COMBINATION OF DEPOT MEDROXY PROGESTERONE ACETATE AND JAVANESE LONG PEPPER EXTRACT ON BODY WEIGHT, HEMATOLOGY, AND BLOOD BIOCHEMISTRY AS A SAFE CONTRACEPTION MODEL

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Abstract

The development of male hormonal contraception is based on a decrease in sperm concentration without affecting libido and sexual potency. The combination of depot medroxy progesterone acetate (DMPA) + extract of Javanese long pepper (JLP) with dosages of 0.94 mg and 1.88 mg decreases the concentration of spermatozoa. However, it remains unknown whether the combination influences body weight, hematology, and blood biochemistry. Therefore, it is necessary to investigate the effect of DMPA + JLP extracts on the body weight, hematology, and blood biochemistry of male rats (*Rattusnorvegicus* L.) using Sprague-Dawley strains. The research uses a completely randomized design (CRD); one group control and two treatment groups. In the first group, the castrated rats were given oral administration extracts of JLP (CJ) with doses of 0, 0.94, 1.88, 2.82, and 3.76 mg. In the second group, the rats were injected with 1.25 mg DMPA and given an oral administration extract of JLP. Injection was given in week-0 and 12. Administration was conducted every day from week 7-18. Analysis of the normality and homogeneity of data is done before the ANOVA test. Data that is abnormal and not homogeneous are tested with non-parametric statistical Kruskal-Wallis. This study shows that the combination of minimal doses of DMPA and administration variousdoses of extracts of JLP does not affect body weight and hematology (erythrocyte, hemoglobin, hematocrite), and the blood biochemistry of rats, such as the values of SGPT, SGOT, HDL, and triglycerides ($p < 0.05$), but rather the total cholesterol and LDL ($p < 0.05$). Furthermore, it is concluded that the combination of the minimal dosage of DMPA and weaned various dosages of JLP extracts affect the total value and LDL cholesterol but do not influence body weight, nor hematology and blood biochemistry. Such combinations can be drawn on for asafe male contraceptive model t by taking into account the value of the total cholesterol and LDL during its use.

Keywords: blood biochemistry, body weight, DMPA, hematology, JLP

1. Introduction

The number of Indonesian citizens is predicted to reach around 285 million between 2020 and 2025 [1]. The Indonesian government will reinforce the family planning program (FPP) for productive couples to manage population growth. Active participation from productive couples is necessary to ensure the success of the program [2]. The FPP is our responsibility since most methods require the husbands' participation [3]. In Indonesia, the husbands' involvement in the program has always been nominal which means husbands, who were not really considered at first, are now the new focus for the family planning program [2].

From so many male contraception models developed, the hormonal approach is one way that can be drawn on for clinical application. Male hormonal contraception works by suppressing spermatogenesis, thus inhibiting the sperm's mobility or immobilizing it. The goal of male hormonal contraception is to change the endocrine environment in the body so that it will inhibit the spermatogenesis process. Spermatogenesis requires stimulation from two gonadotropin hormones in the pituitary which are the luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH functions by stimulating Leydig cells and constantly producing high testosterone in the testes which is needed for spermatogenesis. FSH is also known to maintain spermatogenesis qualitatively [4].

An androgen intake, one of which is testosterone, can suppress gonadotropin and the amount of sperm in normal men. However, additional progestin can increase the effectiveness of hormonal contraception. Additional depot medroxy progesterone acetate (DMPA) in the use of testosterone enanthate (TE) in a single dosage can induce azoospermia. Besides that, additional progestin can inhibit spermatogenesis and minimize the use of testosterone [5].

Based on Moeloek *et al*., [6] research, injecting Indonesian men with 500 mg testosterone undecanoat (TU) within a 6 week interval, and 250 milligram (mg) DMPA within 12 weeks tresulted in a drastic decrease in sperm concentration of about 99.95% with 80% severe oligozoospermia and about 20% with less than 0.1 million/mL sperm concentration.

Based on the conducted researches, the DPMA single dosage is effective in inhibiting spermatogenesis, but the problem was that DMPA also inhibits the testosterone secretion in the testes. As a result, the compositon in the plasma would decrease, and libido and sexual potential would be disturbed. This observation made experts interested in combining DMPA with androgen, such as TE. DMPA inhibits spermatogenesis by inhibiting gonadotropin pituitary secretion, while TE replaces the missing endogen testosterone—as a result of DMPA which would reinforce the inhibition of gonadotropin secretion [7-8]. In Yurnadi *et al*., [9] research, the minimal dosage of DMPA injections in male rats of the Sprague-Drawley strain can decrease fertility and positively impact the sperms' concentration, viability, and testosterone hormonal level. Moreover, the minimum dosage of DMPA injections given to the male rats did not influence body weight and blood chemicals, such as eritrocytes, hemoglobin, hematocrite, HDL, LDL, total cholesterol, SGOT, SGPT, and triglicerydes [10].

