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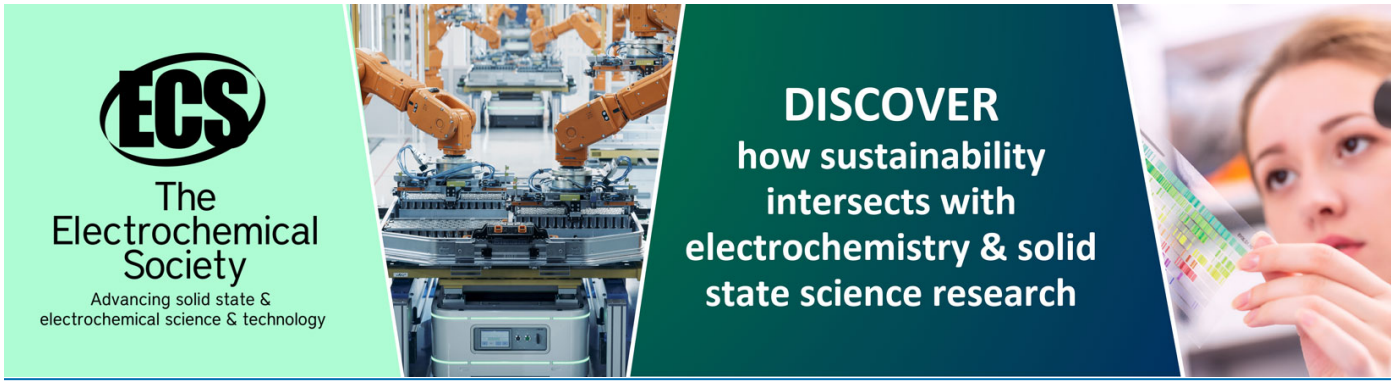

Histological changes in the testis if the guinea pig during administration of methanol extract of bitter melon (*Momordica charantia* L.) seed and DMPA

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Abstract. Various attempts were made to get male contraceptive drugs continued. One of them is a combination of herbal medicine with patent medicine. The combination of pare seeds with DMPA is still being studied to obtain the best results. Therefore, the use of guinea pigs as experimental animals was carried out by using experimental methods with complete randomized design (CRD). The study was divided into four control groups and four treatment groups. C0; Control group of dimethyl sulphoxide (DMSO) for 0 week (four hours), group T0; Bitter melon seed extract of 50 mg/100g Body Weight/day for 0 week (four hours), C4; Control group of dimethyl sulfoxide (DMSO) for four weeks, group T4; Bitter melon seed extract of 50 mg/100g BW/dayfor four weeks + Depot medroxy Progesterone Acetate (DMPA), C8; Control group of dimethyl sulfoxide (DMSO) for eight weeks, group T8; Bitter melon seed extract of 50 mg/100g BW/day for eight weeks + DMPA, C12; Control group of dimethyl sulfoxide (DMSO) for 12 weeks, T12 group; Bitter melon seed extract of 50 mg/100g BW/day for 12 weeks + DMPA. Results showed that there was a significant effect ($p<0.05$) methanol extract of bitter melon seed to decrease composition spermatogenic cell from spermatogonia to spermatid, as well as minimizing the diameter of the seminiferous tubules, and the thickness of the tubular epithelial seminiferous walls.

1. Introduction

Based on the 2010 population census of Indonesia is 237.6 million people. The target population growth rate of 1.49%, was higher than the expected target in 2010 of 1.27%. This allows the population explosion due to constant fertility rates. One of the best ways (effective and safe) in regulating the rate of population growth is the Family Planning Program (KB). The reality in the field of family planning program has not run optimally according to the government's expectation. Family planning programs are still dominated by women and are less attractive to men. This is due to lack of male contraceptives so it is difficult to find fitness with the body. Another thing contrasting tools that exist still have shortcomings such as immunoglobulin disorders in vasectomy and condoms there is still a possibility of leaking [1]. The above statement opens the opportunity to look for contraceptives that are more effective, safe, comfortable and accepted by the public.

