Quantity and quality of guinea pig *(cavia porcellus)* spermatozoa after administration of methanol extract of bitter melon (*momordica charantia*) seed and depot medroxy progesterone acetate (DMPA)

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Abstract. The discovery of male contraceptive drugs continues to be pursued, due to the few participation of men associated with the lack of contraceptive options for men. The combination of bitter melon seed methanol extract and DMPA are the options that currently apply to men. Therefore, the use of guinea pigs as experimental animals conducted research using experimental methods with complete randomized design (CRD). There are 4 control groups and 4 treatment groups. The first group, control group of dimethyl sulphoxide (DMSO) for 0 week (K0), The second one, bitter melon seed extract of 50 mg/100g Body Weight/day for 0 week (P0), the third one, control group of dimethyl sulfoxide (DMSO) for 4 weeks (K1), the fourth one, bitter melon seed extract of 50 mg/100g BW/day for 4 weeks + Depot medroxy Progesterone Acetate (P1), the fifth one, control group of dimethyl sulfoxide (DMSO) for 8 weeks (K2), the sixth one, bitter melon seed extract of 50 mg/100g BW/day for 8 weeks + DMPA (P2), the seventh one, control group of dimethyl sulfoxide (DMSO) for 12 weeks (K3), the eighth one, bitter melon seed extract of 50 mg/100g BW/day for 12 weeks + DMPA (P3). Methanol extract of bitter melon seed to decrease the quantity and quality of guinea pig spermatozoa decreased significantly, i.e. viability and normal morphology of spermatozoa ($p<0.05$).

Keywords: antifertility, bitter melon *(Momordica charantia*), guinea pig, sperm concentration

1. Introduction

According to the Survey of Indonesia Health Demographic at the 2002, family planning participation is still very low, only 4.4%, which include: condom use (0.9%), vasectomy / male surgery method (0.4%), intermittent intercourse (1.5%) and periodic abstinence (1.6%) [1]. The participation rate as a family planning acceptor still lower from Islamic countries, such as Bangladesh at 13.9% in 1997, and Malaysia at 16.8% in 1998.

Male was less interested in becoming "family planning" acceptor because there were not many contraceptive options available. Therefore it is necessary to develop from herbs one of the seeds of bitter melon (*Momordica charantia*) [2,3]. Combination of bitter melon seed with DMPA was better able to suppress spermatogenesis in mice [4,5,6,7] and rabbits to decrease the quantity and quality. The content of flavonoids in bitter melon seeds can lower serum testosterone levels so that it can cause decreased libido. Therefore, DMPA able to benefit as a source of testosterone in serum [8].

The workings of bitter melon seed extract and DMPA through the hormonal mechanism of hypothalamus-pituitary-testis [4]. Decreased intratesticular testosterone levels disrupt to spermatogenesis, so reduced spermatozoa or no one in the testis. Testosterone may enter the seminiferous tubule and bind by Androgen Binding Protein (ABP) so that it can be used for the growth and development of spermatogonia as its stem cell spermatozoa. Spermatogonia transformation become to spermatocytes, spermatids, and spermatozoa in the seminiferous tubules used to testosterone [9,10].

2. Methods

The study subjects used healthy male healthy guinea pigs aged 8-11 months (proven to have once-a-year offspring when mated with a female marmot) weighing 400-450g and placed in a clean cage. The study has received permission from the Research Ethics Committee of Health with no. 085 / KEPH-FMIPA / 2017.

2.1 Spermatozoa concentration

The spermatozoa suspension previously was homogenized by vibrating by hand or carefully stirred with a stirring glass. Removal of dilution liquid to 1.01 mark after sucking 0.005 mL spermatozoa suspension. Laying the spermatozoa suspension right on the edge of the cover glass to spread into the Neubauer Heumocytometer under a microscope with 400 times magnification. Then determine the concentration of spermatozoa in the field of view A, B, C, D. The total calculation of spermatozoa using the formula of determination of spermatozoa concentration in ml suspension cauda epididymis as follows: spermatozoa concentration = (N=1+2+3+4) x 50.000 sperm/mL. Calculating the total number of spermatozoa using the formula by computing boxes A, B, C, D [11].

2.2 Spermatozoa normal morphology

Calculation of spermatozoa morphology by taking ten µL of cement on top of object glass. Then fixation with ethanol 70% for 10-15 minutes and color with Giemsa for 15-20 minutes. Abnormalities or abnormal forms of spermatozoa seen primary and secondary deformities of 100-200 normal spermatozoa. The percentage of normal spermatozoa is the result of normal spermatozoa with the total number of normal and abnormal spermatozoa then multiplied 100% [11].

2.3 Spermatozoa viability

Checking of spermatozoa viability were performed with supravital painting, ten µL of semen added a 0.5% Eosin-Y solution then mixed on top of the object glass and covered with a glass cover. Spermatozoa that live colorless and colored dead. Then performed by microscope at 400x magnification and calculated against 100-200 spermatozoa. Express regarding percent of life gained from the results of live spermatozoa with the total number of live and dead spermatozoa multiplied by 100% [11].