Besides modern medicines that have been used as androgen substitutes, natural kinds of androgen in herbs could also be used. One herbs which has an androgen composition is Javanese long pepper (JLP) (*Piper retrofractum* Vahl). Empirically, JLP has been used to treat impotency, weak stomach, and sweat secretion [11]. A number of herbal manufacturers have been using JLP as its herbal ingredient. The amount of JLP used as an ingredient is around 10-15% [12].

Based on the research conducted by Sa'roni *et al*., [12] infusion of JLP with 2.1 mg per 10 grams (g) of the rats' body weight showed there were androgenic and anabolic effects. The research conducted by Isnawati *et al*., [13] also showed that JLP extracts which were tested with the Ames method did not show a mutagenic effect so they were safe to be consumed. Next, Wahjoedi *et al*., [11] studied the 70% ethanol extracts of JLP regarding its androgenic effect on male rats with a dosage of 3.75 mg/100 g to body weight to see whether it has more or less the same response with the metiltestosterone (Andriol) standard material with a 500 mg/100 g dosage to body weight.

Moeloek *et al.*, [14] conducted clinical trials on JLP extracts on hypogonadalmen and showed that a dosage of 100 mg/day of JLP extract scan increase the blood testosterone levels in 7 out of 9 male volunteers and increase the frequency of coitus. The results of counting the number of sperm demonstrated at day-30 an increase in the concentration of sperm in the male volunteers given JLP extracts. The concentration of sperm remained high after treatments were stopped (day 60). The monitoring of prostate specific antigen (PSA) and blood bio chemicals of revealed that the safety level of JLP extract usage is at a dosage of 100 mg/day for nine hypogonadalmen.

Giving a single dosage of DMPA could suppress spermatogenesis and did not influence body weight, hematology, and blood biochemistry. However, it could also inhibit testosterone secretion, and thus result in a decrease of libido and create potential sexual disorders. On the other hand, JLP was proven effective in increasing the level of testosterone hormone in the blood. Therefore, further research is required to discover whether the combination of the minimum dosage of DMPA (1.25 mg) and optimal dosage of JLP could affect body weight, hematology, and blood biochemistry by using rats as the animal model.

2. Methods

Material and research tools. Fertile two-month old healthy male rats of the *Sprague-Dawley* strain weighing 200-250 g, food and drinks for rat, cages, sawdust bedding, drinking bottles, physiolocical NaCl, DMPA (depogeston 50 mg), testosterone hormonal kits, haematology and biochemistry kit, George'ssolution, aquabides, shaved ice, measuring glasses, therumo syringe 1 mL and 5 mL, improve Neubauer, surgical instrument 1 set, 1.5 mL Eppendorf tube, 15 mL falcon tube, Finn pipette, yellow and blue tip, transfer pipettes, rat body weighing, refrigerator, rat holder, microscope, labels, calculators, stationery, computers, and so forth.

Experimental design. The research used a completely randomized design (CRD) with equal size samples consisting of two major treated groups and one control group without treatment. The first treatment group is castrated rats and JLP extracts weaned at a dosage of 0 mg (placebo), 0.94 mg, 1.88 mg, 2.82 mg and 3.76 mg. In the second treatment group, the rats were injected with a dosage of 1.25 mg DMPA and combined with various doses of JLP extracts i.e. 0 mg (placebo), 0.94 mg, 1.88 mg, 2.82 mg and 3.76 mg. The number of

Table 1. Experimental Design

Repeated	Control Rat	Castrated Rat					Rat injected with DMPA (1.25 mg)				
		CJ 0	CJ 1	CJ 2	CJ 3	CJ 4	CJ 0	CJ 1	CJ 2	CJ 3	CJ 4
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

(Notation: CJ 0 = JLP 0 mg; CJ 1 = JLP 0.94 mg; CJ 2 = JLP 1.88 mg; CJ 3 = JLP 2.82 mg; CJ 4 = JLP 3.76 mg)

replications is 55 rats with 5 rats per group [15]. The design of the study can be seen in Table 1.

Treatment of experimental animals. The rats were injected with 1.25 mg DMPA at a pre determined dosage. Injection was into the rat's right or left thigh. Injections were done twice. The first injection was don eat week-0 and the second injection was performed at week 12. Treatments of JLP extracts started at the7th week in accordance with dosages as listed in Table 1. Weaned JLP extracts were given until the 18th week and the amount of JLP extracts weaned were adjusted with the weight of the rats. Then the rats were cared for until the 18th week to optimize the androgenic effects of JLP on those that had been castrated and injected with DMPA.

