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To cite this article: Nursasi Handayani and Abdul Gofur 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **276** 012056

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# Does Propolis Extract Alleviate Male Reproductive Performance Through Gonadotropic Hormone Levels and Sperm Quality?

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**Abstract.** Nowadays, the exploration of natural substances as an antifertility agent is the new paradigm in controlling the production and physiological performance of male and female germ cells. Natural antifertility agents have fewer side effects compared to chemical synthetic antifertility. Among that, the propolis extract was improved the reproductive performance through increasing sperm and male gonadotropic hormone activity. However, the discrepancy remains. Even though the action of testosterone has an essential role during spermatogenesis. However, the higher level of this hormone was also suggested to correlate with infertility. Thus, the circulating testosterone levels can be used as an additional parameter for monitoring reproductive system disorders. The purpose of this study was to evaluate the effect of Propolis extract on testosterone hormone levels of Balb C mice (*Mus musculus*). Male Balb C strain mice (*Mus musculus*) with age of 10-12 weeks and weight of 25-29 g were used. There were six kinds of treatments were approved, including a control group and five serial dilutions of the propolis extract (2.5%, 5%, 7.5%, 10%, and 12.5% ). The propolis extract was given peroral for 18 days. Mice were dissected on the 19<sup>th</sup> day, and its blood was taken to count the levels of testosterone. The quantification of circulating testosterone levels was carried out by using the ELISA test. The data were analyzed by one way ANOVA and followed by LSD test. Testosterone level began to increase at 10% concentrations of propolis extract. It can be concluded that Propolis Extract can increase testosterone levels. Thus, it is expected to serve as a potential male anti-fertility agent.

**Keywords:** Propolis extract, antifertility, testosterone level, BALB/C Mice

## 1. Introduction

The use of propolis is shared among the people at present. The propolis that is used is propolis extract, both in liquid and in solid form. Propolis is commonly used as a medicine for skin wounds, antibacterial, antiviral, antifungal, antiparasite, and antioxidant. It also could improve body immune. A research result by Alencar *et al.* found that propolis contains flavonoids, terpenes, phenolics and esters, sugar, hydrocarbon elements and minerals [1]. The chemical content of the propolis varies by the region as well as the pollen of the tree collected by the bees [2]. The research on propolis reported



was addressed for antibacterial activity [3]. According to Harfouch *et al.*, Syrian propolis extract shows antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii* [4]. Also, Ruffato *et al.* found that Red propolis is a potential natural agent as antibacterial, anti-inflammatory, antioxidant, cytotoxic and healing agent [5]. However, there was limited information on whether propolis extract could also potentially have another effect especially as an antifertility agent in the male individual.

The potential for natural ingredients as antifertility can be identified through phytochemical screening from several plants. Plant extracts administration to the male animal model can be proposed as an antifertility agent. For instance, the plant extract results in antispermatogenic effects, antifertility of post testis, spermicide, decreased sperm immobilization, and antiandrogenic effects [6]. The previous studies showed that the extract of *Albizia lebbeck* (Linn.) Benth, *Aloe barbadensis* Mill. Syn., *Acalypha indica*, *A. littoralis*, and *A. vera* can act as antiandrogenic activity on in vivo model [6].

Moreover, *Hibiscus rosasinensis* with steroids, tannins, saponins, and flavonoids contents also showed as antispermatogenic and antiandrogenic activity [7]. The presence of these components is a primary indication that the substance has potential as an antifertility agent. Plants that contain saponins, flavonoids, and phenols mostly have spermicidal or sperm immobilization effects [8]. Propolis contains flavonoids, which is one of the most important compounds found in propolis [9]. The results of the study showed that administration of propolis in mice could decrease sperm motility, number, and increase abnormal sperm morphology even not significant statistically [9]. The report by Reda *et al.* also showed that the administration of propolis in male rats tends to cause the decrease in sperm motility, number, and viability while corroborating sperm abnormality [10].

Importantly, the antifertility compound can work in two ways, by the effect of cytotoxic or cytostatic and hormonal impact [11]. In case the compound is toxic, cells that are exposed to these compounds could be died immediately, especially for the developing cells. The similar chemical structure between this natural compound and the hormone could inhibit the action of a hormone in organs targeted at the same receptor. In specific, for the natural hormone in the body, negative feedback of testosterone can be caused by exogenous administration of testosterone. As a result, the level of LH and FSH secretion decreased significantly. The administration of AAS (Anabolic Androgenic Steroids) can cause infertility in males, abnormalities that appear can include oligozoospermia or azoospermia, sperm motility, and morphology [12].

Importantly, one of the antifertility parameters used to determine that a material has the potential to be antifertility is the level of testosterone hormone. The levels of testosterone can affect spermatogenesis [13], and androgen hormones also play a role in regulating the function of reproductive organs and their structures [14]. Testosterone is a steroid hormone in the group of androgens produced by the testes, precisely in the Leydig cells located between the seminiferous tubules. Testosterone is an essential hormone that regulates spermatogenesis or sperm formation, so can be used as a potential indicator of the success of spermatogenesis. The decreasing of sperm formation also influenced by the testosterone levels and closely associated with fertilization rate. According to the previous investigation, this study aimed to explore the primary contribution of propolis extract on spermatogenesis and do confirmation whether this extract has an additional effect on fertility related to sperm activity in the reproductive organs.

