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Effectivity of honey to regenerate the production of testosterone by induction of endogenous stem cells of rat with low libido due to malnutrition

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ABSTRACT

The aim of the study was to know effectivity of honey from bee forest (*Apis dorsata*) to regenerate the production of testosterone by induction of endogenous stem cells of rat with low libido due to malnutrition. 40th rats as animal model were devided 4th groups: normal male rats without honey (C-) and 3th groups rats with low libido due to malnutrition were consists of without honey (C+), with 30% (v/v) honey (T1) and 50% (v/v) honey (T2) during 10 days. Low libido condition was induced by fasting without food during 5th days. Results: The improvement of libido was based on immunoreactivities of testosterone were detected in testis tissue. Testosterone expression as an immune response for regenerate signal in group C-, C+, T1 and T2 respectively is 2.95^{c} ± 0.15; 0.19^{a} ± 0.55; 0.27^{a} ± 0.45 and 2.05^{bc} ± 0.35. T1 and C+ were decreased significantly ($p < 0.05$) compared with C- and T2, while T2 didn't show decrease significantly ($p > 0.05$) compared with C-. The libido level were based 3 category of libido: the number of copulations, time of reaction and period of copulation. The T1 group was not increased of libido, while in the T2 group, there was an increased of libido that significant different ($p<0.05$) compared with T1 and C+. The conclusion is the 50% (v/v) honey from bee forest (*Apis dorsata*) can to be use regenerate the production of testosterone by induction of endogenous stem cells and improvement of libido of rat.

Keywords: Honey, *Apis dorsata*, testosterone, libido.

1. Introduction

One of the major cases of low libido in male rat can be caused by malnutrition with the testis tissue degeneration (Safitri[a] et al., 2016; Prasetyo and Safitri, 2016). Therapy for degenerative testis tissue was using stem cells in now and next few decades is very interesting and greatly increased sharply, because stem cells potential is very promising for utilization as a treatment (Caplan and Correa, 2011). Nevertheless, the complexity of the isolation method, in vitro culture process and transplant procedure with booster treatment are very expensive (Hariadi and Safitri, 2019). The innovation therapy is needed as an effort to degenerative testis tissue in male. The honey as bee product is a nutrient gotten from bees (Safitri[b] et al., 2016; Hasib et al., 2017). It could serve as an antioxidant and as an antibacterial (Naqvi et al., 2013). Antioxidant is an important substance that protects individuals from free radicals such as reactive oxygen species (ROS). Adequate consumption of an antioxidant can reduce the prevalence of cardiovascular failure, cancers, digestive tract disorder, cataract and other degenerative diseases (Silva et al., 2013; Prasetyo and Hestianah, 2017), It also leads to degenerative testicular (Safitri[a] et al., 2016). Consumption of honey is also effective in the treatment of diarrhea with low immune response (Prasetyo and Safitri, 2016) and the reproductive system disorder as well (Safitri[b] et al., 2016; Parwata et al., 2010).

As a conceptual solution, further study is needed to explore the benefits of the honey from the use of bee forest (*Apis dorsata*), to induce of endogenous stem cells as an alternative for stem cells therapy.

2. Materials and methods

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2.1. Low libido condition

Low libido model of male rat was available through food fasting 5th days long, but drinking of water still administrated (Safitri[a] et al. 2016). The experiment animals was used in this study are healthy, 8-10 weeks old male rats (*Rattus norvegicus*), Wistar strain with a body weight of 250-300g each. They were placed in an individual plastic cage in experimental animal laboratory at Faculty of Veterinary Medicine, Universitas Airlangga. The study was approved by ethical committee vide Ethical Clearance (*Komisi Etik Penelitin, Fakutas Kedokteran Hewan Universitas Airlanga*), Animal Care and Use Committee (ACUC). They were divided into 4 groups, each group consist of 10 rats. Group C-: normal male rats without honey, Group C+: low libido male rats without honey, Groups T1 and T2: the low libido of rats were given 30% (v/v) and 50% (v/v) of honey product from bee forest (*Apis dorsata*) for 10 days.

2.2. Immunoreactivity of testosterone by Immunohistochemical (IHC)

The testosterone expression was performed by immunohistochemical (IHC) method. Before IHC observation were made histological preparation, by way of a incision was made transversely of testicular tissue from paraffin block. Furthermore, examination through IHC method using a monoclonal testosterone antibody (Monoclonal, P2G1, Thermo Fisher Scientific, Pittsburgh, PA, USA). That was done to determine the expression of testosterone. Observation of testosterone were made with 200x magnification of microscope (Nikkon H 600L Microscope; digital camera DS Fi2 300 megapixel), and the expression of each variable was indicated by score of cells with brownish discoloration in each incision (Hariadi and Safitri, 2019).

