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THE EFFECT OF ETHANOL EXTRACTS RED BEET (*BETA VULGARIS L.*) INCREASE SERUM LEVELS OF 17B-ESTRADIOL AND FOLLICULOGENESIS IN FEMALE RATS EXPOSURED BY CIGARETTE SMOKE

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ABSTRACT

The free radicals contained in cigarette smoke can improve oxidative stress. The state of oxidative stress results in decreased levels of 17β-estradiol. Oxidative stress is also able to reduce on the work of ovarian folliculogenesis *Rattus norvegicus* Exposured by cigarette smoke. Importance sought natural alternatives that are rich in antioxidants and anti-inflammatory. This study aims to prove the influence provision of Ethanol Extract Red Beet (*Beta vulgaris L.*) To increased Levels17β-estradiol and folliculogenesis Sample use female rats exposed to cigarette smoke 2 sticks per day for 56 days. Red beet (Beta vulgaris L.) containing betalain potential as an antioxidant and anti-inflammatory. Experimental studies using post-test only control group design, Sample use female rats (*Rattus novergicus*) as many as 25 animals. Rats were divided into 5 groups that group control negative (no treatment), control group positive (exposed to cigarette smoke), the group P1 (exposed to cigarette smoke and were given ethanol extract of red beet dose of group 1 (exposed to cigarette smoke and red beet extract ethanol dose of 125 mg.Kg.WB^{-1}/day), group P2 (exposed to cigarette smoke and red beet extract ethanol dose of 250 mg.Kg.WB^{-1}/day) and group P3 (exposed to cigarette smoke and red beet extract ethanol dose of 500 mg.Kg.WB^{-1}/day). The results showed that red beet extract Increased level of 17β-estradiol and folliculogenesis with p-value = 0.000 <α. It can be concluded that the extract of ethanol red beet (*Beta vulgaris L.*) can increase the levels of 17β-estradiol and folliculogenesis Sample use female *Rattus norvegicus* to cigarette smoke exposed

KEYWORDS: Cigarette smoke, red beets, 17β-estradiol, folliculogenesis.

INTRUDUCTION

The prevalence of infertility increased globally and the number of infertile couples reach 48.5 million in 2010.[1] Factor lifestyle such as illicit drug use, alcohol consumption, caffeine, and smoking can affect infertility.[2] Cigarette smoke contains particulate and gaseous components which are highly reactive free radicals and easy to react with other compounds such as proteins, DNA and lipids[3]. Oxidative stress caused by cigarette smoke also can inhibit GnRH pulses through the GABAA receptor system. Inhibition of GnRH pulses will cause interference with the synthesis and secretion of *Follicle Stimulating Hormone* (FSH) and *Luteinizing Hormone* (LH).[4] Oxidative stress generated by cigarette smoke are also capable of interfering with the process of folliculogenesis, one antral follicles that have an important role in the process of folliculogenesis as it can predict the quality of their ovaries to achieve ovulation.[5] In addition, exposure to secondhand smoke is also associated with early menopause and loss of ovarian follicles via cell death pathways. In a study use rats, cigarette smoke decreases the number of ovarian follicles by increasing oxidative stress and the formation of autophagosome on granulosa cells without inducing apoptosis. Disorders of ovarian follicles causing low fertility condition characterized by a low number of

oocytes in the ovary and damaged preantral oocyte development.[6]

Free radicals can be dealt with by the body with a special compound that is endogenous and exogenous antioxidants. The enzyme superoxide dismutase (SOD) is one of the main or primary endogenous antioxidants that counteract free radicals in the body. In a state of oxidative stress predicted SOD concentration will decrease and it required exogenous or secondary antioxidants from food either from natural or synthetic sources to assist in the control of free radicals in the body.[7,8]