The solution sought could be contraceptive devices or ingredients derived from herbs such as bitter melon [2,3,4] seed ethanol extract combined with DMPA (Depo medroxy progesterone acetate). In some studies reported achievement of change or suppression of spermatogenesis resulting in spermatozoa with no amount or a little (oligozoospermia) [5,6,7].

The development of spermatogenic layers is a complex and orderly process and is called a spermatogenic cycle, which is divided into a number of specific cells of the cell stages or associations. The mouse and mouse respectively consist of 12 and 14 association stages. Spermatogenic development is synchronized and undergoes cytokinesis during mitotic division that leads to a cytoplasmic bridge maintained between germ cells. RNA proteins and messenger are exchanged through cytoplasmic bridges and can assist in coordinating synchronized cell development. Each stage is characterized by a combination of spermatogonia, spermatocytes and spermatids simultaneously progressing through a spermatogenic process.

As a native cucurbitaceae, pare also contains ingredients belonging to triterpen or cucurbitacin glycosides. The active compound cucurbitacin allegedly works to inhibit the development of spermatogenic cells through cytotoxical effects and through hormonal effects [8], stated that the administration of pare extract in male rats during one cycle of spermatogenesis can decrease sperm motility and increase abnormal morphology of spermatozoa male rat wistar strain.

2. Materials and Methods

2.1 Animals

The experimental animals used in this study were 30 guinea pigs of 30 animals weighing 300-400 g. Guinea pig in good health, no defects or injuries.

2.2 Methods

Methods of research was complete randomized design (CRD). The study was divided into four control groups and four treatment groups. C0; Control group of dimethyl sulphoxide (DMSO) for 0 week (four hours), group T0; Bitter melon seed extract of 50 mg/100g Body Weight/day for 0 week (four hours), C4; Control group of dimethyl sulfoxide (DMSO) for four weeks, group T4; Bitter melon seed extract of 50 mg/100g BW/dayfor four weeks + Depot medroxy Progesterone Acetate (DMPA), C8; Control group of dimethyl sulfoxide (DMSO) for eight weeks, group T8; Bitter melon seed extract of 50 mg/100g BW/day for eight weeks + DMPA, C12; Control group of dimethyl sulfoxide (DMSO) for twelve weeks, T12 group; Bitter melon seed extract of 50 mg/100g BW/day for twelve weeks + DMPA.

2.3 Intake of genital organs

After the guinea pig are sacrificed, the genital organs are taken and weighed like testicular organs, epididymis cauda, vas deferen, seminal vesicles and prostate. The organ was taken in the treatment group (giving pare seed extract every four weeks and DMPA at week 0).

2.4 Histopathology of the testis

The testes are fixed in the Bouin solution, and then rinsed with water. Eliminated water with stratified alcohol to albosolut. After dehydration, cleared in xylene, grown in paraffin, cut to five μm size and stained with hematoxyline and eosin. Observed under a light microscope of testicular tissue with magnification of 400x.

3. Result and Discussion

Based on the results of research that has been done, obtained some test parameters as in **Table 1**, **Figure 1** and **Figure 2**. In **Table 1** we found the relative weight of genital organs of testes, epididymis, vas deferens, seminal vesicles and prostate.

Table 1. Relative weight of genital organs (g/100 g bw) after administration seed extract of bittle melon and DMPA

Genital organs (g/100 g bw)	Time (weeks)			
	0	4	8	12
Testis (T)	0.48±0.03^{ns}	0.45±0.02^{ns}	0.40±0.02*	0.30±0.03*
Testis (C)	0.47±0.03	0.48±0.05	0.46±0.04	0.45±0.05
Epididymis (T)	0.13±0.03^{ns}	0.10±0.0^{ns}	0.08±0.0^{ns}	0.04±0.01*
Epididymis (C)	0.14±0.03	0.12±0.02	0.09±0.03	0.08±0.02
Vas deferens (T)	0.04±0.01^{ns}	0.03±0.01^{ns}	0.02±0.01^{ns}	0.01±0.01^{ns}
Vas deferens (C)	0.04±0.01	0.04±0.01	0.03±0.01	0.02±0.01
Seminal vesicle and prostate (T)	0.58±0.04^{ns}	0.55±0.04^{ns}	0.39±0.01*	0.36±0.02*
Seminal vesicle and prostate (C)	0.56±0.04	0.54±0.03	0.54±0.01	0.52±0.01