Data collecting. Six weeks post injection of the second DMPA, the rats were anesthetized with ether and prepared for data retrieval. Blood sampling used a 5 mL terumo syringe in the jugular vein. Parameters observed in this study include body weight, hematology (erythrocyte, hemoglobin, hematocrite) and blood biochemistry (total cholesterol, HDL, LDL, SGOT, SGPT, and triglycerides).

Statistical analysis. Samples of each parameter were evaluated by using the following statistic analysis: 1) Kolmogorov Smirnov test for normality and homogeneity of variance test, 2) Data were analyzed using analysis of variance (ANOVA) test for normally distributed and homogeneneity variance. If the obtained value of $p < 0.05$ Bonferroni test is carried out. Data that is not normally distributed and/or not homogenous in variety (after data transformation) are performed under a non-parametric Kruskal-Wallis test. If the obtained value is $p < 0.05$, post hoc analysis will be conducted with the Mann-Whitney test [16].

3. Results and Discussion

Rats' body weight during treatments (In the first, second**,** and third treatment**).** The result of the average body weight of rats in the first months of the combination of DMPA and JLP extracts can be seen in

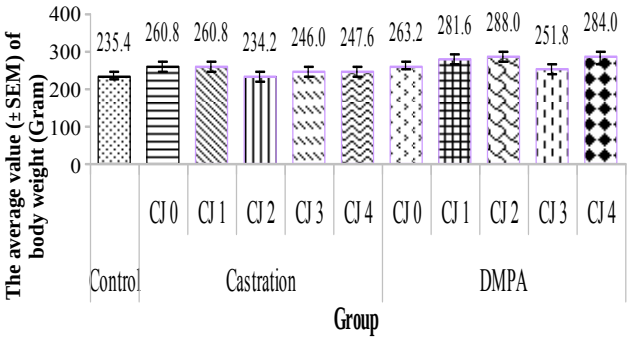

Figure 1. The Average Body Weight of Rats in the First Month Within the Controlled Rat Group, Castration Group, and DMPA at 18 Week Post-injection of DMPA and Weaned Various Dosages of JLP Extracts. Description: CJ 0 = 0 mg JLP Extracts; CJ 1 = 0.94 mg JLP Extracts; CJ 2 = 1.88 mg JLP Extracts; CJ 3 = 2.82 mg JLP Extracts; CJ 4 = 3.76 mg JLP Extracts, Kontrol (▨), Kastrasi CJ0 (▤), Kastrasi CJ1 (▧), Kastrasi CJ2 (▥), Kastrasi CJ3 (▦), Kastrasi CJ4 (▩), DMPA CJ0 (▩), DMPA CJ1 (▦), DMPA CJ2 (▧), DMPA CJ3 (▥), DMPA CJ4 (▨)

Figure 1. Based on the test for normality and homogeneity of data, it is known that the body weight of rats in the first months of treatment is distributed normally ($p = 0.070$) and is of homogeneous variance ($p = 0.271$). Result of ANOVA tests obtained $p = 0.018$. Furthermore, the Bonferroni test of the average data known showed that there is no significant difference in the average body weight of rats between the controlled group and the treatment group ($p > 0.05$).

Figure 2 shows that the calculation of the average body weight of rats in the second month is a combination of DMPA and JLP extracts. Normality and homogeneity test results indicate that the body weight of rats in the second month of treatments were distributed normally ($p = 0.200$) and were homogeneous in variety ($p = 0.362$). Results of ANOVA test obtained $p = 0.047$. Furthermore, the Bonferroni test of the average data shows that there is no significant difference in the average body weight of rats among the controlled group and the treatment group ($p > 0.05$).

The result of the average body weight of rats in the third months of combination DMPA and JLP extract scan be seen in Figure 3. Based on the test for normality and homogeneity of data, it is known that the body weight of rats in the first months of treatment is distributed normally ($p = 0.200$) and homogeneously ($p = 0.640$). Results of ANOVA tests obtained $p = 0.072$. Furthermore, the Bonferronitest of the average data shows that there is no significant difference in the average body weight of rats between the controlled group and the treatment group ($p > 0.05$).

Hematologyvalues (**erythrocytes, hemoglobin, hematocrite**). Figure 4 shows results from the calculation of the average value of rat erythrocytes. Normality and homogeneity test results show that the erythrocytes value was distributed normally ($p = 0.200$) and it was homogeneous in variety ($p = 0.051$). Results of ANOVA test obtained $p = 0.000$. Furthermore, the Bonferroni test of the average data shows that there is no significant difference in the average erythrocyte of rats between the controlled group and the treatment group($p > 0.05$).