Fruits and vegetables have been found in many studies to protect the body from some diseases such as cancer, kardiovaskiler disease, and immune dysfunction. This natural protective effect is due to a variety of components such as carotenoids, lycopene, vitamins, polyphenols, betalains and other fitokoimia. Betalain, betacyanins, and betaxanthin is pigment vacuolar nitrogen-containing water-soluble. Betalain has antiradical and antioxidant activity as high. Betalain also used as additives in the food industry because of its natural coloring properties, high solubility in water and absence of toxicity. Red beet (*Beta vulgaris L.*) is the most important food products that contain dyes of this type.[9]

Bit-owned red Compounds include polyphenols, flavonoids, betalain, vitamins and folic acid. Most abundant antioxidant in red Bit is betalain compound which forms the red pigment in fruits Bit and useful for controlling free radicals.[10] Research Cho et al.[8] Bit extract of red states that not only stimulate cell proliferation but also minimize damage to DNA. Mice treated with the extract of red Bit showed an increase in hematocrit and hemoglobin and red blood cell counts. The purpose of this study was to determine the effect of ethanol extract of red beet against increasing levels of 17β-estradiol serum and Folliculogenesis in female rats exposed to cigarette smoke.

MATERIALS AND METHOD

The research was carried out experimentally with the study design group Post Test Only Control Design conducted in the laboratory, to see the effect of red beet extract against 17β-estradiol serum levels and folliculogenesis in female rats Eksposured by cigarette smoke. Subjects in this study were female rats (*Rattus novergicus*) were divided into five groups and each group consisted of 5 rats. 5 groups include the following: 1 negative control group, 1 positive control group were only exposed by smoke 2 cigarettes/day for 56 days, and 3 treatment groups exposed to smoke two cigarettes/day and given extracts of Red beet by oral in three doses different for each group (125; 250; 500 mg.Kg.WB^{-1}/day) for 56 days.

After treatment, Rats dissected during proestrus phase. Before surgery rats injections of 0.2 mg ketamine IM to obtain blood until the heart. Blood from the heart centrifuged to obtain blood serum, then examined using ELISA Rat E2 to measure levels of 17β-estradiol. Meanwhile, the ovarian tissue is cut crosswise then made slide histopathology and staining HE After that, to counted of number of the primary follicle, secondary follicles and antral follicle using Dotslide microscope Olympus XC 10 at the cross-whole or all of the follicles that exist in all passenger ovaries and identified more with 400x magnification.

RESULTS

17β-estradiol

The results of statistical tests on observation group showed a significantly different level of 17β-estradiol serum *Rattus norvegicus* in all groups of observations with *p-value* = 0.000> α.

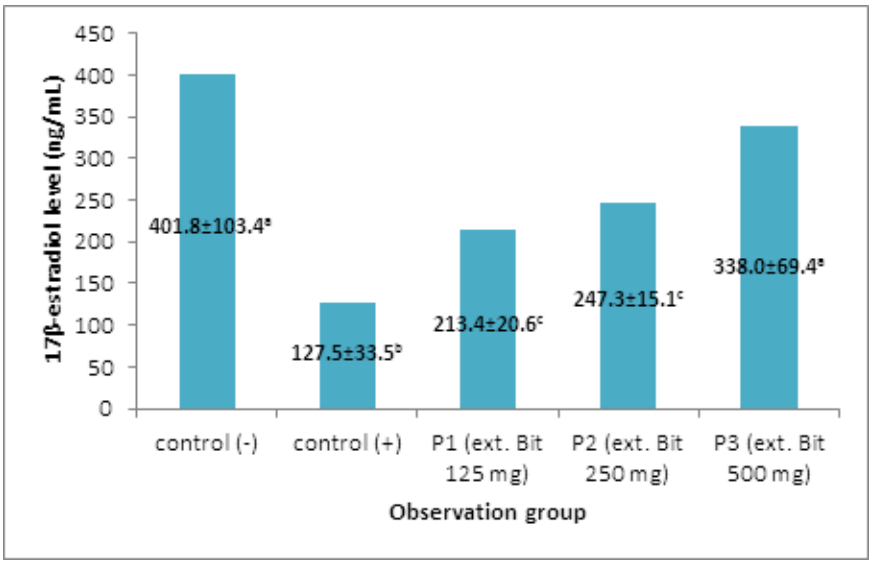

Figure 1: Histogram average levels of 17β-estradiol in all study groups.