*$^{T\ vs\ C}$<0.05 = treatment (T) *vs* control (C); ^{ns}p>0.05 = not significant

The weight of testicular guinea pig treated as compared to controls at week 0 and fourth observations was not significantly different (p>0.05), whereas at weeks eighth and twelveth were significantly different (p<0.05). This is due to the influence of cucurbitacin content in pare seed and the effect of DMPA injected at week to 0. Cucurbitacin can cause cytotoxic effect (poison the cells) on spermatogenesis so that the number of spermatogenic cells decreases when compared with control. The testicular organ in the treatment group containing fewer spermatogenic cells would have an effect on lower testes weight if compared with the weight of the control group's testes (p<0.05). This also occurred in the lower treatment group epididymal weight of the control group after twelve weeks (p<0.05). This is because epididymis is the site for spermatozoa maturation of spermatogenesis and temporary storage before removal from the male genital organ. As Souza et al.,[9] that epididymis is a complex organ in which spermatozoa acquire the motility and ability to fertilize an egg.

The vas deferens is a continuation of the epididymal ducts. The vas deferens channel contains fewer spermatozoa than the epididymal ducts. So the effect of cucurbitacin from seed extract of bitter melon and DMPA on spermatozoa in vas deferen in treatment group did not significantly different with control group (p>0.05). This is in contrast to the results of Mohomed et al., [10] research, that mice given nicotine (the main ingredient of cigarette smoke) intraperitoneally for 30 days, epididymal epithelium and vas deferens have a relatively lower weight and decrease compared with controls. This is due to changes in their biochemical content such as low levels of protein, nucleic acids, cholesterol and phosphatase acid activity as well as higher levels of alkaline phosphatase activity. This change can lead to an unfavorable environment in the epididymis and vas deferens for sperm maturation and motility.

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The weight of seminal vesicles and prostate at weeks 8 and 12 was significantly different ($p<0.05$) between treatment and control. This is due to the influence of testosterone derived from progesterone (DMPA) which can reduce the weight of seminal vesicles and prostate. Testosterone inhibits the incorporation of the amino acid leucine with the prostatic ribosome and testosterone inhibits RNA polymerase activity. This inhibition of the enzyme prevents the formation of RNA so that new cell-forming proteins are not formed which result in reduced seminal and prostate vesicle cells. Finally, the weight of these two organs becomes lighter than the group without giving DMPA (testosterone precursor). As Souza et al., [9] points out, that the weight of the prostate and seminal vesicles is reduced by induction by testosterone. This occurs because of the resistance of testosterone to the incorporation of leucine into the prostatic ribosome of the mouse thus inhibiting the stimulation of RNA polymerase activity.

