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The effect of ethanol extract of beetroot (L.) on the number, morphology spermatozoa and testis weighin *Male Mice (Mus Musculus)* by exposure to heat

Dillasamola D.*, Helmi A. and Dhila S. M.

Faculty of Pharmacy, Andalas University, Padang, Indonesia

ABSTRACT

This research aimed to know the influence of ethanol extract of beetroot (L.) on the number, morphology spermatozoa and testis weighin male mice (Mus musculus) by *by exposure to heat. This research was conducted by using Post Test Only Control Group Design. Data was statistically analyzed by using onewayANOVA followed by Duncan Post Hoc Test (P<0.05).The study used 30 male mice aged 2-3 months, weight 20-35 g and were randomly divided into 5 groups with 6 mice each groups. Group K- (negative control) were given distilled water, Group K+ (positive control) is exposed to temperatures of 40 ° C for 60 minutes/day, third group (P1)is exposed to temperatures of 40 ° C for 60 minutes/day and a solution of tuber extract bits dose of 100 mg / kgBB / day, fourth group (P2)is exposed to temperatures of 40 ° C for 60 minutes/day and a solution of tuber extract bits dose of 200 mg / kgBB / day, fifth group (P3))is exposed to temperatures of 40 ° C for 60 minutes/day and a solution of tuber extract bits dose of 400 mg / kgBB / day. This research lasted for 36 days, at 37 th day all mice were terminated, then the sperm count, morphology spermatozoa and* testis weigh was done. *The results showed significant differences in the number, morphology of spermatozoa and testis weight between groups (P <0.05). But Duncan's test based on the number and morphology did not show any significant difference. Where the ethanol extract of root beet (Beta vulgaris L.) with a dose of 100 mg / kgBB / day, 200 mg / kgBB / day and 400 mg / kgBB/day can not fix and increase the amount of morphologically normal spermatozoa in mice induced heat for 60 minutes. And testis weight at a dose of 200 mg / kgBB / day and 400 mg / kgBB / day showed a significant difference where the results showed that the ethanol extract of the tuber bit heavy testes capable of repairing damaged by exposure to heat.*

Keywords: extract, beetroot, spermatozoa ,exposure to heat

INTRODUCTION

Infertility toward male is caused by the derangement of sexual reproduction, spermatozoa quality and transportation that one of factors which determines the quality of spermatozoa to reach ovum, ovum membrane and penetrates in the fertilization. Spermatogenesis within seminiferous tubule is affected by many factors such as hormonal factors, the obstruction of epididymis function factors and temperature factors [1].The one of those factors that is a high temperature that causes the oxidative stress. It is an imbalance between the production of free radical and antioxidants, while amount of free radical is higher than antioxidants. Free radical is believed causing the oxidative stress in the cell membrane [2].

The high temperature will cause the obstruction of epididymis function in maturing spermatozoa and distributing the nutrition of food specially glucose as a substrate for spermatozoa metabolism. The maximum activity for more human enzyme occurs when human body temperature is around 37°C because in the higher temperature, the denaturation (the loss of secondary and tertiary) happens. French researchers reported that within 2000 people who drive a car more than 2 hours, their scrotal temperature increase to be more than 2°C.The similar impact also can be found in the daily routines and activities while the heat increase from the environment happens, likely: the use of tight underwear, hot water bath (sauna) and doing a job that should sit during hours.

Sailer [3] did a research toward mice by giving 38°C, 40°C and 42°C temperature shelf as along as 60 minutes. This research aims to discover the effect of temperature shelf toward testicle cells and the structure of chromatin in the spermatozoa. The result of the research is learnd the significance effect on the 40°C group and 42°C, but 38°C group shows insignificance effect than the control.

According to Ermiza [4], based on her research showed that the exposure of 40°C temperature as long as 60 minutes during 36 days affect amount of mice's spermatozoa. Thus, a mature male has to consume the nutrition food namely folic acid that much content within beet. It functions to repair the damage of cells and its role in supporting cells growth.