## 2. Methods

This research was conducted at the Zoology Laboratory, Department of Biology, Universitas Negeri Malang. Propolis extract in the liquid form was diluted using dilution 40 times, 20 times, 13.33 times, 10 times, and 8 times. There were six treatments in this study: control group and the 2.5%, 5%, 7.5%, 10%, 12.5% concentrations (control, dilution 40 times, 20 times, 13.33 times, 10 times and 8 times) of Propolis Extract. Male Balb C mice (*Mus musculus*), aged 10-12 weeks and weighed 25-29 g, were used as experimental animals.

Oral treatments were performed by using a gavage tool and were repeated five times for each treatment. Propolis Extract was given for 18 days. On the 19<sup>th</sup> day, mice's cervical was dislocated and

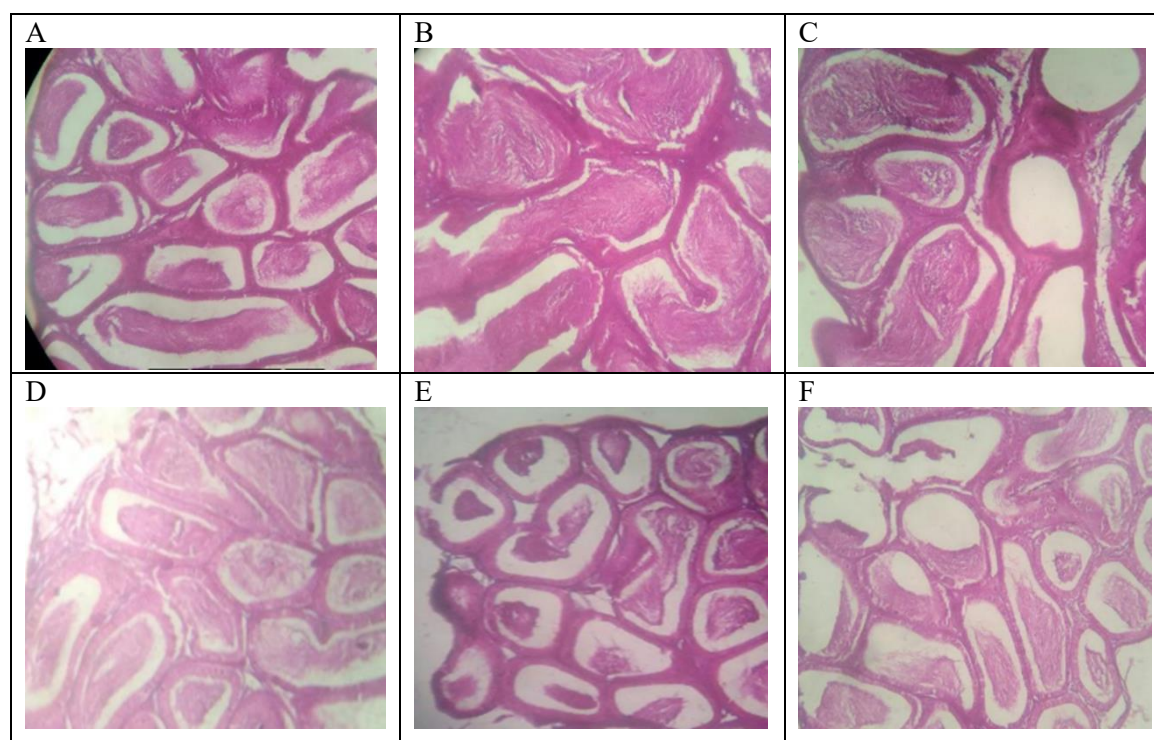
dissected; then its blood was taken from its heart. The separation of serum and plasma is performed by centrifuging blood as much as 1 mL at a speed of 3000 rpm for 15 minutes. The measurement of testosterone levels was carried out by the ELISA method. One-way ANOVA test was applied with a significance level of 5%, which was used to examine the influence of Propolis Extract. The significant data were tested by the Least Significant Different model with the same significance level.

### 3. Results and Discussion

The baseline data of testosterone levels in all experimental group are listed in Table 1. Testosterone levels of the mice pre-administrated by the propolis extract significantly increased compared to the control group. When testosterone increased or decreased, then spermatogenesis was affected, because spermatogenesis depends on testosterone hormone and mice sperm motility.

