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Study of fasting effect in holy Al-Qur'an on testosterone hormones in mice using Elisa method

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Abstract. Fasting is one of the routine worship performed by a Muslim. It has many benefits including controlling lust (Hadith of Bukhari and Muslim). Lust of an organism is controlled by the testosterone hormone. Fasting has the possibility to control the levels of testosterone in the blood so it does not exceed normal levels that be able to manage the organism's libido. The method that is used to determine hormone levels in the blood is the ELISA method (enzyme-linked immunosorbent assay). This study aims to determine the fasting effect in the Qur'an on testosterone levels in mice using the ELISA method. This study utilized 5 treatments (n=3). The types of treatments were not fasting (control), fasting in the middle of the Hijri month for 3 days; fasting on Mondays and Thursdays; Dawood fasting, and fasting every day for 36 days. Based on the type of fasting performed, fasting on Monday and Thursdays generated the highest increase in hormone levels followed by Dawood fasting then fasting every day for 36 days. Increased levels of testosterone did not reach excessive levels; thus it can be concluded that fasting can curb lust in the organism that was accordance with Hadith of Bukhari and Muslim.

1. Introduction

The millennial generation, or commonly called Y Generation which is always keeping up the current development although sometimes is not accompanied by their psychological development, especially in terms of maintaining the desire to act immorally. Lust or libido is controlled by the hormone testosterone[1]. One way to control these hormone levels is by fasting as written in Hadith of Bukhari and Muslim, Prophet Rasulullah said "O young men, whoever of you has a family should be married because he can control his eyes and keep his genitals. Anyone who has not been able to fast because he can maintenance his lust". Based on the hadith, it is explained that for young people who can not get married should fast because fasting can hold lust in the normal condition[2].

Health problems that occur in humans can be treated using 2 ways, namely pharmacological therapy (using drugs) and non-pharmacological therapy (without drugs). Fasting is one way that can be used for non-pharmacological therapy for abnormalities in the testosterone levels in the blood. The influence of pharmacological and non-pharmacological therapies before being carried out in humans (clinical trials), must be done in experimental animals (preclinical testing) to ensure safety and side effects. This study is still in the preclinical testing phase using mice in the form of mice. Mice are normally used as experimental animals because these animals belong to the class of mammals so the reproductive, respiratory, and circulatory systems resemble human[3]. In addition, mice have a relatively short life



cycle, many children per birth, variations in characteristics are high, easy to handle, anatomical characteristics and physiology are well characterized[4].

The hormone can be analyzed by various methods such as radioimmunoassay (RIA), enzyme immunosorbent assay (EIA), and enzyme-linked immunosorbent assay (ELISA). The advantages of the ELISA method compared the other two methods are simple, easy, economical and have a high enough level of sensitivity so it is suitable for research to test the types of hormones that are not too specific as in this study[5].

Fasting is one of the worship carried out by a Muslim and is done by refraining from eating, drinking, and things that cancel fasting from the dawn until the sunset. In Islam, there are several kinds of fasting such as obligatory fasting, namely fasting every day on the month of Ramadan (Surat al-Baqoroh: 183) and fasting of the Sunnah such as fasting on Mondays and Thursdays, Dawood fasting (Hadith of Bukhari and Muslim), and fasting 3 days in the middle of the month. Fasting has several benefits, such as the study which shows that fasting on Monday can increase body temperature[6]. Explains that Dawood's and Monday-Sunnah fasting can increase achievement motivation in students[7]. Dawood fasting can also stabilize human emotions[8]. Fasting on Monday can reduce anxiety levels in humans[9]. Thursday's fasting can reduce blood sugar levels[10]. Based on the results of the above research, the study of the effects of fasting in the Qur'an on testosterone levels in mice using the ELISA method has never existed, so this research has the urgency to finish it.

2. Materials and Methods

The materials used in this research were a plastic tub, wire mesh cover, plastic bottle for drinking of mice with the volume of 50 ml, digital scales (Acis, max: 500 g, d: 1 g) to balance the mice weight, syringe of 1 mL for taking blood samples of mice, Eppendorf tube of 1,5 ml to storage the blood sample, freezer (-20 °C) to maintain the temperature of blood sample, centrifuge (80-1 Table Top Low Speed Centrifuge), ELISA (Zenix-320 Microplate Reader) blood samples for analysis the testosterone hormones, ELISA kit (DRG, Germany), wood shavings as mats cages, tap water for drinking of mice, pellets type 511 broilers produced by PT. Japfa Comfeed Indonesia Tbk. for food of mice and. The experimental animals used in this study were male Balb / C strain mice with an initial weight of 23-26 grams and an age range of 8-10 weeks obtained from the Farma Veterinary Center Surabaya, East Java, Indonesia.

The research began by adapting mice to the treatment site for 1 week. Mice were placed in 30 cages (each cage containing 1 mouse) made of plastic tub surrounded by wire mesh, and given a wood shaving which was replaced every 4 days. Mice were given feed in the form of pellets and tap water which were given in *ad libitum*. Mice were weighed every day using digital scales to determine the body weight before being treated. Mice were set fasting (not fed and not given a drink) starting at 4 AM until 6 PM for 36 days.

