The Effectivity of Red Dragon Fruit Skin Extract (*Hylocereus polyrhizus*) Against Leydig Cells, Sertoli Cells, Testosteron Levels, and *Malondialdehyde* Levels of White Rat (*Rattus norvegicus*) Exposed with High Temperatures

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Abstract

High temperature exposure is one form of free radicals that can reduce fertility. High temperature exposure which are included in the form of free radicals can be counteracted by antioxidants. The aim of this study was to determine the effectiveness of red dragon fruit skin extract on Leydig cells, Sertoli cells, testosterone levels and *malondialdehyde* levels white rats exposed to hight temperatures. A total of twenty white rats randomly divided into 5 groups, the control group given CMC Na 1%. 0.5 ml, the P0 group given CMC Na 1%. 0.5 ml and heat exposure 40° C for 45 minutes, group P1 was given red dragon fruit peel extract at a dose of 150 mg/kg BW, group P2 at a dose of 300 mg/kg BW, group P3 at a dose of 600 mg/kg BW and all three groups were exposed to heat 40° C for 45 minutes, with 4 repetitions for each group. The conclusion showed administration of red dragon fruit skin extract dose of 600 mg/kg BW can efficiently of Leydig cells (8.40 ± 1.07), Sertoli cells (13.25 ± 1.15), testosterone levels (3.26 ± 0.80) and *malondialdehyde* levels (0.60 ± 0.08) in white rats exposed to high temperatures.

Keywords: *Antioxidant, Dragon Fruit, Leydig cells, Sertoli cells, Testosterone, Malondialdehyde*

Introduction

Free radical compounds are inseparable in everyday life. Exposure to high temperatures is a form of free radical that can reduce fertility (1). Animal bodies respond to discomfort due to exposure to high temperatures by producing excessive Reactive Oxygen Species (ROS). Excess ROS induces oxidative stress, which disrupts the structure and function of important molecules such as nucleic acids and proteins (2). Oxidative stress due to heat exposure can affect testicular somatic cells, causing germ cell depletion which results in damage to Sertoli cells and Leydig cells as well as a decrease in the testosterone hormone (3).

Exposure to high temperatures which are included in the form of free radicals can be counteracted by antioxidants. Antioxidants are substances that the body needs to prevent or slow down oxidation, such as inhibiting nucleic acids, fat oxidation and other molecules, thereby reducing the risk of damage to body cells (4). Lack of antioxidants in the body can increase the effects of free radicals, making it easier for ROS to carry out oxidative damage (3).

One of the herbal ingredients that is useful for maintaining fertility is red dragon fruit skin. The skin of red dragon fruit contains several ingredients such as alkaloids, terpenoids, flavonoids, tianine, niacin, pyridoxine, cobalamin, phenolics, carotene, and phytoalbumin as well as polyphenols as antioxidants (5).

The skin of red dragon fruit contains high levels of antioxidants compared to the flesh of the fruit, including phenolics, flavonoids and polyphenols (6). Therefore, it is necessary to know the effectiveness of red dragon fruit skin extract on Leydig cells, Sertoli cells, testosterone levels and malondialdehyde levels of white rats exposed to high temperatures.

Research Materials and Method

Materials and Equipments

The experimental units used in this research were 20 healthy male white rats (*Rattus norvegicus*) weighing around 150 grams body weight, 12 weeks old.

Materials that used in the research included f red dragon fruit skin extract, 96% ethanol, distilled water, NaCl 0,9%, 1% CMC Na, formalin liquid, and ELISA-Kit for examining testosterone levels and malondialdehyde (MDA) levels.

The equipment that used in this research included of: rotary evaporator, rats cage, drinking bottles for rats, cables, plugs, incubator containing three 5watt lamps, thermometer, husks, rubber gloves, 1 ml tuberculin gastric probe, jar, support board for installation, safety pins, scalpel, clamps, tweezers, scissors, 1 ml syringe, vacutainer tube and tube rack.

Rats were divided randomly into five groups, C, T (0), T (1), T (2) and T (3). Each treatment consisted four rat. Then all group of rats were adapted for one week, given pelleted food and drinking water in the morning and evening.

Research Design

This research using Completely Randomized Design. In this design there is only one source of variability, that is the random effect of treatment on the rats, so the different result of the treatment only caused by the treatment's effect and random effect. Then this research using five groups and four replicates for each group.

Red Dragon Fruit Skin Extract Preparation

Dried red dragon fruit skin will first be crushed using a pollinator machine. Red dragon fruit skin powder (1 kg) soaked in 96% ethanol solution and stirred for 3 x 24 hours. Filtration was done to separate the dregs from the solution. Then it was evaporated using a rotavapor at 40^0 C with 50 rpm obtain a viscous extract.