2.3. Libido observaton

The libido observation of male rats through single mating with the female rat. Estrus synchonization was performed on the female rat using the PMSSG-hCG hormone (Bioworld, 220606401-MIH9827, Thermo Fisher Scientific, Pittsburgh, PA, USA), this was done in order to provide same reproductive organs in estrus phase. The detection of the capability of the libido of male rats were done by the close monitoring of the female rats. The detection of the libido is done after 1 cycle of spermatogenesis process which takes about 35days and they were fed normally. The libido data was taken based on the observations of the male rats and the female rats in the cage. (Safitri et al., 2014; Canale and Postonia, 2000). The libido level were based 3 category of libido: the number of copulations, time of reaction and period of copulation These observations were made on the 4th day, after a one hour mating, after which the male rats were separated from the female rats.

2.4. Statisticl analysis

Expression of score of testosterone and male rat libido that classified into 3 categories (the number of copulations, time of reaction and period of copulation) were statistically analyzed using SPSS 15 for Windows XP with the level of significance 0.05 (=0.05) and the confidence level 99% (α=0.01). Test data normality with the Kolmogorof Smirnov test, homogenecity of variance test, Anova and post-hoc test using the Tukey HSD 5%.

3. Results

The improvement of libido was based on immunoreactivities of testosterone were detected in testis tissue. Testosteron expression as a immune response for regenerate signal of Leydig cells in testis in group: C-, C+, T1 and T2 respectively is 2.95^{c} ± 0.15; 0.19^{a} ± 0.55; 0.27^{a} ± 0.45 and 2.05^{bc} ± 0.35 (Table 1, Figure 1).

The observations of the male rat libido were classified into 3 categories (Safitri et al., 2014; Canale and Postonia, 2000): Number of mating per unit time, reaction time, and the mating period (time between one mating with the next mating). The result of the libido test after being observated was based on the statistics as shown on Table 2.

4. Discussion

The results of this study showed that the administration of a dose of 50% (v/v) honey product from *Apis dorsata* in drinking water for 10 days in the T2 group can be used to treat low libido of male rats. Observation for libido from the male rats, are divided into three categories (Safitri et al., 2014; Canale and Postonia, 2000): number of mating per unit time, reaction time or time at the introduction of the male and female animals until the first mating; and the mating period or time of one mating with the next mating. The control positive group (C+) experienced a decreased libido, because the adrenal cortex became ineffective in producing dehydroepiandrosterone (DHEA) due to malnutrition. Low levels of DHEA in the blood can be acause of decreased body stamina, fatigue, and also decreased libido. DHEA was produced by the renal adrenal cortex (Heckbert and Heian, 2002) and the Leydig cells (Hafez and Hafez, 2013) is the most potent precursor of steroid

hormones such as testosterone. It also occurs in the degenerative group of testes who have received 30% honeybee product (T1) for 10 days. This suggests that 30% dose of honey has not been able to restore libido as in the negative control group (C-).

Table 1

Score of Testosterone expression in rat testis with IHC method in several treatments.

No	Treatments	Average score of testosterone ± SD
1.	Negative control group (C-) : no fasted (fed standard ad libitum) and without honey	$2.95^{c} \pm 0.15$
2.	Positive control group (C+) : fasted for 5th days and without honey	$0.19^{a} \pm 0.55$
3.	Trial group 1 (T1) : fasted for 5th days and 30% (v/v) honey during 10th days	$0.27^{a} \pm 0.45$
4.	Trial group 2 (T2) : fasted for 5th days and 50% (v/v) honey during 10th days	$2.05^{bc} \pm 0.35$

^{a,b,c,d} Different superscripts in the same column was significantly different ($P<0.05$).

Table 2

Libido test based on 3 category of libido.

No	Treatments	3 Category of Libido		
		Number of mating per unit of time (60 minutes) ±SD	Reaction time or time at the introduction of the male and female animals until the first mating (in 60 minutes observation)±SD	Mating period or time of one mating with the next mating (during 60 minutes) (average in minutes) ± SD
1.	Negative control group (C-) : no fasted (fed standard ad libitum) and without honey	$5.2^{c} \pm 0.6$	$2.495^{b} \pm 0.37$	$13.176^{b} \pm 1.102$
2.	Positive control group (C+) : fasted for 5th days and without honey	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$
3.	Trial group 1 (T1) : fasted for 5th days and 30% (v/v) honey during 10th days	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$
4.	Trial group 2 (T2) : fasted for 5th days and 50% (v/v) honey during 10th days	$3.1^{b} \pm 0.497$	$5.56^{c} \pm 1.75$	$18.64^{c} \pm 1.245$

^{a,b,c,d} Different superscripts in the same column was significantly different ($P<0.05$).