The mice that have been fasted then were taken the blood through the heart \pm 1 ml and put it in an Eppendorf bottle. Then, the collected blood was centrifuged at 3,000 rpm for 20 minutes to get the plasma. The plasma obtained was stored in a freezer with a temperature of -20°C[11]. The blood plasma sent to the Physiology Laboratory, Faculty of Veterinary Medicine, Gajah Mada University to calculate testosterone levels using the ELISA method. Data were analyzed using one-way ANOVA with a significance level of 5%. The statistical program used for analysis is the SPSS 16 program.

3. Results and Discussions

3.1 Effect of fasting on body weight

The body weight of an organism can be influenced by the amount of food intake in the body, including mice. Fasting is done by refraining of eating and drinking from dawn to sunrise. Mice given fasting treatment will affect their weight. Data on the average body weight of mice were presented in Table 1. Based on the results of the analysis indicate that the value of the average body weight of mice is 0.317 which means that the average body weight of mice between treatment groups was not statistically significantly different ($P > 0.05$). Nevertheless, the average body weight of mice in treatment group B

decreased by 1.57% when compared to the control group. However, the average body weight of mice in the treatment group E, C, and D increased when compared to the control group, which amounted to 1.47%; 1.36% and 1.23%.

Table 1. Average body weight of mice

Treatment	n (the total of mice)	Body weight mice (gram) \pm SD
A	6	26,92 \pm 1,38 ^a
B	6	25,35 \pm 2,42 ^a
C	6	28,28 \pm 3,42 ^a
D	6	28,14 \pm 3,96 ^a
E	6	28,33 \pm 2,16 ^a

Information: a = no significant difference at the level of 5%
 n = number of experimental animals
 SD = Standard Deviation
 A = not fasting (control)
 B = fasting in the middle of the Hijri month for 3 days
 C = fasting on Mondays and Thursdays
 D = Dawood fasting
 E = fasting every day for 36 days

According to previous studies, fasting could cause weight loss so fasting is one way that can be done if you want to avoid a state of obesity or excessive weight [12–14]. In this study, the statement is contrary because fasting carried out in mice actually increases weight. Although the increase in body weight of mice does not cause obesity but makes mice having the ideal body weight which is close to the range of 30 grams. This fact is in accordance with the statement that the body weight of adult male mice ranges from 20-40 grams. 30 grams of body weight is the ideal body weight because it is not close to thin (20 grams) and also not close to obesity (40 grams) [15].

The increase in body weight of mice in this study was caused by observations that fasted mice had improved appetite. This was indicated by the mice who were fasted to always eat food when they are given food after fasting, is in the afternoon until the morning before they have fasted again. It is very different from the control group, which is not fasting mice. They tend to waste food given and actually their appetite decreases. One of the benefits of fasting is that it can maintain the digestive organs to work properly. If the digestive organs work well, the absorption of food will run well and the metabolic processes in the body will run well. This will cause the body to be healthy, be able to carry out activities well and also have an ideal body weight as the results shown in this study [16].

3.2 Effect of fasting on testosterone hormone levels

The hormone testosterone is formed from cholesterol originating from food intake so that there is a possible correlation between the treatment of differences in fasting with testosterone levels. Data on the average testosterone levels of mice were presented in Table 2. Based on the results of the analysis indicate that the value of the significant average testosterone of mice was 0.308 which means that the average testosterone hormone mice between treatment groups were not statistically significantly different ($P > 0.05$). Nevertheless, the average testosterone hormone in mice in the C, D, and E treatment groups rose by 73.51%; 53.21% and 28.86% when compared to the control group. However, the average testosterone level of mice in treatment group B decreased compared to the control group, which was 14.74%.

Table 2. Average testosterone hormone levels of mice

Treatment	n (the total of mice)	Testosterone hormone levels of mice (ng/ml) \pm SD
A	6	0,2423 \pm 1,139 ^a
B	6	0,2066 \pm 0,099 ^a
C	6	0,9146 \pm 0,346 ^a
D	6	0,5179 \pm 0,658 ^a
E	6	0,3406 \pm 0,296 ^a

Information: a = no significant difference at the level of 5%
 n = number of experimental animals
 SD = Standard Deviation
 A = not fasting (control)
 B = fasting in the middle of the Hijri month for 3 days
 C = fasting on Mondays and Thursdays
 D = Dawood fasting
 E = fasting every day for 36 days

Based on the results of this study, testosterone levels in the treatment group namely fasted mice showed an increase compared to the control group of mice that did not fast. The hormone testosterone is a hormone produced by Leydig cells in the testicles. The process of synthesis of this hormone is stimulated by the hormones GnRH and LH. GnRH produced by the hypothalamus stimulates the anterior pituitary to produce LH. LH stimulates Leydig cells to produce testosterone. The testosterone hormone has two roles, namely affecting spermatogenesis in the testes and going into the blood vessels and then circulating throughout the body to control secondary sex in male organisms. The process of forming this hormone is strongly influenced by the presence of energy from respiration (burning food using oxygen). In the discussion above, it was explained that fasting can improve appetite, improve the performance of digestive organs, and metabolic processes can run well so that food absorption runs optimally. Food that is sufficiently available will produce very enough energy, one of which is for the formation of the hormone testosterone[17]. In addition, explains that the hormone testosterone is formed from cholesterol which initially in the form of LDL in the blood and LDL is also derived from food intake that enters the body of the organism[18].