Treatments

White rats (*Rattus norvegicus*) were captived in cages placed in Laboratory Experimental Animal at Faculty of Veterinary Medicine Airlangga University, randomized by lottery and divided into five groups. Then were adapt to the environment for one week. After the adaptation period, exposure to high temperatures treatment phase is carried out for 14 days. The details are as follows:

a. C : without high temperature exposure and given 0.5 ml CMC Na 1%
b. T (0) : high temperature exposure 40°C for 45 minutes and given 0.5 ml CMC Na 1%
c. T (1) : high temperature exposure 40°C for 45 minutes and given red dragon fruit skin extract 150 mg/kg BW in 0.5 ml CMC Na 1%
d. T (2) : high temperature exposure 40°C for 45 minutes and given red dragon fruit skin extract 300 mg/kg BW in 0.5 ml CMC Na 1%
e. T (3) : high temperature exposure 40°C for 45 minutes and given red dragon fruit skin extract 600 mg/kg BW in 0.5 ml CMC Na 1%

Each group was given treatment at the same time every day for 14 days. Blood samples were taken intracardially, then stored in a vacutainer tube with a gelcloth activator to obtain blood serum. After taking blood, the rats are sacrificed by cervical dislocation method and the testes of rats collected.

Microscopic Examination

Testicular histology preparations were examined to determine the condition of Sertoli cells and Leydig cells in white rats (*Rattus norvegicus*) after treatment. Examination the number of Leydig cells and Sertoli cells used a microscope with 200x magnification.

ELISA Examination

The blood serum that has been taken is tested using ELISA to determine testosterone levels and malondialdehyde (MDA) levels. Readings of optical density (OD) and absorbance values will be used as analysis results.

Data Analysis

Data for each group were analyzed statistically using the ANOVA statistical test followed by the Duncan Test to compare the effect of treatment for each group.

Result

Leydig Cell and Sertoli Cell Examination

Data was obtained from examination of testicular histology preparations carried out at a microscopic magnification of 200x. The number of Leydig cells and Sertoli cells was calculated based on the average of five visual fields from each replication of the treatment group. The results of the average number were then analyzed using the ANOVA test. If there is a significant difference ($p<0.05$) between treatment groups then proceed with the Duncan test to determine significant differences in each treatment group.

The analysis results for the average number of Leydig cells and Sertoli cells can be seen in Table 1.

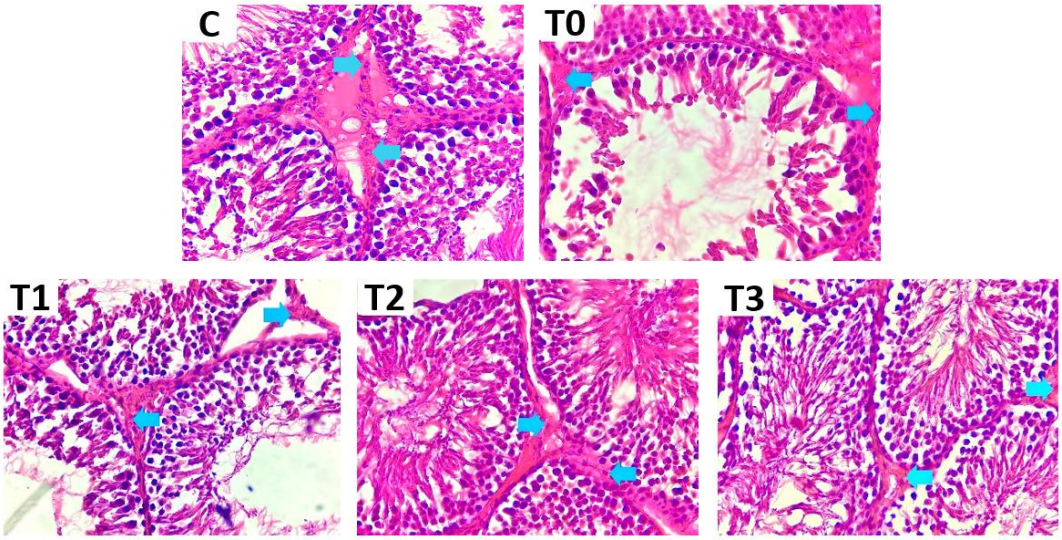

Figure 1. The blue arrow shows a microscopic image of male rat Leydig cells in a histology preparation at 200x magnification. C (control group), T0 (without extract group), T1 (150 mg/kg bw of extract), T2 (300 mg/kg bw of extract), T3 (600 mg/kg bw of extract)