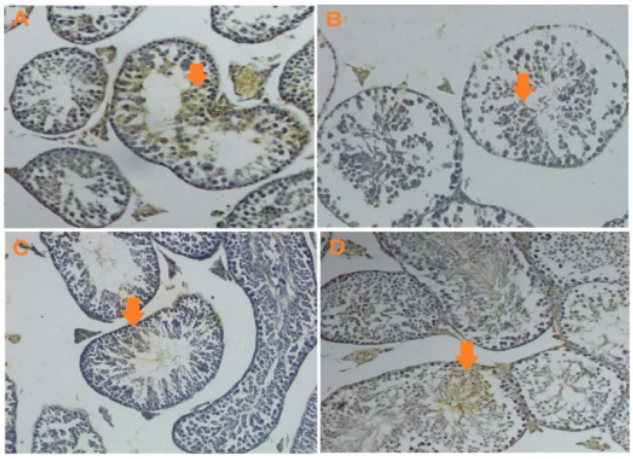

Fig. 1. Immunohistohemical analysis from response immune signal of testicular tissue based on Testosterone expression (brown chromogen) on several treatments 200× magnification (Nikkon H600L Microscope; digital camera DS Fi2 300 megapixel). The different superscripts indicate significant difference at $p < 0.05$. A. the negative control group (C−) was on the score $2.95^{c} \pm 0.15$; B. The positive control group (C+) was on the score $0.19^{a} \pm 0.55$; C. The group 30% (v/v) honey (T1) was on the score $0.27^{a} \pm 0.45$; D. The group 50% (v/v) honey (T2) was on the score $2.05^{bc} \pm 0.35$.

DHEA was increased as a precursor of steroid hormones (testosterone), it is also responsible for fat metabolism and acts as an enzyme inhibitor of glucose 6-phosphat dehydrogenase, which has a role as a biocatalyst that changes glucose to fat. The increase in DHEA allows for an increase in the amount of free ATP in the body, thereby, increasing the body stamina (Joshi et al., 2018), libido and fertility (Hafez and Hafez, 2013).

The libido process begins from the stimulation in the hypothalamus, where dopamine is produced as neurotransmitters and neurohormones that affect the activities and sexual behavior in individuals. The stimuli received by the sensory nerve triggers the acetaminoline in the stimulation of the nitric oxide secretion from endothelial cells to activate the cGMP. Activation of the cGMP causes the muscles of the corpus cavernosus, and the penis to be relaxed. This causes the occurrence of dilatation of the penis arteriole so that the blood will flow in erectile tissue of penis. The erection of erectile tissue from the penis by the blood will cause the veins to be depressed and inhibit the release of blood and water so that there is an increase of tugor in the penis, which leads to erection (Guyton and Hall, 1997).

Lack of glucose in rats with malnutrition and degenerative testicles, were found in the positive control group (C+) and the 30% honeye bee product group (T1). This caused less fuel and energy sources. Glucose is universal for all cells proliferation including spermatogenesis process. The unavailability of glucose leads to the absence of carbon source for synthesis of most other compounds such as amino acids, nucleic acids, fatty acids, cholesterol, and steroid hormones such as testosterone. In addition, glucose is needed as a precursor for a variety of other glucoses such as glycosaminoglycans, lactose and nucleotides (Marks et al., 2000).

However, the existence of malnutrition due to the control positive group (C+) means there is a deficiency of vitamin B complex and unavailability of glucose from the food ration, plus imperfect metabolic process, it can be assumed as free energy or ATP in the body. This would also decrease the stamina of the male rat so that there would be a manifestation of decreased libido.

The effectivity of honey from bee forest (*Apis dorsata*) to regenerate the production of testosterone by induction of endogenous stem cells of rat with low libido due to malnutrition and increase of libido of rat based on 3 categories could be improvement.

5. Conclusion

The conclusion is the 50% (v/v) honey from bee forest (*Apis dorsata*) can to be use regenerate the production of testosterone by induction of endogenous stem cells and improvement of libido of rat based on 3 category (the number of copulations, time of reaction and period of copulation)

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