Information: Control (-) is the group that fed and watered / without treatment; Control (+) is a group that is exposed to smoke / red beet extract was not given; P1 is the group that was given red beet extract 125 mg.Kg.WB^{-1} / by exposure to tobacco smoke; P2 is a group that was given red beet extract 250 mg.Kg.WB^{-1} / by exposure to tobacco smoke; P2 is a group that was given red beet extract 250 mg.Kg.WB^{-1} / by exposure to cigarette smoke.

In the histogram looks mean17β-estradiol levels supreme the negative control group (401.8 ± 103.4^{a}) and the low on the rod average levels of 17β-estradiol in the positive control group (127.5±33.5^{b}). This That exposured by cigarette smoke in rats resulted in decreased levels of 17β-estradiol. While the average levels of 17β-estradiol appear to be increasing in the P1 group, P2, and P3 when compared to the positive control group. Increased levels of 17β-estradiol along with increased doses of ethanol extracts of red beet (*Beta vulgaris L.*) were given.

Folliculogenesis

Folliculogenesis in this study calculates the number of primary follicles, secondary follicles and antral follicles. The mean number of primary follicles on the fifth of the sample group presented in full looks in the image histogram (bar chart) below.

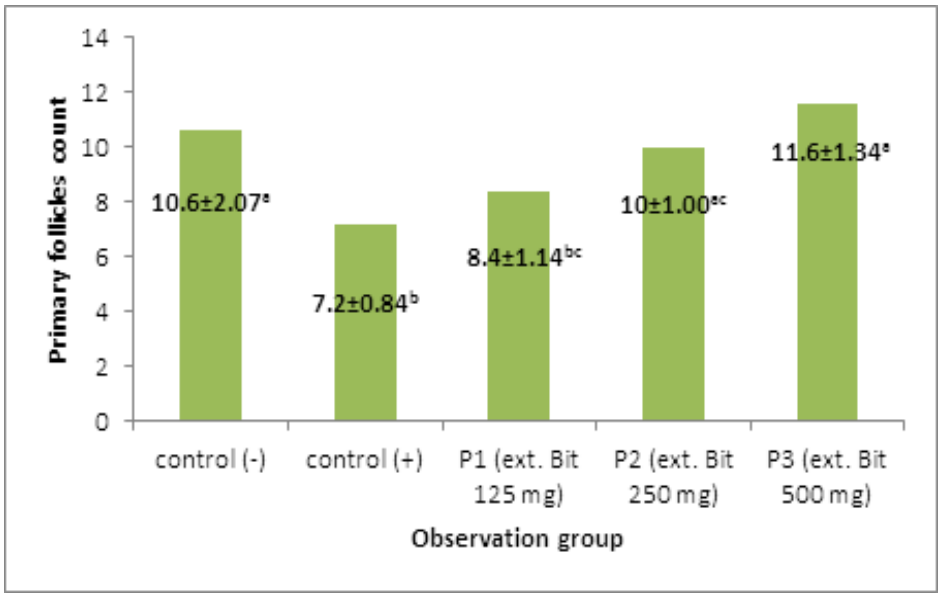

Figure 2: Histogram average number of primary follicles in all study groups.

Information: Control (-) is the group that fed and watered / without treatment; Control (+) is a group that is exposed to smoke / red beet extract was not given; P1 is the group that was given red beet extract 125 mg.Kg.WB^{-1} / by exposure to tobacco smoke; P2 is a group that was given red beet extract 250 mg.Kg.WB^{-1} / by exposure to tobacco smoke; P2 is a group that was given red beet extract 250 mg.Kg.WB^{-1} / by exposure to cigarette smoke.