The result of the average hemoglobin of rats can be seen in Figure 5. Based on the test for the normality and the homogeneity of data, it is known that the value of hemoglobin is distributed normally ($p = 0.200$) and is homogeneous in variety ($p = 0.515$). Results from the ANOVA tests obtained $p = 0.002$. Furthermore, the Bonferroni test of the average data shows that there is no difference in the average value of hemoglobin of rats significantly between the controls with the treatment group ($p > 0.05$). From the Bonferroni test it is known that castrated rats in the CJ 0 group have hemoglobin levels that were significantly lower than the DMPA group CJ 0 ($p = 0.026$), DMPA CJ 1 ($p = 0.033$), and DMPA CJ 2 ($p = 0.002$).

Figure 6 shows the results of the calculation of the average value of the hematocrite of rats. The results from the normality test showed that the hematocrite value is not distributed normally ($p = 0.000$). Once the data is transformed with $1\checkmark x$, the obtained data are distributed normally ($p = 0.256$). From the results of the homogeneity test, the data is homogeneous in variety

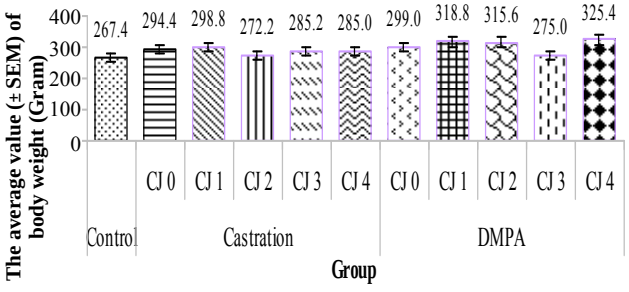

Figure 2. The Average Body Weight of Rats in the Second Month of Control Group Rat, Castration, and DMPA at 18 Week Post-injection of DMPA and Weaned Various Dosages of JLP Extracts. Description: CJ 0 = 0 mg JLP Extracts; CJ 1 = 0.94 mg JLP Extracts; CJ 2 = 1.88 mg JLP Extracts; CJ 3 = 2.82 mg JLP Extracts; CJ 4 = 3.76 mg JLP Extracts. Kontrol (▨), Kastrasi CJ0 (▤), Kastrasi CJ1 (▧), Kastrasi CJ2 (▥), Kastrasi CJ3 (▧), Kastrasi CJ4 (▨), DMPA CJ0 (▨), DMPA CJ1 (▦), DMPA CJ2 (▨), DMPA CJ3 (▨), DMPA CJ4 (▨)

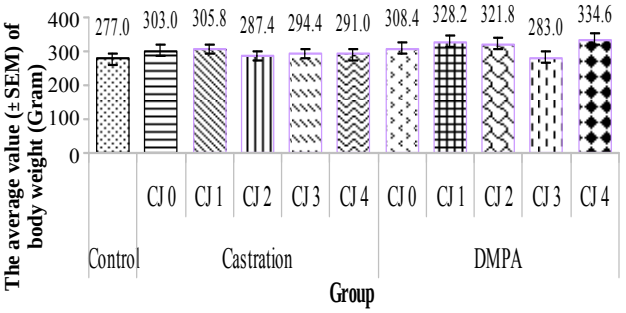

Figure 3. The Average Body Weight of Rats in the Third Month of Control Group Rat, Castration, and DMPA at 18 Week Post-injection of DMPA and Weaned Various Dosages of JLP Extracts. Description: CJ 0 = 0 mg JLP Extracts; CJ 1 = 0.94 mg JLP Pepper Extracts; CJ 2 = 1.88 mg JLP Pepper Extracts; CJ 3 = 2.82 mg JLP Extracts; CJ 4 = 3.76 mg JLP Extracts. Kontrol (▨), Kastrasi CJ0 (▤), Kastrasi CJ1 (▧), Kastrasi CJ2 (▥), Kastrasi CJ3 (▧), Kastrasi CJ4 (▨), DMPA CJ0 (▨), DMPA CJ1 (▦), DMPA CJ2 (▨), DMPA CJ3 (▨), DMPA CJ4 (▨)