Researcher is interested to do the research about the effect of giving ethanol extract of Beet (Beta vulgaris L) toward number and morphology of spermatozoa and the weight of white mice's testicles that is given the heat exposure; 40°C temperature.

MATERIALS AND METHODS

This research was coducted by using Post Test Only Control Group Design method. The data was gotten and worked statisticaly by using ANOVA Hoc Test ($P<0,05$). This research used 2-3 month old white male mice with 20-30 weight grams as many as 30 mice those were devided into 5 groups of treatment test that for each group consists of 6 mice.

Sampling

The sample was 4 kg beets got from a fruit store around *Pondok* area, Padang City.

The Identification of Sample

Sample identification was conducted in the Herbarium ANDA of Biology Department, Math and Natural Science Faculty, Andalas University

Making Beet Extract

4 kg beet were washed, cut, dried and then mashed. The sample was extracted with maceration method by using 70% ethanol solvent in the 2,5 L capacity dark bottle. The comparison between sample and solvent is 1:10. During first 6 hours, sample was stirred occasionally, and then deposited along 18 hours (Supplemen Farmakope Herbal, 2010). Maceration process was done three time repetition. The maceration was screened and evaporated in vacuous with rotary evaporator in order to get the tick extract.

Dose Plan

The doses that will be given to the animal test are 100 mg/kg BB, 200 mg/kg BB and 400 mg/kg BB [6][9].

Methodology of the Research[8]

1. Preparing Animal Test

a. 36 mice are prepared, 30 mice as sample and 6 mice for reserve.
b. The mice are grouped as showed in (attachment 1, picture 6).
c. Before the test, the mice are cared as long as a week for adaptation process.
d. The exposure is done by putting the mice into incubator with stable temperature about 40°C and given the different doses.
e. There are 5 different treatments based on the groups of mice, where there are 6 mice for each group. The exposure is done every day during 60 minutes for 36 days.
f. The test of sperm quality is in the 37^{th} day of treatment. The mice are sacrificed by an anesthesia with *eter*, then

doing the laparotomy.
g. Cutting the ductus deferens. Spermatozoa is taking by massaging both cutting ductus deferens then the result is lodged within the watch glass and added 0,5 ml 0,9% NaCl [7]..