Figure 2 shows the histogram of the average number of primary follicle in rats that were not given any (negative control), rats with cigarette smoke exposure (positive control), and three groups of rats *Rattus norvegicus* exposed by cigarette smoke and the provision of extracts ethanol red beet (Beta vulgaris L .) at a dose of 125 mg.Kg.WB^{-1}/day, a dose of 250 mg.Kg.WB^{-1}/day and a dose of 500 mg.Kg.WB^{-1}/day, looks mean the number of primary follicles bottommost the positive control group. This means That exposured by cigarette smoke on rats decreases the number of primary follicles. While the average number of primary follicles in the treatment group P1, P2, and P3 appears to be increased when compared to the positive control group. Increasing the number of primary follicles in the ovaries *Rattus norvegicus* along with increased doses of ethanol extracts of red beet (*Beta vulgaris L.*).

Furthermore, the average number of secondary follicles on the fifth of the sample group presented in full looks in the image histogram (bar chart) below.

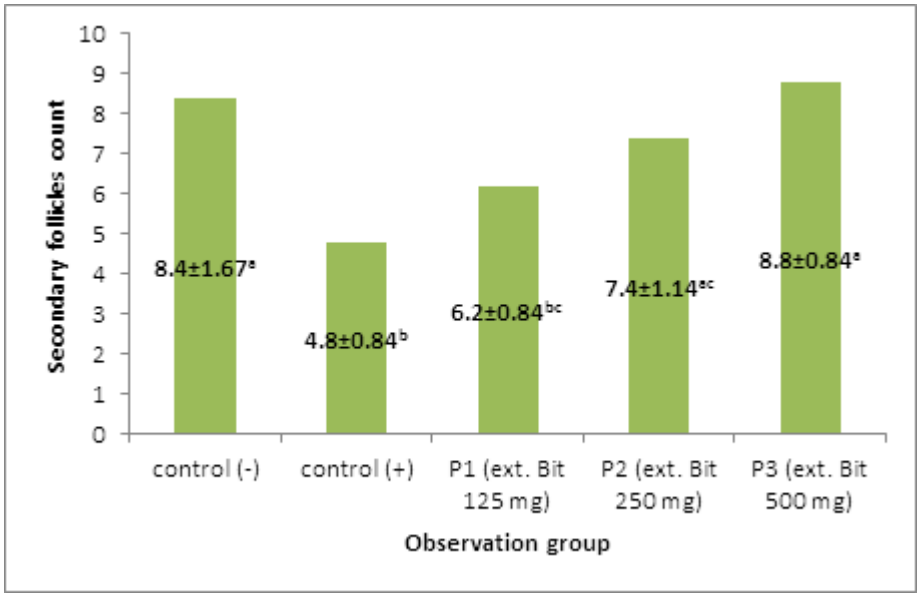

Figure 3: Histogram average number of secondary follicles in all study groups.

Information: Control (-) is the group that fed and watered / without treatment; Control (+) is a group that is exposed to smoke / red beet extract was not given; P1 is the group that was given red beet extract 125 mg.Kg.WB^{-1} / by exposure to tobacco smoke; P2 is a group that was given red beet extract 250 mg.Kg.WB^{-1} / by exposure to tobacco smoke; P2 is a group that was given red beet extract 250 mg.Kg.WB^{-1} / by exposure to cigarette smoke.

Figure 3 shows the histogram of the average number of secondary follicles in rats that were not given any (negative control), Rats with cigarette smoke exposure (positive control), and three groups of rats *Rattus norvegicus* exposured by cigarette smoke and the provision of extracts ethanol red beet (*Beta vulgaris L* .) at a dose of 125 mg.Kg.WB^{-1}/day, A dose of 250 mg.Kg.WB^{-1}/day And a dose of 500 mg.Kg.WB^{-1}/day, looks mean the number of secondary follicles bottommost the positive control group. This means that exposure to cigarette smoke in rats decreases the number of secondary follicles. While the average number of secondary follicles in the treatment group P1, P2, and P3 appears to be increased when compared to the positive control group. The increase in the number of secondary follicles in the ovaries *Rattus norvegicus* treatment groups P1, P2, and P3 along with increased doses of ethanol extracts of red beet (*Beta vulgaris L.*) were given.