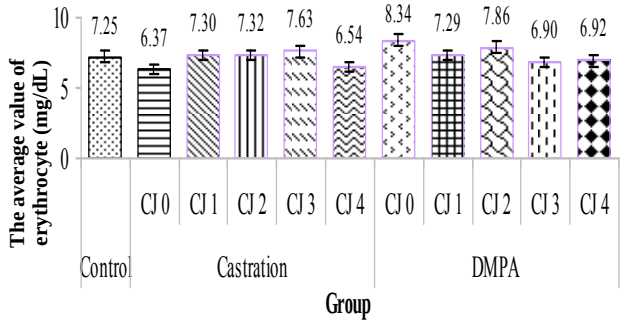

Figure 4. The Average Erythrocyte Value of Control Group Rat, Castration, and DMPA at 18 Week Post-injection of DMPA and Weaned Various Dosages of Javanese Long Pepper Extracts. Description: CJ 0 = 0 mg JLP Extracts; CJ 1 = 0.94 mg JLP Pepper Extracts; CJ 2 = 1.88 mg JLP Pepper Extracts; CJ 3 = 2.82 mg JLP Pepper Extracts; CJ 4 = 3.76 mg JLP Extracts. Kontrol (▨), Kastrasi CJ0 (▤), Kastrasi CJ1 (▧), Kastrasi CJ2 (▥), Kastrasi CJ3 (▧), Kastrasi CJ4 (▨), DMPA CJ0 (▨), DMPA CJ1 (▦), DMPA CJ2 (▨), DMPA CJ3 (▨), DMPA CJ4 (▨)

($p = 0.304$). Results of ANOVA test obtained $p = 0.000$. Furthermore, the Bonferroni test of the average data is significantly different in the average number of the hematocritic rats between the controlled group and the treatment group CJ 0 castrated group has a hematocrite value that is significantly lower than the CJ 1 castrated group 1 ($p = 0.029$), DMPA CJ 0 ($p = 0.001$), DMPA CJ 1 ($p = 0.020$), and DMPA CJ 2 ($p = 0.000$). CJ 2 hematocrite values in the castrated group were significantly lower than the DMPA group CJ 0 ($p = 0.017$) and DMPA CJ 2 ($p = 0.002$). CJ 2 DMPA group had hematocrite values that were significantly higher than the CJ 3 DMPA group ($p = 0.032$).

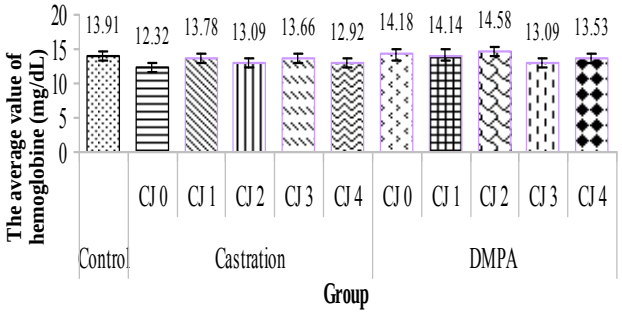

Figure 5. The Average Hemoglobin Value of Control Group Rat, Castration, and DMPA at 18 Week Post-injection of DMPA and Weaned Various Dosages of JLP Pepper Extracts. Description: CJ 0 = 0 mg JLP Pepper Extracts; CJ 1 = 0.94 mg JLP Pepper Extracts; CJ 2 = 1.88 mg JLP Pepper Extracts; CJ 3 = 2.82 mg JLP Pepper Extracts; CJ 4 = 3.76 mg JLP Pepper Extracts. Kontrol (▨), Kastrasi CJ0 (▤), Kastrasi CJ1 (▧), Kastrasi CJ2 (▥), Kastrasi CJ3 (▦), Kastrasi CJ4 (▩), DMPA CJ0 (▦), DMPA CJ1 (▦), DMPA CJ2 (▧), DMPA CJ3 (▥), DMPA CJ4 (▶)

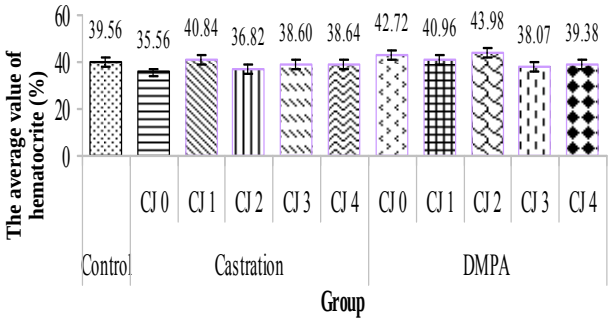

Figure 6. The Average Hematocrite Value of Control Group Rat, Castration, and DMPA at 18 Week Post-injection of DMPA and Weaned Various Dosages of JLP Extracts. Description: CJ 0 = 0 mg JLP Extracts; CJ 1 = 0.94 mg JLP Extracts; CJ 2 = 1.88 mg JLP Extracts; CJ 3 = 2.82 mg JLP Extracts; CJ 4 = 3.76 mg JLP Extracts. Kontrol (▨), Kastrasi CJ0 (▤), Kastrasi CJ1 (▧), Kastrasi CJ2 (▥), Kastrasi CJ3 (▦), Kastrasi CJ4 (▩), DMPA CJ0 (▦), DMPA CJ1 (▦), DMPA CJ2 (▧), DMPA CJ3 (▥), DMPA CJ4 (▶)

Analysis of blood biochemicals (SGPT, SGOT, total cholesterol, HDL, LDL, and triglycerides). The results of the calculation of the average value of SGPT is shown in Figure 7 below. Based on the test for normality and homogeneity, it is known that the value of SGPT is normally distributed ($p = 0.070$) and is homogeneous in variety ($p = 0.409$). Results from the ANOVA test obtained a significant value of 0.063. This means that there is no significant difference in SGPT values between all groups.