2. Spermatozoa Test

a) Spermatozoa Number Test

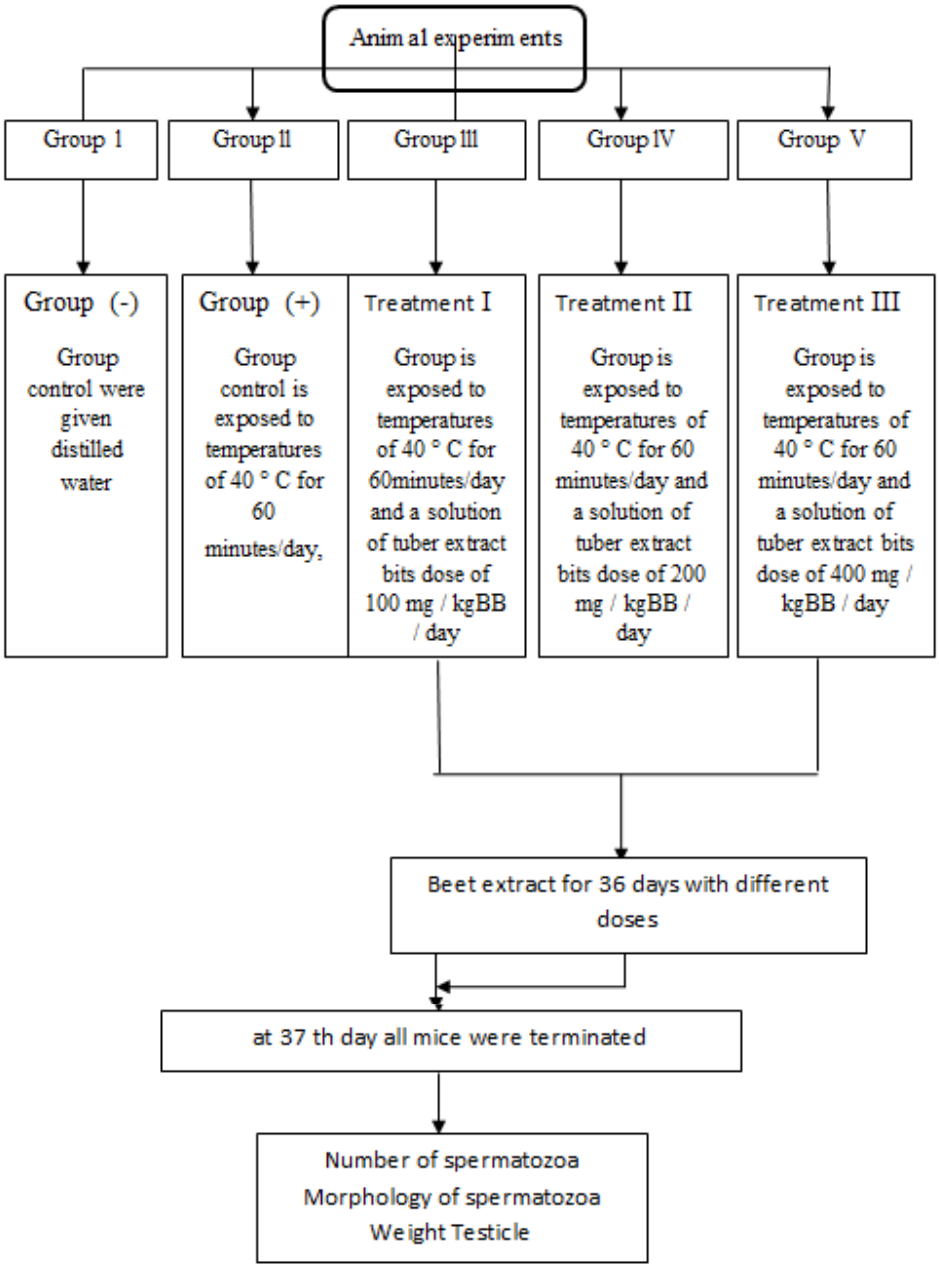
- Make sperm reserves/stock (0,5 ml 0,9 % NaCl liquid + spermatozoa) that is lodged into watch glass.
- Fill the reaction tube with the reserve of 90 George liquid micro + 10 stock micro liquid.
- Drop on the Improved Neubauer count room as many as 5 micro
- Close with cover glass, count number of spermatozoa in the count room beneath the microscope.

b. Morphology of Spermatozoa Test

- Make a new stock liquid (0,5 ml 0,9% NaCl+ spermatozoa)
- Drop a stock of sperm liquid on the object glass.
- Pull with another object glass on the 45 degree position.
- Fixation by wet with methanol, after that dried.
- Then, drop the Giemsa liquid to color the cells, dry about 20 minutes after that wash hand with flow water, dry.
- Next, count underneath microscope with 10 fields of view in a zigzag.
- Count normal and abnormal spermatozoa.

c. Testicle Weight Test

- Measuring the weight of testicle is conducted by weighing testicle organ with analytical weight balance.
- Next, the result of mice's testicle weight that got the treatment is compared withcontrol mice's.



Picture. Scheme Treatment of Animals

RESULT AND DISCUSSION

Table I. Results of examination of the number of spermatozoa

Group	Mean	*SD*	P
K (-)	39.75	6.626	
K(+)	27.383	4.135	
P1	30.667	5.870	0.010
P2	31.1	5.0015	
P3	32.55	5.538	