While the average number of antral follicles show significant differences between the mean antral follicles in the fifth research group. The differences are evidenced by the *p-value* = 0.000 <α.

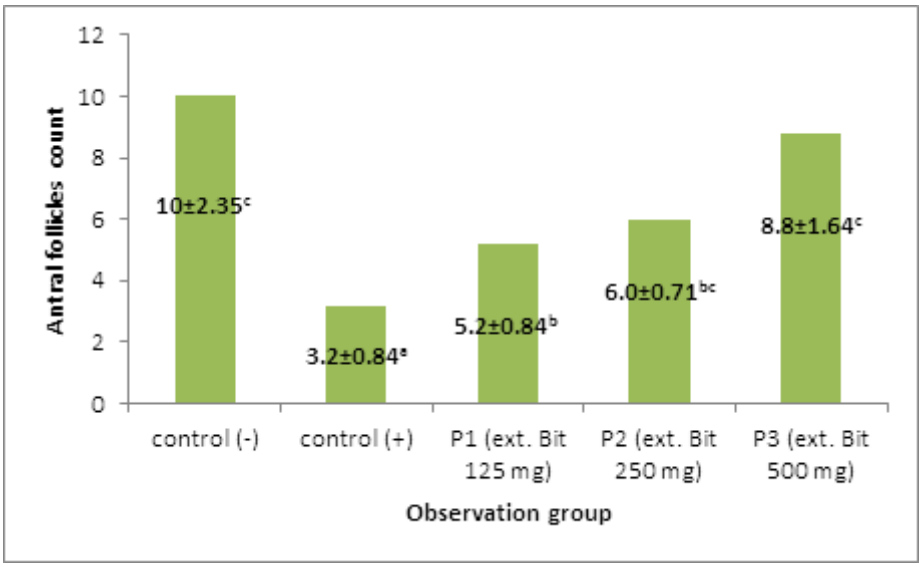

Figure 4: Histogram average number of antral follicles in all study groups.

Information: Control (-) is the group that fed and watered / without treatment; Control (+) is a group that is exposed to smoke / red beet extract was not given; P1 is the group that was given a red beet extract 125 mg.Kg.WB^{-1} / by exposure to tobacco smoke; P2 is a group that was given a red beet extract 250 mg.Kg.WB^{-1} / by exposure to tobacco smoke; P2 is a group that was given a red beet extract 250 mg.Kg.WB^{-1} / by exposure to cigarette smoke.

Figure 4 load on the effect of red beet extract the five treatment groups. We can see that the average value of the positive control group (3.2 ± 0.84^{a}) Has the smallest average than other groups. Meanwhile, red beet extract therapy at a dose of 500 mg.Kg.WB^{-1}/day with a mean (8.8 ± 1.64^{c}) Is capable of approaching a group of normal rats (10 ± 2.35^{c}). Increasing the number of antral follicles in the ovary *Rattus norvegicus* along with increased doses of ethanol extracts of red beet (*Beta vulgaris L*.) were given.

DISCUSSION

The effect ethanol extracts red beet to increase serum levels of 17β-estradiol in female rats Smoke exposed

There is a significant difference in the mean levels of 17β-estradiol between the negative control group were Healthy rats with the positive control group were rats give exposured by cigarette smoke 2 sticks per day without being treated ethanol extract of red beet, where the average levels of 17β-estradiol positive group is lower than negative control group. It is proved that exposure to cigarette smoke 2 sticks per day for 8 weeks in female rats can reduce levels of 17β-estradiol. Attia and Supreme[11] in their study also mentions that serum estradiol concentration results in lower passive smokers than non-smokers. It is also consistent with other studies conducted by Kapoor & Jones[12] found that women exposed to secondhand smoke exposure have antiestrogenic effects.

