready to be released into the uterus. Luteinizing hormone (LH) itself, is a hormone produced by the pituitary gland to stimulate the ovaries to release eggs. If cigarette smoke affects GnRH levels in blood serum, it will also affect FSH and LH production by hypnosis in the brain.

H2O2 also penetrates the ovarian organs, in the ovary, there are follicles that play a role for ovulation. follicles have theca cells, granulosa cells, and oocyte cells. The three cells communicate with each other and affect the growth and development of follicles, namely from primordial follicles to degraft follicles. H2O2 which is increased due to cigarette smoke will penetrate the ovaries and affect theca cells. Increased H2O2 causes oxidative stress which can trigger apoptosis, but apoptosis can be prevented by HSP 70. HSP 70 is responsible for cell homeostasis. HSP acts as a means to adapt to environmental changes that cause oxidative stress such as changes in temperature, PH, oxygen radicals, metabolic disorders[4,26,27], It is hoped that HSP 70 is still high so that it can prevent apoptosis in cells.

Materials and methods

This study uses design *True Experimental* with a *post-test control group design.* The subjects used in this study were 20 mice (Mus Musculus) Balbb / c females aged around 8-10 weeks with a bodyweight of 25-30 grams. The independent variable is exposure to cigarette smoke, while the dependent variable is the MDA level, GnRH, HSP70, theca cell apoptosis, and the number of follicles. The research was divided into 2 groups: Group 1: Control group without the provision of cigarette smoke, Group 2: the treatment group by giving 1 cigarette smoke dose per day for 20 days using a smoking pump. The study was conducted in the embryology department of the Faculty of Veterinary Medicine, University Airlangga. Data analysis using the *independent Mann Whithney*.

Results

MDA levels were measured in blood serum using the ELISA test, showing that the treatment group exposed to cigarette smoke had twice the MDA levels compared to the control group. Exposure to cigarette smoke reduced GnRH levels in blood serum 2 times lower than in the control group. Likewise with HSP70 expression, apoptosis of theca cells and the number of follicles as seen in Table 1.

Variable	Group	Mean	SD	Min	Max	p
MDA	control	156.4	36.8651	91.61	216.26	
	Treatment	128.44 205.22 575.44		98.43	321.92	.003
GnRH	control	53.56025 185.22 366.92	32,34	92.22	259.49	
	Treatment	35.79497 74.81 189.64	21.61	76.23	148.21	.000
HSP70	control	7.1010	11,270	2.3	22.3	
	Treatment	1.39	0.9158	0.2	2.6	,002
Apoptosis theca cells	control	.0	.11		0.00	
	Treatment	.70	.21	0.4	1.10	.000
follicles	control	.004	3.985	15	26	
	Treatment	13.70	5.519	6	21	.90

Table 1. MDA levels, GnRH, HSP70 Expression, theca cell apoptosis index, and total follicles.

Tabel 1 showed Variabel MDA Control have Mean 156,4±36,8651, Treatment Group have Mean 128,4±36,8651 (*P*= .003). Variabel GnRH Control have Mean 53,56±32,34 Treatment Group have Mean 35,7±21.61 (*P*= .000), Variabel HSP70 Control have Mean 7,1±11,27 Treatment Group have Mean 01,39±0.91 (*P*= .002), Variabel Apotosis Theca Cells Control have Mean ,0±0.11 Treatment Group have Mean 0,7±0.21 (*P*= .000)Variabel Follicles Control have Mean .004±3,985, Treatment Group have Mean 13,70±5,519 (*P*= .9)

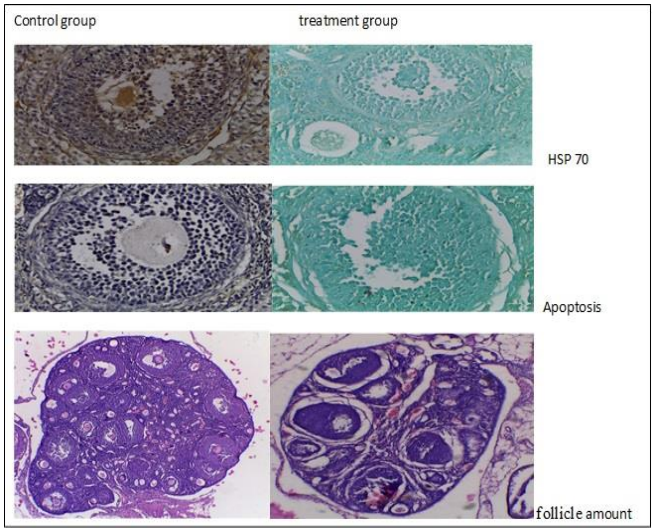

Figure 1. HSP 70 expression, theca cell apoptosis, and number of follicles in the ovary.