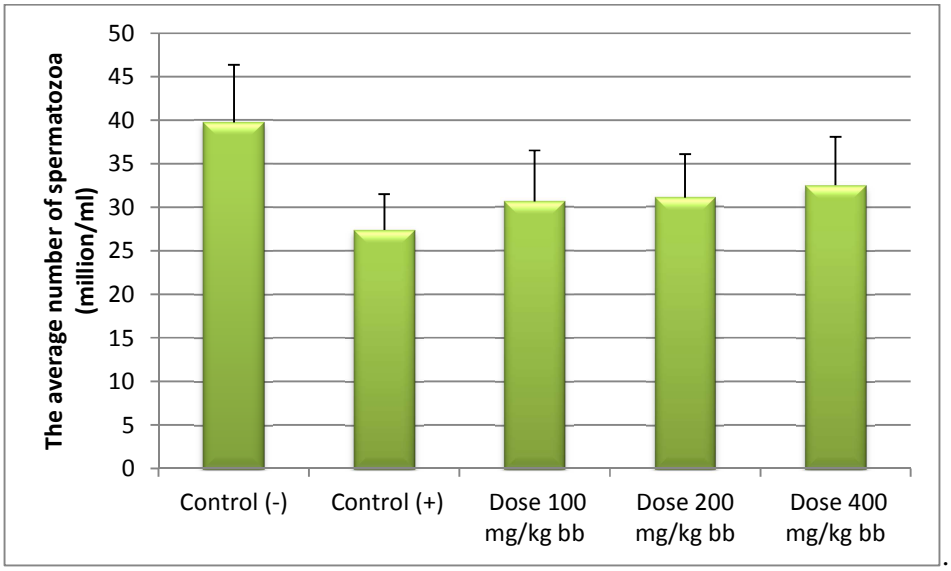


Figure . The bar chart the effect of tuber extract bits of the number of mice spermatozoa

Table II. Test results morphologically normal spermatozoa

Group	Mean	*SD*	P
K (-)	89.78	5.32	
K(+)	65.73	9.96	
P1	74.96	10.34	0,003
P2	76.12	3.12	
P3	75.82	12.78	

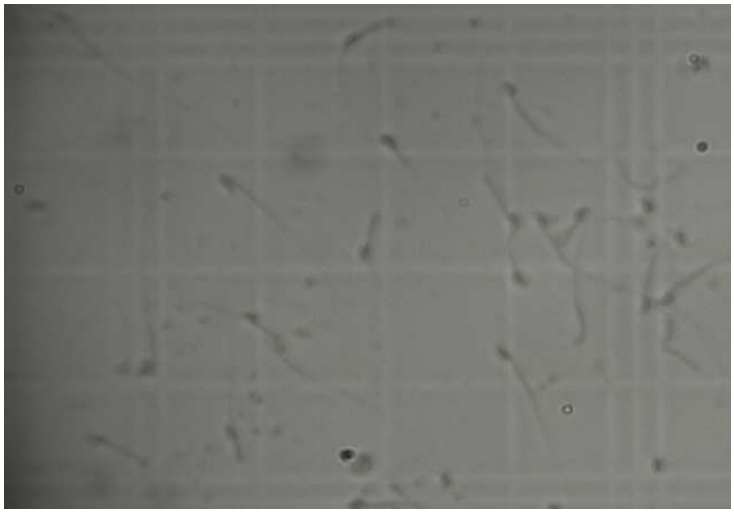


Figure. spermatozoa of mice in the hemocytometer (room count) (magnification 40x10)

Based on the result is acquired that there is differentiation numbers of sperm between negative and positive control groups. Moreover on the negative control is acquired 39,75 million/ml average number of sperm and on the positive control is gotten 27,383 million/ml average number of sperm. Furthermore, it can be concluded that the heat exposure affects the quality of sperm. It is also showed from P1, P2, and P3 with the average numbers of sperm are 30.667 million/ml, 31.1 million/ml and 32.55 million/ml. It can prove that ethanol extract of beet can repair the quality of damage sperm because of the heat exposure.

Meanwhile on the statiscial test by using one way anove test is acquared the result is (α< 0.05) where on the spermatozoa number count there is significance differentiation between control groups and dose groups. The average numbers of spermatozoa between positive control and dose one have significane differentiation. The Anova table is discovered α<0,010 where the acquared data has significance differentiation. After Duncan test, the data shows that between control groups and dose groups are in the two subsets that means there is significance data between control groups and dose groups.