The mean levels of 17β-estradiol in the positive control group 127.5 ± 33.5 ng / mL, a significant difference to the treatment group P1 exposed to smoke + ethanol extract of red beet dose of 125 mg.Kg.WB^{-1}/day (213.4 ± 20.6 ng/mL), P2 exposed smoke + red beet ethanol extract dose of 250 mg.Kg.WB^{-1}/day (247.3 ± 33.5) and exposured by cigarette smoke P3 + red beet ethanol extract dose of 500 mg.Kg.WB^{-1}/day (338.0 ± 69.4). This means that exposure to cigarette smoke 2 sticks per day for 8 weeks in female rats leads to decreased levels of serum 17β-estradiol and ethanol extract of red beet (*Beta vulgaris L.*) of the third dose can increase levels of serum 17β-estradiol on female rats exposed by cigarette smoke previous. While the dose of ethanol extract of red beet (*Beta vulgaris L*.) which is considered the fastest able to reduce levels of 17β-estradiol is a dose of 500 mg.Kg.WB^{-1}/day. This is because of the red beet extract containing betalain[13] and other antioxidant compounds.[14] Based on in vitro studies ever conducted by Wettasinghe et al.[15] betalain of beet red indicates antiradical properties and high antioxidant. The content betalain on red beets is also believed to be very useful to prevent cancer.

The effect ethanol extracts red beet (*Beta vulgaris L.*) to folliculogenesis in female rats Smoke exposed

The results showed that the number of primary, secondary and antral follicles decreased as a result of exposure to cigarette smoke. The results of this study are supported by research about the dangers of cadmium compounds in cigarette smoke are known to cause retraction of the cytoplasm to the culture medium granulosa cells of human follicles.[16] This experiment in vitro follicular granulosa cells of mice proved cadmium also inhibits the activity of SOD.[17] Cadmium also reduced the growth of follicles and increase the number of oocytes atresia in ovarian African frogs.[18] Cigarette smoke has harmful effects on the ovarian cycle and work to cause infertility. The content of cigarette smoke as BAP able to inhibit and affect the size of the follicle. Besides cigarette smoke is closely related to the disruption of the process of folliculogenesis for compounds of cigarette smoke can induce mutations in the DNA of target genes that can increase the formation of oocytes abnormal,[19] and is able to retain the toxic compounds that can increase the pathway of oxidative stress to affect the process of meiosis and cell death pathways. The process is capable of causing adverse effects on the impaired growth and maturation of follicles.[16]

Administration fruit Bit (*Beta vulgaris L*)with the average number of primary follicles, secondary and antral follicles is higher than the positive control group. From the results of these studies showed that does fruit Bit (*Beta vulgaris L*) Faster increase the number of primary follicles and secondary follicles in female rats exposed by cigarette smoke is a dose of 500 mg.Kg.WB^{-1}/day (treatment P3) compared doses of anthocyanin 125 mg.Kg.WB^{-1}/day and a dose of mg.Kg.WB^{-1}/day.

Red beet is one of the tubers commonly known as a plant that is rich in antioxidants and has many benefits for health. Red beet contains many antioxidant compounds such as flavonoids, nitrite and betalain. Red beet extract also contains rich in nitrates and nitrites. Nitrate contained in red beets can function improve blood perfusion, restore endothelial function and improve performance.[20] In addition, extract red beet proven to prevent apoptosis and necrosis in the renal tubules compared with the control group. The process can inhibit pro-apoptosis such as caspase 3 in addition to the chemical activity of the extracts from red beet can interfere with NF- κ B which induce apoptosis as a result of oxidative stress.[21]

The absence of research related to the effects of the extracts from red beet reproductive organs. This study was able to prove that the red beet extract is also able to increase the number of primary follicles, secondary

follicles and antral follicles on ovarian exposed by cigarette smoke.

CONCLUSION

The ethanol extract of red beet (*Beta vulgaris L.*) dose of 125 mg.Kg.WB^{-1}/day, 250 mg.Kg.WB^{-1}/day and 500 mg.Kg.WB^{-1}/day are proven to increase levels of 17β-estradiol serum and folliculogenesis in female rats (*Rattus novergicus*) exposed by cigarette smoke 2 cigarettes/day for 8 weeks.

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