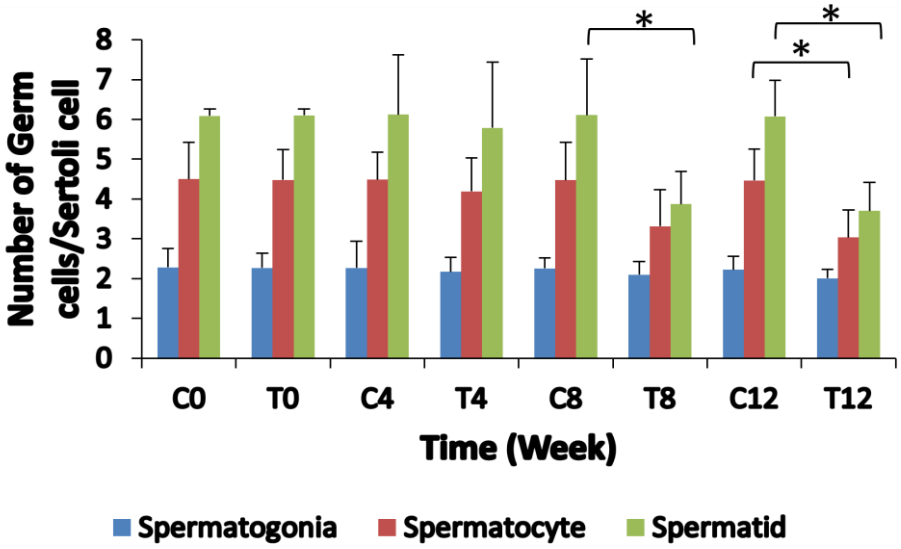

Figure 1. Number of germ cells/Sertoli cell of guinea pig at the several time (weeks) administration of bittle melon seed extract and DMPA. C=control, T=treatment. *$p<0.05$.

Figure 1 shows the amount of comparison between the number of germ cells with Sertoli cells at each observation time (0, 4, 8, and 12 weeks) and the comparison of control analysis with treatment (Cx *vs* Tx). The results of the analysis were a significant difference ($p<0.05$) between the germ cell ratio (spermatid) / Sertoli cells in C8 *vs* T8 and C12 *vs* T12 and between the germ cell ratio (spermatocyte)/Sertoli cells in C12 vs T12 (**Figure 2**). This is because cucurbitacin (such as triterpen) pare seed or DMPA as a precursor testosterone, can inhibit the formation and development of spermatogenic cells.

Cucurbitaceae of the nation, pare contains material belonging to triterpen or cucurbitacin glycosides [11,12,13,14]. According to Souzaet al., [9] and Ilyas et al., [4] the cucurbitacin active compound is thought to be working to inhibit the development of spermatogenic cells through the cytotoxic site and through hormonal effects.

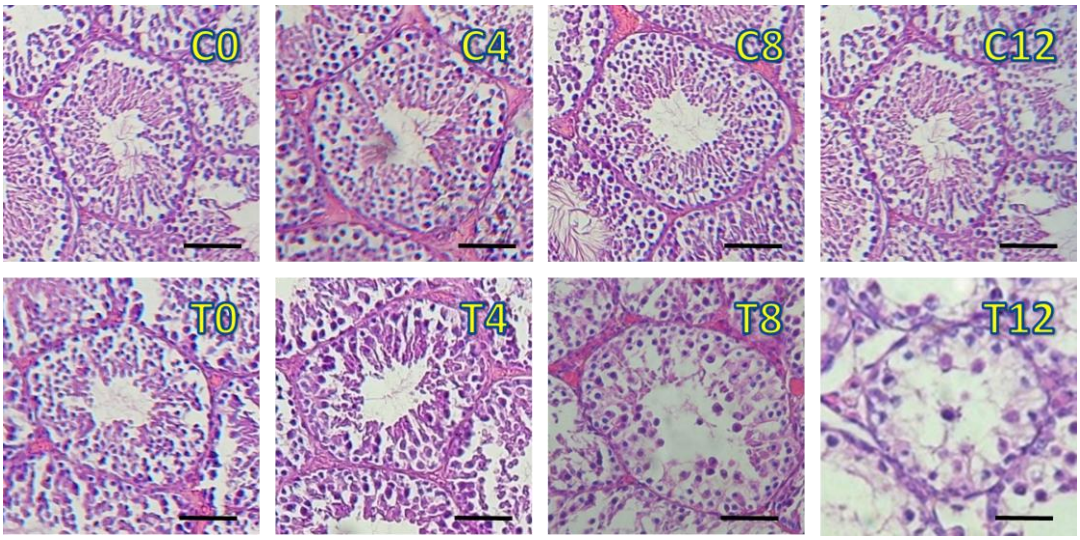

Figure 2. Germ cells of guinea pig at the several time (weeks) administration of bittle melon seed extract and DMPA. ▬ = 250 µm

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