3. Results and Discussion

Research was conducted in Medan, and various parameters was observed as follows: (1). Spermatozoa concentration, (2) Spermatozoa normal morphology, Spermatozoa normal morphology, and (3) Spermatozoa viability.

3.1. Spermatozoa concentration

The concentration of spermatozoa after giving of biter melon seed extract able to see Figure 1 below. There are different between treatment and control groups significantly ($p<0.05$) at the 12 and 16 weeks for administration of methanol extract of bitter melon seed. Testicles of guinea pig produced suppressing of spermatozoa concentrations.

The process of spermatogenesis or stages of spermatozoa cell formation ranging from spermatogonia and spermatocytes and spermatids greatly determine by concentration of spermatozoa. Provision of bitter melon seed extract allows decreasing testosterone levels in the testes (intratesticular testosterone) through repeated administration. Bitter melon seed extract contains β-sitosterol with a chemical structure similar to cholesterol. Cholesterol is the source of testosterone and enters the testicles so that the intratesticular testosterone is increased and consequently causes negative feedback to the hypothalamus and pituitary. The hypothalamus reduces the production of FSHRH (Follicle Stimulating Hormone Realizing Hormone) and LHRH (Luteinizing Hormone Realising Hormone) to affect the pituitary so that production of FSH and LH decreased. The reduction of FSH affects Sertoli cells in producing ABP (Androgen Binding Protein) or testosterone receptors. LH reduction suppresses Leydig cells to produce testosterone. These two ingredients are very important in the development and growth of spermatozoa cells. So that production interruption will reduce the production of spermatozoa cells in the testes.

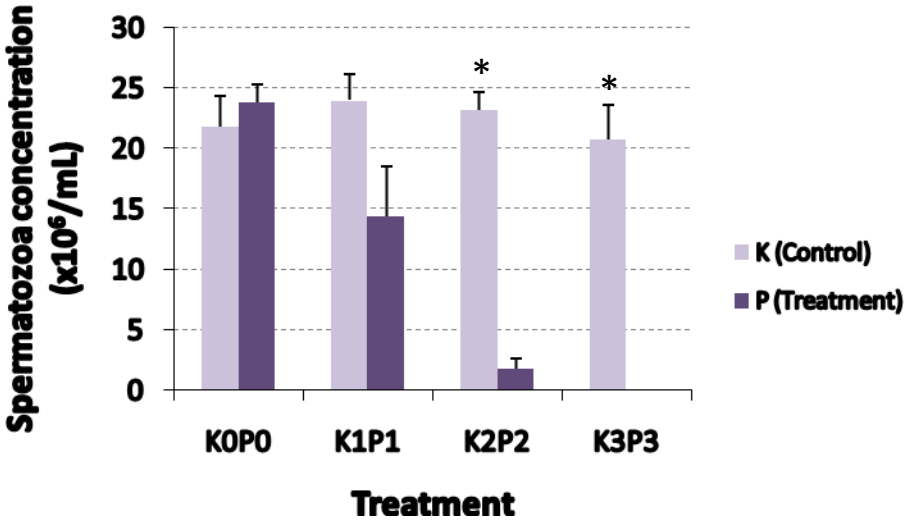

Figure 1. Bar graphic of spermatozoa concentration of guinea pig (x±SD). $p^{*}<0.05$=the same pair between control and treatment, K1P1=control and treatment on week 4th, K2P2=control and treatment on week 8th, K3P3=control and treatment on week 12th.

Beta sitosterol D-glucoside is a phytosterol contained in *Momordica charantia* Linn., and *Withania somnifera* (L.) Dunal and several other plants. Beta sitosterol D-glucoside has many pharmacological activities [12,13] such as androgenic, antiadenomic, anticancer [14,15] antiedemic, antiinflammatory [16]. Sitosterol has a structure such as cholesterol that later in the body can become the precursor of testosterone [2,17,18], so that if eating the extract of *Momordica charantia* seeds will cause increased testosterone levels to the highest culmination point and will eventually lead to decrease in testosterone serum body and testes [2,3]. DMPA replaces the decreased testosterone in the body, so there is no decrease in libido [4].

3.2. Spermatozoa normal morphology

In Figure 2 below we able to see the morphology of guinea normal spermatozoa after administration of methanol extract of bitter melon seed. Provision of bitter melon seed extract at weeks 8 (P2) and 12 (P3) showed significant results ($p < 0.05$) on morphology percentage of guinea pig spermatozoa. It is seen if we compare the research treatment with each control. This suggests that there are inhibitory effects of FSH and LH production due to negative feedback of testosterone on the hypothalamus (indirectly) or directly on the pituitary. Thus the functioning FSH in spermatogenic cell-form changes ranging from spermatogonium-spermatocytes-spermatids and spermatozoa are impaired. As a result, abnormal spermatozoa or defective spermatozoa (teratozoospermia) are present. According to Gordetsky [20], FSH levels have statistically significant associations with abnormal and morphological spermatozoa concentrations but are not related to the volume of semen (spermatozoa + liquid spermatozoa).