The results of the calculation of the average value of SGOT are shown in Figure 8. The results of the normality tests show that the value of SGOT data is not

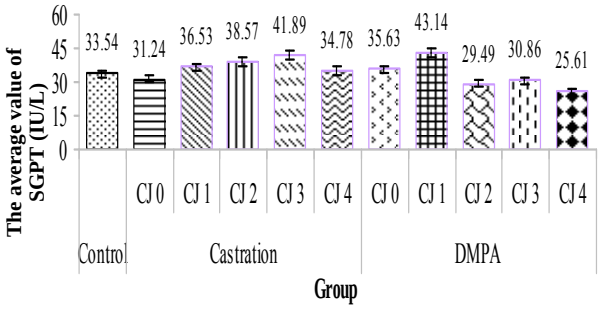

Figure 7. The Average SGPT Value of Control Group Rat, Castration, and DMPA at 18 Week Post-injection of DMPA and Weaned Various Dosages of JLP Extracts. Description: CJ 0 = 0 mg JLP Extracts; CJ 1 = 0.94 mg JLP Extracts; CJ 2 = 1.88 mg JLP Extracts; CJ 3 = 2.82 mg JLP Extracts; CJ 4 = 3.76 mg JLP Extracts. Kontrol (▨), Kastrasi CJ0 (▤), Kastrasi CJ1 (▧), Kastrasi CJ2 (▥), Kastrasi CJ3 (▦), Kastrasi CJ4 (▩), DMPA CJ0 (▦), DMPA CJ1 (▦), DMPA CJ2 (▧), DMPA CJ3 (▥), DMPA CJ4 (▶)

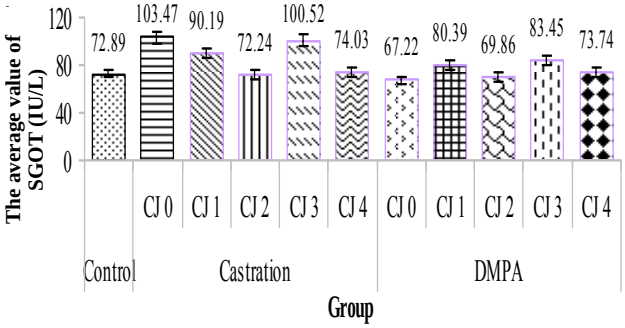

Figure 8. The Average SGOT Value of Control Group Rat, Castration, and DMPA at 18 Week Post-injection of DMPA and Weaned Various Dosages of JLP Extracts. Description: CJ 0 = 0 mg JLP Extracts; CJ 1 = 0.94 mg JLP Extracts; CJ 2 = 1.88 mg JLP Extracts; CJ 3 = 2.82 mg JLP Extracts; CJ 4 = 3.76 mg JLP Extracts. Kontrol (▨), Kastrasi CJ0 (▤), Kastrasi CJ1 (▧), Kastrasi CJ2 (▥), Kastrasi CJ3 (▦), Kastrasi CJ4 (▩), DMPA CJ0 (▦), DMPA CJ1 (▦), DMPA CJ2 (▧), DMPA CJ3 (▥), DMPA CJ4 (▶)

Distributed normally ($p = 0.002$). The results of the transformation of data with $1\checkmark x$ produces data that SGOT value sare distributed normally ($p = 0.200$). Based on the homogeneity test, it is known that the data of SGOT value is homogeneous in variety ($p = 0.373$). Results of ANOVA test obtained $p = 0.000$. Furthermore, the Bonferroni test shows that there is no significant difference in the average value of SGOT in rats between the controlled group and the treated group ($p > 0.05$). The Bonferroni test showed CJ 0 castrated group has a value of SGOT that is significantly higher than the CJ 2 castrated group ($p = 0.025$), DMPA CJ 0 (0.006), and DMPA CJ 2 (0.022). CJ 3 SGOT value castrated group was significantly higher than the DMPA group CJ 0 ($p = 0.020$).

The results of the calculation of the average value of the total cholesterol of rats (Figure not shown) and the results of the normality tests show that the value of total cholesterol data is not distributed normally ($p = 0.005$). After transforming the data with $1\checkmark x$, the total cholesterol value data obtained are distributed normally ($p = 0.200$). The homogeneity test of the data value shows that the total cholesterol is homogeneous in variety ($p = 0.365$). Results of ANOVA test obtained $p = 0.000$.