The results of ANOVA analysis from this study indicated that fasting could increase testosterone levels even though statistically it was not significantly different. The normal testosterone level in adult male mice is 3.06 ng/mL³. The testosterone hormone in addition to controlling the process of spermatogenesis can also control lust or libido in organisms. If the hormone levels rise above the normal limit, it will cause lust also to increase excessively. Based on the results of this study, the level of male mice testosterone hormone fasted rose from the control group but did not exceed the normal limit of more than 3.06 ng/mL[1].

Based on the type of fasting performed, fasting on Mondays and Thursdays had the highest effect on the increase in testosterone, then followed by Dawood fasting and fasting every day for 36 days. Whereas the middle fasting month of the Hijri actually decreases testosterone levels compared to controls. This shows that the type of fasting performed to control testosterone levels should not be too much, such as fasting every day or not too little, such as fasting 3 days in the middle of the Hijri month. Dawood fasting is still not optimal for controlling testosterone levels because the distance between fasting with one another is too close, which is only 1 day. The best type of fasting to control testosterone levels is seen on fasting on Mondays and Thursdays. This is because the distance between fasting with one another is not too close and not too long. The process of forming testosterone from cholesterol is probably best to occur with a distance of 2 to 3 days as in the treatment of fasting on Mondays and Thursday. The biosynthesis process of testosterone hormones occurs from cholesterol in the mitochondria of testis cell. It consists of many steps, including transferring cholesterol from mitochondria membrane pass through StAR protein. After that, cholesterol will be changed to be

testosterone hormones using enzymes including 3β -hydroxysteroid dehydrogenase, 17α -hydroxylase/ $C17$ - 20 -lyase, and 17β -hydroxysteroid dehydrogenase, as shown in Figure 1 below[19].

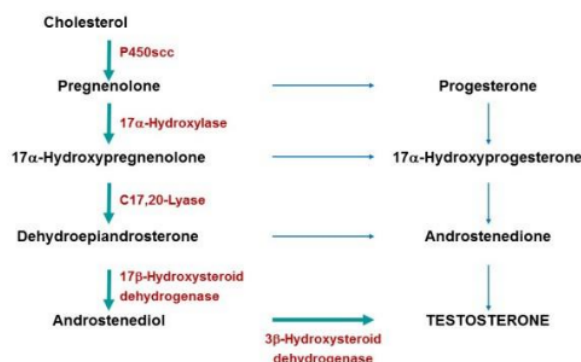


Figure 1. Biosynthetic pathway of testicular Testosterone synthesis

The method used to measure testosterone levels in the blood of mice in this study used the ELISA method. ELISA (enzyme-linked immunosorbent assay) is one technique designed to detect and quantify peptides, proteins, antibodies, and hormones on a plate-based. This polystyrene plate in ELISA will bind antibodies and proteins, making this technique easy to design and do. The working principle of ELISA is employing antibodies associated with enzymes complexed with antigens that are immobilized on a solid surface. While the enzyme activity on the substrate. It can be used as a method of detection in this technique. The necessary procedures in ELISA are four as in Figure 2 below (direct, indirect, sandwich, or competitive). ELISA is directly carried out by direct immobilization of the antigen by absorption on the plate. Whereas ELISA is not immediate, immobilization of antigen is not directly absorbed in the plate but on antibodies on the plate. In sandwich ELISA, antigens can interact with 2 types of antibodies so this ELISA technique tends to be specific to antigens. It has at least 2 antigenic sides (interactions with antibodies) or multivalent antigens such as polysaccharides or proteins. While Competitive ELISA is done by adding competitors to the micro titer holes on the plate[5,20].

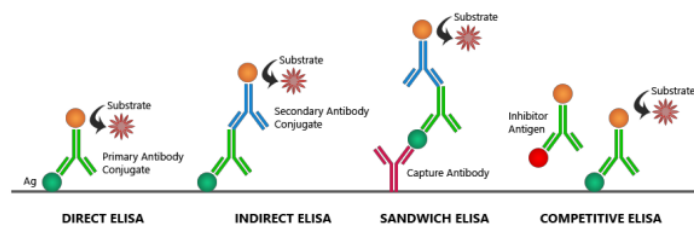


Figure 2. The types of ELISA

4. Conclusions

The results showed that fasting in the holy of Al-qur'an caused an increase in testosterone levels in mice. Although statistically the increase was not significantly different (P value > 0.05). Based on the type of fasting performed, fasting on Monday Thursday caused the highest increase in hormone levels followed by fasting every day for 36 days. Whereas the middle fasting month of the Hijri actually decreases testosterone levels compared to controls. Increased levels of testosterone did not reach excessive levels.

Thus, it could be concluded that fasting has ability to curb lust in the organism in accordance with the word of Rosululloh in Hadith of Bukhari and Muslim.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7