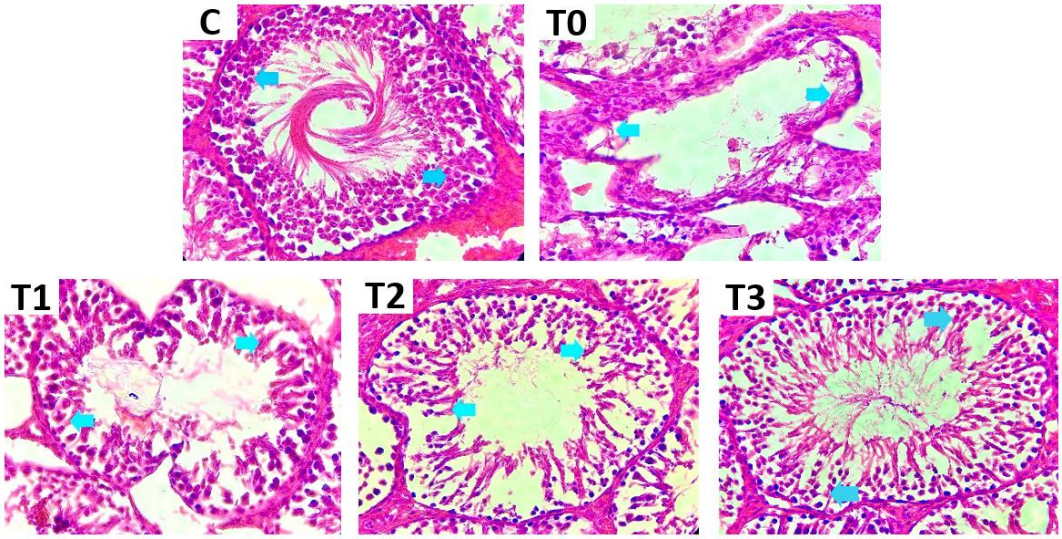

Figure 2. The blue arrow shows a microscopic image of male rat Sertoli cells in a histology preparation at 200x magnification. C (control group), T0 (without extract group), T1 (150 mg/kg bw of extract), T2 (300 mg/kg bw of extract), T3 (600 mg/kg bw of extract)

Table 1. Mean and standard deviation the number of Leydig cells and Sertoli cells in each treatment group.

Treatment	Leydig Cell Count ± SD	Sertoli Cell Count ± SD
C	$10,95^{d} \pm 1,33$	$16,05^{c} \pm 1,33$
T0	$3,30^{a} \pm 1,50$	$4,95^{a} \pm 1,50$
T1	$5,95^{b} \pm 0,87$	$8,75^{b} \pm 0,87$
T2	$7,35^{bc} \pm 1,02$	$10,65^{c} \pm 1,02$
T3	$8,40^{c} \pm 1,15$	$13,25^{d} \pm 1,15$

Different superscripts in the same column indicate significant differences (P<0.05)

Examination of Testosterone Levels and Malondialdehyde Levels

Testosterone levels were examination using the Competitive ELISA-Kit method and malondialdehyde levels were examination the Sandwich-ELISA method. The results of the examination are known by looking at the intensity of the color produced which is inversely proportional to the concentration of testosterone and malondialdehyde in the sample. Testosterone and malondialdehyde concentrations in the samples were then determined by comparing the optical density (OD) of the samples with a standard curve.
The results of the analysis of total testosterone levels and malondialdehyde levels can be seen in Table 2.