It is supported by the data of normal spermatozoa morphology that on the negative control, the average normal of spermatozoa morphology is 89.78% and positive control groups is 65.73%, but on the P1 group is 74.96%, P2 group is 76.12% and P3 group is 75.82% .

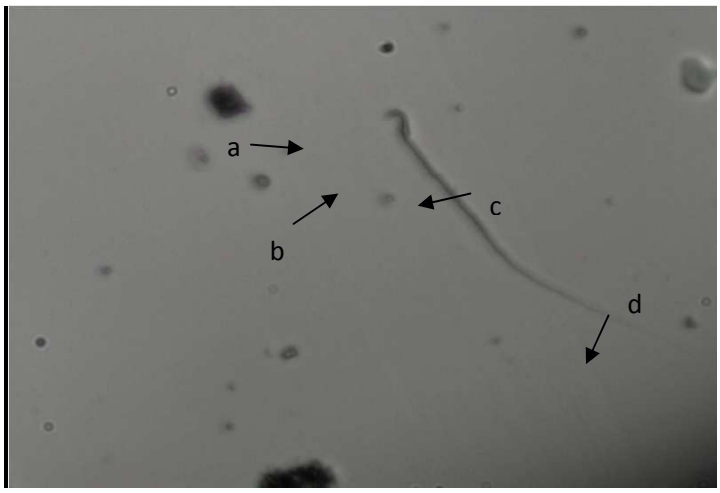


Figure . Morphology of normal spermatozoa, ga: the hook-shaped head, bh: neck, c: the central part, d: tails (magnification 40x10)

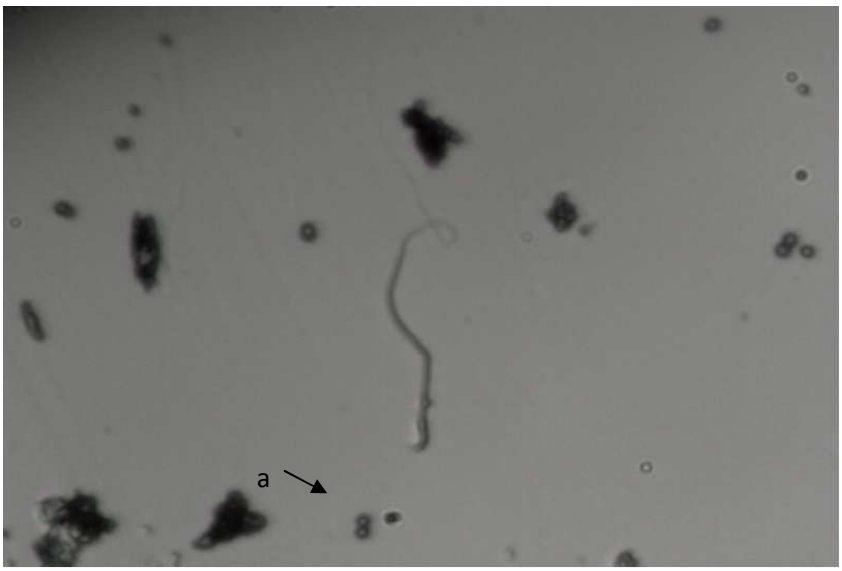


Figure . Morphology of abnormal spermatozoa, a: flattened head (magnification 40x10)

