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Effect of Ethanol Extract of Red Beetroot (*Beta vulgaris L.*) on the Follicle Stimulating Hormone Levels among Wistar Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

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A B S T R A C T

Ethanol extract of red beetroot *(Beta vulgaris L.)* contains chemical compounds of ascorbic acid, carotenoite, phenolic acid, betalain, and favonoids. Red beetroot supplements can prevent oxidative damage to the structure of DNA, lipids and proteins in vitro. This study aims to prove that the ethanol extract of red beetroot (*Beta vulgaris L.*) has an effect on increasing FSH levels among female white rats (*Rattus norvegicus)* exposed to cigarette smoke. This was an experimental study with the post-test only control group design. The current study involved 25 female rats which were assigned into 5 groups: negative control, positive control, treatment I, II and III. The doses of red beetroot extract used were PI (125 mg/kgBW/day), PII (250 mg/kgBW/day), and PIII (500 mg/kgBW/day). Data were analyzed using One Way ANOVA with p-value of FSH (p = 0.000). The results of the study proved that administration of red beetroot ethanol extract at a dose of 500 mg/kgBW/day could increase the ovarian FSH levels among female rats exposed to cigarette smoke.

*Ekstrak etanol bit merah (*Beta vulgaris L.*) memiliki kandungan senyawa kimia: asam askorbat, karetenoit, asam fenolik, betalain, serta favonoid. Suplemen bit merah dapat menghindari terjadinya kerusakan oksidatif pada lipid, struktur DNA, dan protein secara in vitro. Tujuan riset ini untuk membuktikan bahwa ekstrak etanol bit merah (*Beta vulgaris L.*) memiliki pengaruh terhadap kenaikan kadar FSH pada tikus putih (*Rattus norvegicus*) betina yang dipapar dengan asap rokok. Desain penelitian eksperimen dengan rancangan post-test only control group design. Riset ini menggunakan 25 ekor tikus betina yang dipisah menjadi 5 kelompok: kelompok kontrol negatif, kontrol positif, perlakuan I, II serta III. Dosis ekstrak bit yang digunakan masing-masing ialah PI (125 mg/kgBB/hari), PII (250 mg/kgBB/hari), PIII (500 mg/kgBB/hari). Analisa data menggunakan One-Way ANOVA dengan p-value FSH (p= 0.000). Hasil riset membuktikan jika pemberian ekstrak etanol bit merah dengan dosis 500 mg/kgBB/hari dapat menambah kadar FSH pada ovarium tikus yang dipapar asap rokok.*

Introduction

One of the bioactives that can be used as an antioxidant to counteract free radicals is betacyanin. Betacyanin is a water-soluble pigment, which gives vegetables, fruits and flowers their purple, blue and red colors. Betacyanin is also a type of polyphenol and is included in a group of flavonoids that contain antioxidants (Ramadhan, 2015). A study conducted by Husna (2013) reported that the higher the betacyanin content, the higher the antioxidant effect. Zhao (2013) also revealed that the antioxidant effect on betacyanin from beetroots could increase the expression of antioxidant enzymes (Indu *at al.*, 2017). Betacyanin contained in red beetroots is known to have anti-radical effects and high antioxidant activity (Mastuti, *et al.*, 2010).

Ethanol extract of red beetroot (*Beta vulgaris L.*) contains chemical compounds including ascorbic acid, phenolic acids, carotenoids, betalains, and flavonoids, which also function as antioxidants, anti-cancer, antimicrobial, anti-anaemic, anti-malarial and anti-inflammatory, which have high levels of antioxidant and anti-inflammation both in in vivo and in vitro performed on several animal models. Red beetroot supplements can prevent oxidative damage to the structure of DNA, lipids and proteins in vitro *(Clifford et al, 2015).* Such damange can result in inhibition of GnRH pulses which further cause disturbances in the synthesis and secretion of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) (Armstrong, 2010). Both of these hormones are necessary for the development of male and female gonads and are important for the processes of spermatogenesis and oogenesis. Disruption of the function of the hypothalamus may interfere with endocrine functions, including reproductive hormones, thereby affecting the process of folliculogenesis (Camihort, 2004). In this case, infertility is a reproductive health problem that affects 8-10% of reproductive couples in the world. Based on Basic Health Research data (2014), smoking behavior of the population aged ≥15 years increased from 2007 to 2013, from 34.2% (2007), 34.7% (2010) to 36.3% (2013). Data derived from the National Socioeconomic Survey (Susenas) and the Household Health Survey (SKRT) also showed an increase in the prevalence of smokers aged ≥15 years by 27% (1995), 31.5% (2001), and 34.4% (2004). Based on WHO records, known causes of infertility in women include fallopian tube factor by 36%, ovulation disorders by 33%, endometriosis by 6%, and other unknown factors by 40%. Thus, most infertility problems in women are caused by disorders of the reproductive organs or disturbances in the ovulation process (Kumalasari, 2012). This study aims to prove whether the antioxidants found in beetroot can increase FSH levels in the ovarie of female white rats.