Figure 1 shows that in the control group HSP 70 expression in theca cells is positive, this is not seen in the cigarette smoke exposure treatment group that HSP 70 expression in the theca cell is negative. In Figure 1 it can also be seen that in the control group the theca cell apoptosis index is negative, whereas in the cigarette smoke treatment group it is positive. Whereas the number of follicles in the control group had many more follicles than the treatment group.

Discussion

Exposure to cigarette smoke increases the blood serum levels of MDA in mice, cigarette smoke is an exogenous source of free radicals. Free radicals and ROS in the body can cause oxidative damage to unsaturated fat bonds in membrane phospholipids (lipid peroxidation) that damage the membrane structure and cell organelle function[3,4,18,19,20]. H2O2 produced from the reaction of cigarette smoke will enter the blood vessels and react with the lipid membrane, then lipid membrane peroxidation will occur, causing MDA levels in the blood to increase. MDA levels are oxidative stress biomarkers if MDA levels increase, it indicates that oxidative stress occurs in cells[3,4,5,18,19,20]

The facts obtained in this study were exposed to cigarette smoke proved to reduce the average GnRH levels in mice, in the exposed group was lower than in the control group. Exposure to cigarette smoke affects a variety of metabolic and biological processes in the body including hormone secretion, this is mainly caused by nicotine thereby increasing the effects of oxidative stress due to an increase in free radicals in the body caused by cigarette smoke. Smoking affects the pituitary, adrenal, testicular and ovarian functions. Cigarette smoke contains 4800 kinds of dangerous chemical compounds and one of them is free radicals[1,6,7]. H2O2 caused by the reaction of cigarette smoke causes *ROS to* increase. *ROS* will cause GnR protein to turn into carbonyl protein. Carbonyl protein enters the proteasome, thereby causing the inhibition of serum GnRH secretion. As a result, FSH and LH secretion also decreases. At the beginning of the LH receptor cycle, it is only found in theca cells, whereas FSH receptors are only found in granulosa cells. LH triggers theca cells to produce androgen hormones, then the androgen hormones enter granulosa cells. FSH with the help of the aromatase enzyme converts androgens to estrogen in granulosa cells[5,6]. However, In smoking activity, oral tissues are initially exposed to various toxins contained in cigarette smoke, thus allowing the toxins to spread to other organs in the body, if the secretion of GnRH is low, the LH will also be lowest so that the theca cells in the follicle are not fully formed so that folliculogenesis is disturbed, because of folliculogenesis involves one of the cells, namely theca cells[17,21]

H2O2 which is increased due to cigarette smoke, will penetrate the ovaries and affect theca cells. Increased H2O2 causes oxidative stress which can trigger apoptosis, but apoptosis can be prevented by HSP 70. HSP acts as a means to adapt to environmental changes that cause oxidative stress such as changes in temperature, PH, oxygen radicals, metabolic disorders[4,7,8]. HSP 70 can prevent the process of apoptosis by disrupting the formation of apoptosome by binding to apaphs, so that caspase 9 and caspase 3 activation does not occur[6,7,8,15,16] .But if HSP 70 is low, it cannot prevent apoptosis in the theca cells. Theca cells play a role in follicular development, follicular development is characterized by theca development[7,8,9,10,12]. If theca cells occur apoptosis, it will inhibit the formation of progesterone into androstenedione which is a staple to become estrogen in granulosa cells, thus affecting the number and development of follicles. The maturation process from primordial follicles to preovulatory follicles is called folliculogenesis[8,9,10,11] .In this study, the development of folliculogenesis is seen from the description of the number of follicles[12,13]. In table 1 the comparison of the average number of ovarian follicles between the control group and exposure to cigarette smoke shows that the cigarette smoke exposure group has a lower average. Then a comparative test was performed to see the significance level of the difference between the control and exposure groups, statistically significant there was a decrease in the number of follicles.

Conclusions

The conclusion of this study is that cigarette smoke increases oxidative stress in the body, this can be seen from the increase in MDA

levels in blood serum, besides that cigarette smoke also decreases GnRH levels even though GnRH is a hormone that enhances the production of LH and FSH for the process of folliculogenesis in the ovary cigarette smoke also decreases the expression of HSP 70 in theca cells on HSP 70 is very necessary to reduce oxidative stress on cells so that it can prevent the occurrence of apoptosis in cells, so that as a result in theca cells there is a lot of theca cell apoptosis in follicles in the ovary, theca cell apoptosis causing folliculogenesis disorders thereby reducing the number of follicles in the ovary.

Declaration of Interest

The authors report no conflict of interest.

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