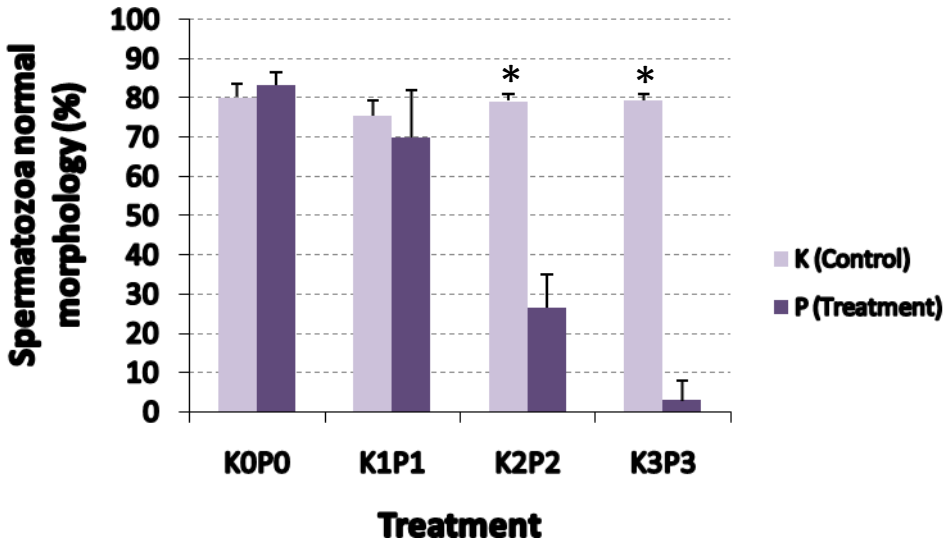

Figure 2. Bar graphic of spermatozoa normal morphology of guinea pig. $p^{*}<0.05$=the same pair between control and treatment. K0P0=control and treatment on week 0, K1P1=control and treatment on week 4^{th}, K2P2=control and treatment on week 8^{th}, K3P3=control and treatment on week 12^{th}.

3.3 Spermatozoa viability

Administration of methanol extract of bitter melon seed and DMPA in guinea pig, obtained by spermatozoa viability data as shown in the following Figure 3.

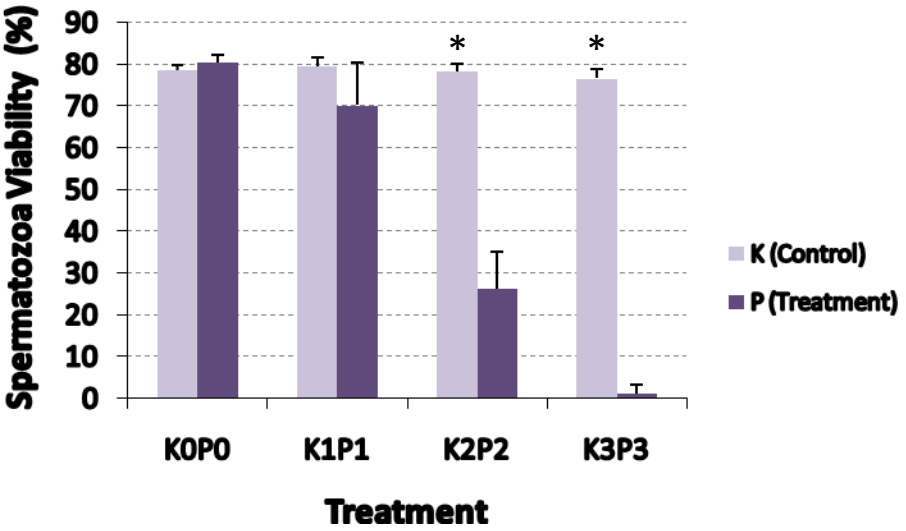

Figure 3. Bar graphic of spermatozoa viability of guinea pig. $p^{*}<0.05$=the same pair between control and treatment, K1P1=control and treatment on week 4^{th}, K2P2=control and treatment on week 8^{th}, K3P3=control and treatment on week 12^{th}.

Provision of bitter melon seed extract and DMPA at weeks zero and fourth did not affect significantly ($p>0.05$), but at weeks 8 and 12 had a significant effect ($p<0.05$). Bitter melon seed extract and DMPA caused by decreased testosterone levels in the testes due to at least LH levels. A small amount of tetosterone causes the production of LH by the pituitary to become to least level. Thus the production of testosterone from the Leydig cells decreases LH levels and causes a disruption to spermatogenesis, so that the production of spermatozoa viability also becomes less.

If the pituitary secrete LH and FSH slightly then the production of testosterone mice also slightly. So the concentration, morphology, and viability of spermatozoa become less. Testosterone is very important at the beginning and end of maturation of spermatozoa in the testes [20]. Lack of the hormone testosterone also triggers the occurrence of spermatogenic cell apoptosis [4,21,22] through the activation of caspase 3. Caspase 3 is the executor of spermatogenic cell death programmatically [23,24,25,26,27].

4. Conclusions

Methanol extract of bitter melon seed to decrease the quantity and quality of guinea pig spermatozoa significantly ($p<0.05$) i.e. viability and normal morphology of spermatozoa. Therefore it was able to be candidate for herbal contraception.

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