**Table 1.** Testosterone level and motility of mice after being treated with propolis for 18 days

No.	Treatment (%) or dilution (times)	Testosterone levels (ng/mL)	Motility (%)
1.	0	$9.441 \pm 2.247^a$	$53.40^d \pm 3.647$
2.	2.5 (40)	$8.276 \pm 1.756^a$	$49.40^{cd} \pm 3.578$
3.	5 (20)	$8.444 \pm 2.998^a$	$47.60^{bc} \pm 5.683$
4.	7.5 (13.33)	$10.482 \pm 4.627^{ab}$	$45.40^{bc} \pm 2.191$
5.	10 (10)	$14.786 \pm 7.087^{bc}$	$43.80^b \pm 3.114$
6.	12.5 (8)	$16.806 \pm 4.205^c$	$36.20^a \pm 4.147$



**Figure 1.** The density of sperm in the lumen of the epididymal ducts of mice after being treated with propolis for 18 days (A) control; (B) concentration treatment 2.5%; (C) concentration treatment 5%; (D) concentration treatment 7.5%; (E) concentration treatment 10%; (F) concentration treatment 12.5%.

The potential of propolis extract as an antifertility could be possibly related to the flavonoid contents. A study by Alencar et al. found that propolis contains flavonoids, terpenes, phenolics and esters, sugar, hydrocarbon elements and minerals [1]. The chemical content of the propolis varies by the region as well as tree utilized by the bees [2]. According to Inge and Jochen, there are four working principles of antifertility agents: cells destruction (cytotoxic or cytostatic effect) and hormonal function disruption (hormonal effects), mutagenicity and functional toxicity. The toxic effects induce cells died after exposure, in particular, the developing cells, such as epithelium germinal cell [11].

One of the ingredients within the propolis extract, flavonoid, can affect the reproductive hormones as anti-estrogen [15]. The report from Yong *et al.*, suggest that three flavonoids (kaempferol, quercetin, and isorhamnetin) from GBE (*Ginkgo biloba* Extracts) synergistically inhibit the aromatase, an enzyme that catalyzes the conversion of androgens to estrogen [16]. Provision of propolis extract increases testosterone hormone so that it can inhibit spermatogenesis. The data of our study showed that the administration of propolis could raise testosterone levels (Table 1). The inhibition of spermatogenesis due to the application of propolis extract may be carried out by flavonoids action. The primary mechanism of this inhibition process may through a negative feedback mechanism from testosterone [17]. Testosterone has receptors in the hypothalamus and pituitary. The higher level of testosterone gives a negative feedback effect on the hypothalamus and pituitary so that high levels of testosterone send signal to the hypothalamus to reduce production of Gn RH and production of LH and FSH by the pituitary.

Besides, the GnRH target that leads to the pituitary also causes LH and FSH production to decrease. LH target cells are Leydig cells, which produce testosterone. Testosterone production is stimulated by LH from the pituitary, so decreasing LH levels causes testosterone produced by Leydig cells to decline; as a result, spermatogenesis is also inhibited. FSH plays a role in controlling the development of germ cells. Together with LH, it has a role in stimulating AR (Androgen Receptor) expression and controlling proliferation and maintaining Sertoli cell function [18,19]. As a result, a decrease in FSH due to negative feedback testosterone can reduce the expression of AR and decrease Sertoli cell proliferation and function, which is thought to cause the number of androgen receptors in Sertoli cells to decrease. Reduction of androgen receptors causes testosterone bond to the receptor to decrease, resulting in much of testosterone in the blood. Since testosterone is bonded to a small number of receptors, the function of Sertoli cells in spermatogenesis will be disrupted. The higher concentration of AR indicates that AR can mediate androgen hormones in the seminiferous epithelium so that spermatogenesis continues [20].

The results of this study showed that the administration of propolis could increase testosterone levels resulting from the presence of flavonoids found in propolis. The high testosterone levels of mice can result in negative feedback, so that GnRH, LH, and FSH decreases, due to decrease in LH it causes Leydig cells not to produce testosterone. Like research from Dohle *et al.*, that giving AAS (Anabolic Androgenic Steroids) can cause infertility in males, abnormalities that appear can include oligozoospermia or azoospermia, their motility and sperm morphology [12]. The sperm motility of mice given propolis also decreased (Table 1). The density of sperm in the lumen of the epididymal duct from treated mice is also laxer than control (Figure 1). The data proved that propolis could cause oligozoospermia. Based on the research of Reda et al. on the cross-section of the testes in experimental animals that were given propolis, the thickness, and density of the germinal epithelium tends to decrease [10].

The decrease in sperm motility of mice may also be caused by a lack of fructose produced by seminal vesicles. The results of the previous study improved that fructose is the primary energy source for sperm and almost all fructose is formed in seminal vesicles [21]. Even though high levels of testosterone in the blood, but through negative feedback from testosterone, cause the AR to form to be reduced. The process of fructose formation in seminal vesicles is initiated and controlled by the testicular androgens [21]. Since the receptor is reduced, the amount of testosterone bound to the

vesicles is also reduced, so that the formation of testosterone-dependent fructose is also disrupted. As a result, the motility of the sperm also decreases.

#### 4. Conclusion

The administration of propolis extract increased testosterone levels of Balb C mice (*Mus musculus*). To sum up, the propolis extract may potentially cause male anti-fertility with a negative feedback mechanism on adenohipophysis hormone.

#### Acknowledgments

We thank DRPM RISTEKDIKTI for the research grant and all colleagues who participated in this study.

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