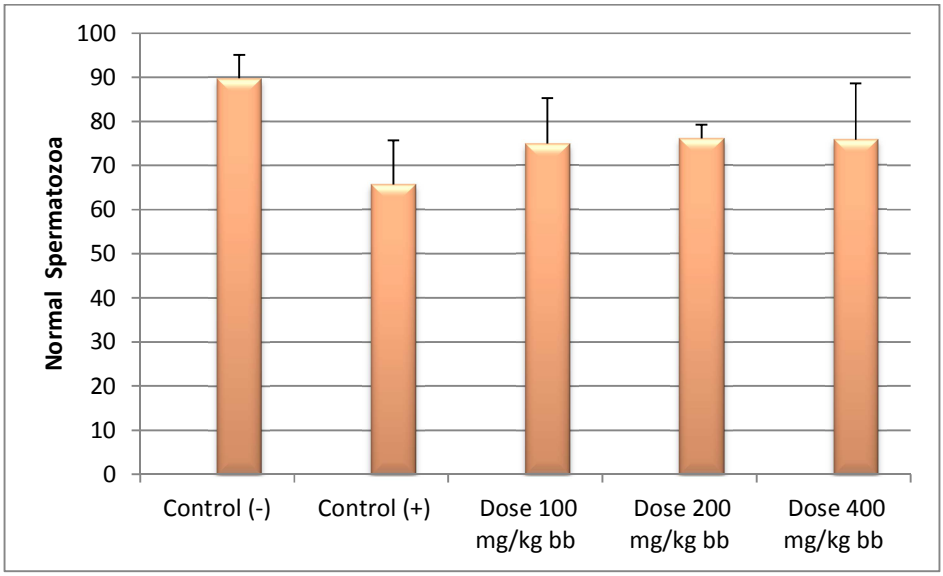


Figure . The bar chart beet extract to mice morphologically normal spermatozoa

Table III. The result of testicle weight measuring

Group	Mean	*SD*	P
K (-)	0.098	0.031	
K(+)	0.067	0.0268	
P1	0.087	0.021	0,012
P2	0.10955	0.010	
P3	0.10950	0.0036	

Moreover, the measuring of the testicle weight average of negative control groups, positive control group and P1, P2, and P3 groups are 0.098 gram, 0.067 gram, 0.087 gram, 0.10955 gram, and 0.10950 gram. The acquired data reveal the differentiation between positive control, P1, P2 and P3 group and negative control group ($p \leq 0,05$). The result of the testicle weight average on the entire groups show the consistency of data because of the treatment groups cause the testicle weight is bigger than control one.

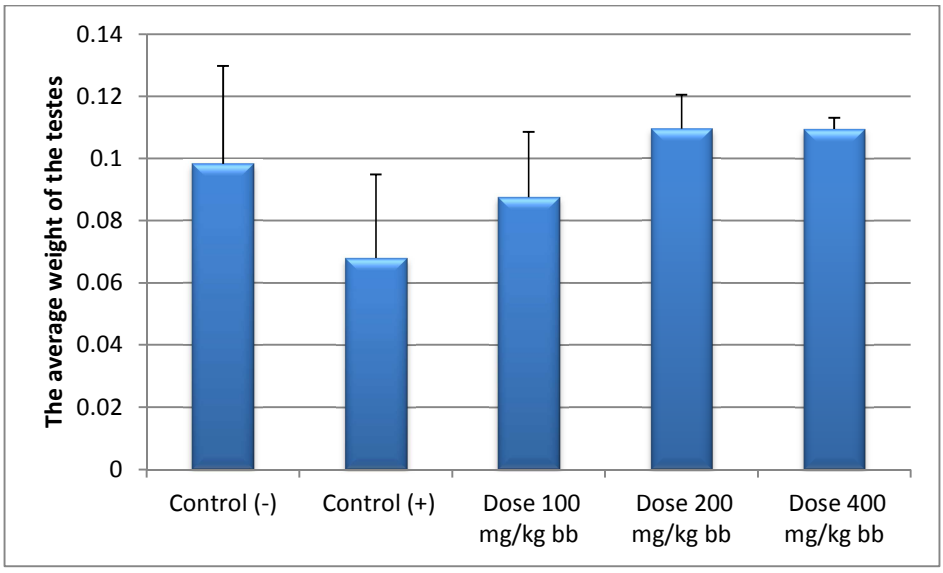


Figure .Diagram stem tuber extract bits to the weight of the testes of mice

CONCLUSION

Based on the research that has been done, the conclusions are:
1. Giving 100 mg/kgBB, 200 mg/kgBB and 400 mg/kgBB dose of beet ethanol extract cannot repair the number and increase the normal morphology of spermatozoa on mice that are induced by heat along 60 minutes.
2. Giving 200 mg/kgBB and 400 mg/kgBB doses of beet ethanol extract can repaire the damage of testicle weight because of the heat exposure.

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