Methods

This was an experimental study with the post-test only control group design. This study was conducted in 3 sites, namely in the Biomedical, Anatomical Pathology and Pharmacology Laboratories, Faculty of Medicine, Brawijaya University. The Pharmacology Laboratory was a site for *Rattus norvegicus* maintenance and treatments of experimental animals namely administration of ethanol extract of red beetroot (*Beta vulgaris L.),* exposure to cigarette smoke, surgery and sampling. Biomedical Laboratory of the Faculty of Medicine was a laboratory for measuring ovarian FSH levels in *Rattus norvegicus* using the Enzyme Linked Immunosorbent Assay (ELISA) of FSH ELISA Kit brand cusabio catalog number CSB-E06869r produced by the United States. This study involved the samples of 30 female white rats (*Rattus norvegicus* wistar strain) with a weight of 150 grams, respectively. The cigarettes used were unfiltered clove cigarettes. The tools used were a smoking pump, gastric tube and FSH antibodies. Twenty-five white rats were acclimatized for 7 days to adapt to the new environment, then the rats were examined and assigned into 5 groups, namely the negative control group (no treatment), positive control group (exposed to cigarette smoke of 2 cigarretes/day without red beetroot extract), treatment I (exposed to cigarette smoke of 2 cigarettes/day along with red beetroot ethanol extract at a dose of 125 mg/kgBW/day), treatment II (exposed to cigarette smoke of 2 cigarettes/day

along with red beetroot ethanol extract at a dose of 250 mg/kgBW/day), treatment III (exposed to cigarette smoke of 2 cigarettes/day along with red beetroot ethanol extract at a dose of 500 mg/kgBW/day). After being treated for 56 days, the rats were killed and blood serum was taken from the heart and ovarian organs. Data analyzed using the One-Way ANOVA test, and if the difference was significant, data were then proceed with the Least Significant Difference (LSD) test.

Table 1. Experimental animal chart

Observational Group	n
Negative control	5
Positive control	5
P1 (Beetroot extract of 125 mg/KgBW/day)	5
P2 (Beetroot extract of 250 mg/KgBW/day)	5
P3 (Beetroot extract of 500 mg/KgBW/day)	5

Results

Table 2. Effects of exposure to cigarette smoke on FSH levels in rat ovaries

Observational group	n	Mean ± SD	P-value
negative control	5	55.23±8.42^{a} ng/mL	
positive control	5	11.07±2.26^{b} ng/mL	
P1 (Beetroot extract of 125 mg/KgBW/day)	5	20.80±7.46^{c} ng/mL	0.000<∝
P2 (Beetroot extract of 250 mg/KgBW/day)	5	26.85±4.30^{c} ng/mL	
P3 (Beetroot extract of 500 mg/KgBW/day)	5	37.82±9.14^{d} ng/mL	

Table 2 was shown that there was a significant difference in the mean FSH levels between the negative control group (55.23±8.42a ng/mL) and the positive control group with rats exposed to cigarette smoke (11.07±2.26b ng/mL). It could be observed that the mean FSH levels in the positive control group was lower than the mean FSH levels in the negative control group. Such finding indicated that rats exposed to cigarette smoke had lower FSH levels when compared to healthy rats. In other words, exposure to cigarette smoke in Rattus norvegicus rats could decrease FSH levels. Likewise, the mean FSH levels of the negative control group (55.23±8.42a ng/mL) was significantly different from the P1 group (20.80±7.46c ng/mL), the P2 group (26.85±4.30 c ng/mL) and the P3 group (37.82±9.14d ng/mL). It could be observed that the mean FSH levels in the negative control group was much higher than that of the other groups.

Discussion

Based on the results of this study, it was found that the positive control group (which was exposed to cigarette smoke of 2 cigarettes per day and without red beetroot (*Beta vulgaris L.*)) showed a significant decrease in the serum FSH levels compared to the negative control group. Armstrong (2010) states that inhibition of FSH hormone production can be due to oxidative stress from cigarette smoke through inhibition of GnRH pulsation through GABA, a receptor system that interferes with the synthesis and secretion of Follicle Stimulating Hormone (FSH). Cigarette smoke is involved in free radical. Unpaired electrons in reactive oxygen compounds make up free radicals.By forming new radicals, these compounds or atoms attempt to reach a stable state by attracting additional electrons. This free radical reaction takes place in a cascade (cascade reaction) so that it can cause an increase in oxidative stress either directly or indirectly (Kelly, 2002; Ratnawati, 2014). As a result of increased

oxidative stress, lipid peroxide will occur which further cause damage to the arcuate nucleus and ventromedial nucleus in the hypothalamus and impaired hypothalamic GnRH synthesis and secretion (Kardi, 2015). Administration of red beetroot (*Beta vulgaris L.*) with several dose levels in this study revealed that the increase in FSH levels in the positive control group at a dose of red beetroot (*Beta vulgaris L.)* of 500 mg/kgBW led to the highest mean FSH levels than the other doses. Based on the results of this study, it was found that the dose of red beetroot (*Beta vulgaris L.*) which increased serum FSH levels more quickly in female white rats exposed to cigarette smoke was 500 mg/kg BW (treatment P3) compared to 125 mg/kgBW and 250 mg/kgBW. The study finding is in accordance with a study conducted by AL-Olayan (2014) concerning the effect of pomegranate juice containing flavonoids including red beetroot (*Beta vulgaris L.*) which could significantly increase the serum FSH levels in male rats exposed to carbon tetrachloride free radicals. The study finding is also in accordance with a study conducted by Sa'adeya (2014) concerning the effect of green tea polyphenols as bioactive similar to red beetroot (*Beta vulgaris L.)* among female rats with oxidative stress which found that there was an increase in FSH levels among white rats with oxidative stress.

Conclusions

lt can be concluded that exposure to cigarette smoke of 2 cigarettes/day without the administration of red beetroot ethanol extract (positive control group) could decrease FSH levels in rat ovaries. Furthermore, there was an increase in FSH levels in treatment I (exposed to cigarette smoke of 2 cigarettes/day along with red beetroot ethanol extract at a dose of 125 mg/kgBW/day), treatment II (exposed to cigarette smoke of 2 cigarettes/day along with red beetroot ethanol extract at a dose of 250 mg/kgBW/day), treatment III (exposed to cigarette smoke of 2 cigarettes/day along with red beetroot ethanol extract at a dose of 500 mg/kgBW/day).

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