Furthermore, the Bonferroni test of the average showed that castrated group CJ 4 has a total cholesterol value that is significantly higher than the controlled group ($p = 0.015$), castrated CJ 0 ($p = 0.029$), DMPA CJ 1 ($p = 0.001$), and DMPA CJ 2 ($p = 0.001$).

The calculation of the average value of HDL rats shows in significant differences in the lowest and highest values (Figure not shown). The normality test data obtained from HDL values are not normally distributed ($p = 0.002$). Once the data is transformed with $1\checkmark x$, the data obtained showed that HDL values are distributed normally ($p = 0.179$). Homogeneity test results indicate that the data value of HDL are homogeneous in variety ($p = 0.881$). Results of ANOVA test obtained $p = 0.936$. This means that there is no significant difference in HDL values between all groups.

The results of the calculation of the average value of LDL (Figure not shown), based on the normality test on data values, shows that LDL is not normally distributed ($p = 0.004$). The results of the transformation of data with $1\surd x$ show that the data obtained for the LDL value is distributed normally ($p = 0.200$). Homogeneity of data obtained from the testis not homogeneous in variety ($p = 0.027$). For that reason, the Kruskal Wallis test was conducted and obtained $p = 0.022$. Results of the Mann Whitney test shows that LDL values of the castrated CJ 4 group rats were significantly higher than the control group ($p = 0.028$), whereas rats in the CJ 0 DMPA group had LDL values that were significantly higher than the control group ($p = 0.009$).

The calculation of the average value of rat triglycerides (Figure not shown) and the results of normality and homogeneity test of the data show that the triglycerides value sare normally distributed ($p = 0.200$), but has a variety of data that is not homogeneous ($p = 0.036$). Once the data is transformed, triglyceride evalues by $1\surd x$, the data obtained homogeneous variety ($p = 0.065$). Results of ANOVA test obtained is $p = 0.000$. Furthermore, Bonferroni tests of the average show that there are no significant differences in triglyceride evalues between the controlled group and the treated group. CJ 2 castrated group had triglyceride values that were significantly higher than the DMPA group CJ 0 ($p = 0.004$) and DMPA CJ 2 ($p = 0.029$). Furthermore, CJ 3 castrated group has a value of triglycerides that were significantly higher than the DMPA group CJ 0 ($p = 0.003$), DMPACJ1 ($p = 0.037$), and DMPA CJ 2 ($p = 0.020$). CJ 4 triglyceride values of DMPA group were significantly higher than the DMPA group CJ 0 ($p = 0.000$), DMPA CJ 1 ($p = 0.007$), and DMPA CJ 2 ($p = 0.004$).

The results of weighing the rats after three months (month-1, to-2, and 3) treatment with combinations of minimal dosages of DMPA and various dosages of JLP extracts showed that there were no significant differences in the body weight of rats between the controlled group and the treated group. This shows that the administration of the combination of the minimal dosage of DMPA and JLP extracts does not affect the body weight of rats. No changes in the body weight of rats were thought to be caused by the administration of DMPA dosages, and JLP extracts do not interfere with the consumption activity of rats during treatment. The dosage of DMPA and JLP extracts also cannot affect the activity and function of enzymes necessary for the protein synthesis processes in the cellular level. As a result, the absence of an increase inprotein synthesis would not have implications for increased body weight. According to Schotelius and Schottelius cit. Yurnadi [10], growth and increase in body weight are influenced by external and internal factors, such as food and hormones. Later in the study by Yurnadi *et al*. [10], in which rats are treated with DMPA injection for 4 months of treatment it showed that DMPA injections to rats did not affect body weight.

Handelsman [17] argued that the side effects arising from the male hormonal contraceptive are discomfort at the injection site and androgenic effects, such as acne, weight gain, hemoglobin and lipids [16]. Therefore, contraceptives that will be given to healthy men should be clearly identified with its effects on a short-term basis and a long-term basis. The most common medical reasons that cause discontinuities of the provision of contraceptive methods is acne (3%), changes in mood, be havior and decreased libido (4%), and incidental and idiosyncratic conditions(<1%) [4].

Based on the research conducted by Mishell [18] on five cross-sectional studies comparing the provision of DMPA and controlled groups, weight gain occurred in volunteers who were given DMPA. Numerous longitudinal studies have also indicated that the use of DMPA showed an average weight gain between 1.5 to 4 kg in the first year and continues to grow in the next year. However, this study did not use a control group for comparison, soweight gain could have been caused by factors other than the use of DMPA. Analysis of the values of rat erythrocyte, hemoglobin and hematocrite showed no significant difference between the control groups with the treatment group. This shows that the injection of DMPA weaned by various dosages of extracts of JLP does not affect rats hematologically.