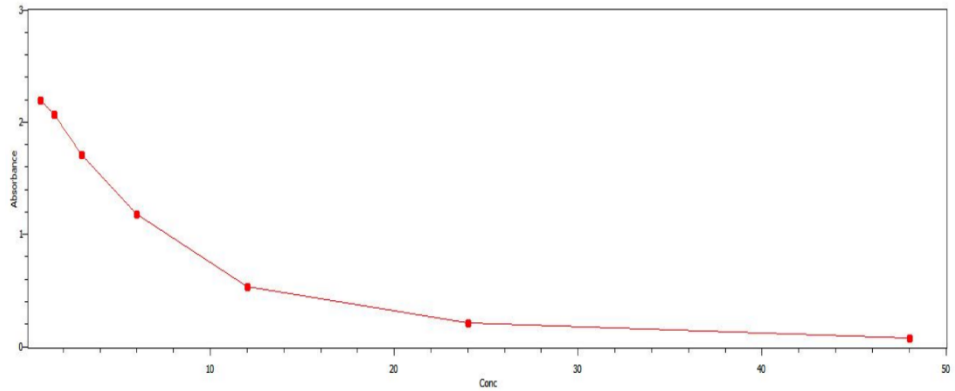

Figure 3. The graph shows the testosterone levels of male rats in nmol/ml

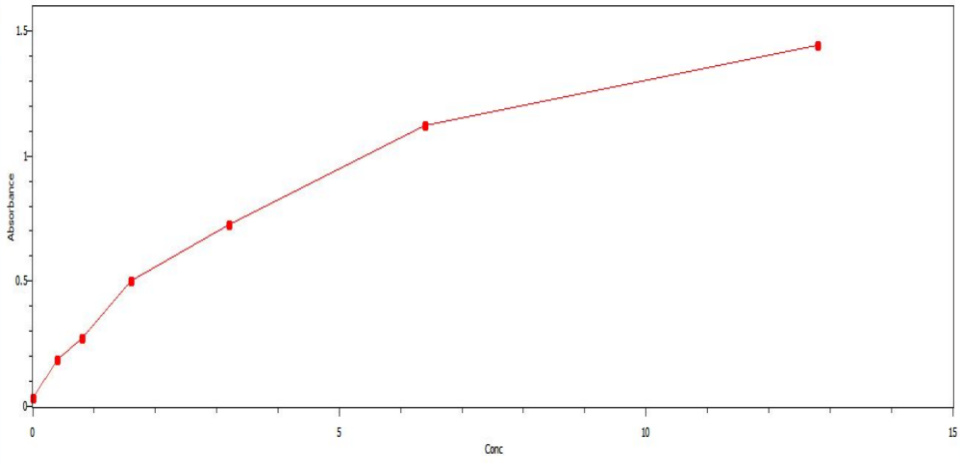

Figure 4. The graph shows the malondialdehyde (MDA) levels of male rats in nmol/ml

Table 2. Mean and standard deviation the number of Testosterone levels and Malondialdehyde levels in each treatment group.

Treatment	Testosterone levels ± SD (nmol/ml)	Malondialdehyde levels ± SD (nmol/ml)
C	$3,93^{e} \pm 0,15$	$0,50^{a} \pm 0,02$
T0	$1,78^{a} \pm 0,09$	$1,04^{d} \pm 0,12$
T1	$2,24^{b} \pm 0,21$	$0,89^{c} \pm 0,14$
T2	$2,73^{c} \pm 0,18$	$0,74^{b} \pm 0,06$
T3	$3,26^{d} \pm 0,80$	$3,60^{ab} \pm 0,08$

Different superscripts in the same column indicate significant differences (P<0.05)

Discussion

The results of examining the levels of Leydig cells, Sertoli cells, Testosterone levels and Malondialdehyde levels showed that the T3 group which was given a dose of 600 mg/kg BW of red dragon fruit skin extract was able to have an effect and significantly different means ($p<0.05$) on the number of Leydig cells, Sertoli cells, Testosterone levels and Malondialdehyde levels compared to the T0 group which exposed to high temperatures without being given red dragon fruit skin extract.

The antioxidant of Vitamin C, Vitamin E, Tannin, Polyphenols and Betacyanin is able to prevent Leydig cell membranes from lipid peroxidation reactions. The mechanism for inhibiting lipid peroxidation reactions in Leydig cell membranes because these compounds are able to donate hydroxyl groups (*OH) to the ring chain structure of free radicals (7, 11, 14). Superoxide released through the process of oxidative phosphorylation is first converted into hydrogen peroxide and then further reduced to produce water. This detoxification pathway is the result of various enzymes, with superoxide dismutase catalyzing the first step and then catalase and various peroxidases removing hydrogen peroxide (8). This mechanism is able to protect Leydig cells from excessive damage to the membrane, structure and cell nucleus due to exposure to high temperatures, thereby reducing the occurrence of death in Leydig cells and reducing the decline in the number of Leydig cells in the treatment group (9).

The flavonoid are able to express endogenous antioxidant genes so that genes that play a role in the synthesis process of endogenous antioxidant enzymes will increase, thereby increasing the number of Leydig cell repairs in the testicles and stimulating the production of the hormone testosterone (10,11). Flavonoids as antioxidants are able to activate Nuclear Erythroid 2 Related Factor (Nrf2) so that compounds that play a role in endogenous antioxidant activity such as glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase increase (12). Flavonoids also act as peroxyl scavenger radicals (ROO*) which will be regenerated back into ROOH and hydroxyl scavenger radicals (*OH) which will be regenerated back into H2O so that they become more stable compounds and become non-reactive to react normally (13). Flavonoids are known to reduce ROS activity by donating electrons, counteracting lipid peroxidation chains and can maintain the plasma membrane so that they can prevent mutagenic alterations that trigger apoptosis in rat exposed to heat (14).

Conclusion

Based on this research, it can be concluded that administration of red dragon fruit skin extract at a dose of 600 mg/kg BW is effective in maintaining the number of Leydig cells, Sertoli cells, Testosterone levels, Malondialdehyde levels and reducing the risk of exposure to high temperatures.

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