Based on a literature review, it was concluded that in general, the chemical or the chemical compound that acts as an aphrodisiac on JLP is an example of steroids, saponins, alkaloids, tannins and other compounds that can improve blood circulation [19].

Based on analysis of blood chemistry of SGPT, SGOT, triglycerides, and HDL values in the 18th week of treatment, there was no significant difference between the controlled groups and the treated group. The value of total cholesterol in the castrated rat group CJ 4 (81.09 ±2.56 mg/dL) was significantly higher than the controls (57.88 ± 0.94 mg/dL). The high value of the total cholesterol was presumably because cholesterol is not used in the synthesis of testosterone. It is known that about 95% of testosterone is synthesized in Leydig cells [20], whereas castrated rats had no such cells. As a result, cholesterol in the body cannot be converted in to testosterone. The high value of total rat cholesterol may also originate from β-sitosterol compounds contained inextracts of JLP [19].

Several researches used a combination of male hormonal contraceptive regimens with progestin that produced 12-28% reduction on levels of high-density lipoprotein cholesterol (HDL). HDL is known to play a role in the fight against atherosclerosis through antioxidant and anti-inflammatory mechanisms as well as eliminating cholesterol from atherosclerotic lesions. However, according to Matthiesson and McLachlan [21], hormonal contraceptive clinical trials so far have a short-term duration, while the pathogenesis of coronary heart disease is long-term. Cit Meriggiola Matthiesson and McLachlan *et al*., [21] also cited a study conducted that showed reduced levels of hemoglobin, hematocrite and red blood cells by using cyproterone acetate (CPA). This may be due to the anti-androgenic effects of CPA on the bone marrow.

Phytosterols are compounds in plants similar to cholesterol in mammals. Both have the same structure, but differ in the side chain. Phytosterols are transported into the plasma and binds lipoproteins, such as cholesterol, and then under go fatty acid esterification by lecithin cholesterol acyltransferase (LCAT). Phytosterol in the liver secretes are efficient, but in other tissues the phytosterols accumulate, especially in the adrenal glands, ovaries and testes. Furthermore, these networks will convert phytosterols into steroid hormones. Cholesterol biosynthesis from acetyl-CoA is a gradual path way that involves 19 enzymatic reactions. Conversion of lanosterol (the first sterol formed) into cholesterol requires several stages. The saturation of double bonds in the C-24 is catalyzed by sterol Δ^{24}-reductase enzyme [22].

The results of the analysis on LDL value showed that the LDL level of the castrated groups CJ 4 (30.42 ± 2.15 mg/dL) and CJ 0 DMPA (27.52 ± 1.40 mg/dL) was significantly higher than in the controlled group (16.37± 0.26 mg/dL). Smith and Mangkoewidjojo [23] reported that the normal value of the LDL limit for rats is 47-82 mg/dL. The value of LDL data of rats in this studyis generally lower than the limit value of LDL reported by Smith and Mangkoewidjojo [23]. Furthermore, if the data value of LDL data is compared with levels of total rat testosterone, it can be presumed that low rat testosterone level sare caused by low LDL values. According to Strauss and Penning [24] and Miller [25], testosterone is synthesized from cholesterol which is mostly derived from plasma proteins of LDL, but can also be synthesized de novo from acetate in Leydig cells.

Gu *et al*. [24] reported that the total cholesterol, LDL, and trigliceride in every treated group returned to their normal level during the recovery time. Matthiesson and McLachlan [20] added that so far the hormonal contraception clinical study has always been conducted over a short period of time, and thus it was safe to be used.

Based on the research of acute toxicity in rats, the injection of JLP was included into the relatively harmless category [25]. Furthermore, Isnawati *et al*. [13] also reported that the simplicia of JLP resulted in negative mutagenic, so it did not cause any genetic mutation.

Traditional medicine is still widely used by the public, especially in the middle and lower classes and also due to the emergence of the ‘back to nature’ trend. The advantage of traditional medicine in comparison with modern medicines, a side from producing relatively low side effects, is that a mixture of different components create a synergistic effect as one plant has more than one pharmacological effect [26].

4. Conclusion

The combination of the minimal dosage of DMPA and weaned various dosages JLP extracts affect the value of

total and LDL cholesterol but do not influence body weight, or hematology (erythrocyte, hemoglobin, hematocrite), and neither blood biochemistry (SGPT, SGOT, HDL, and triglycerides). The combination of minimal dosage of DMPA and JLP extract scan be used as a safe male contraceptive model by taking into account the value of total cholesterol and LDL during its use.

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