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Herbal Medicine Used to Increase the Libido of Male Mice (*Mus musculus*): *Tribulus terrestris* and *Panax ginseng*

(Penggunaan Obat Herbal untuk Meningkatkan Libido Mencit Jantan (*Mus musculus*): *Tribulus terrestris* dan *Panax ginseng*)

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Abstract: *Tribulus terrestris* and *Panax ginseng* are two therapeutic plants that might increase libido. The aim of the study was to see the effects of herbal medicine containing *Tribulus terrestris* and *Panax ginseng* on increasing male mice libido**.** This study used mice divided into two sets of 7 and 14 days. Each group was divided into four parts: control (solvent), comparator (Vitan), and test 1 (herbal medicine). Parameters measured were mice sexual behaviour (introduction, climbing, coitus), sperm concentration, sperm motility, and testicular weight. Except for coitus behaviour, there were no significant changes between groups in the 7 days of treatment. There were significant differences in introduction and climbing behaviour between the control group and test 2 after 14 days of treatment, but not in other parameters. There were no significant variations in any parameters of the mice's libido between 7 and 14 days of treatment with 1x dose of herbal medicine; however, with 2x doses, only introduction behaviour showed a significant difference. Based on the research results, it can be concluded that administering two doses of herbal medicine can increase the frequency of treatment for 14 days.

Keywords : Libido, mice, *Panax ginseng*, *Tribulus terrestris.*

Abstrak: *Tribulus terrestris* dan *Panax ginseng* merupakan tanaman obat yang dapat meningkatkan libido. Penelitian ini bertujuan untuk melihat pengaruh obat herbal yang mengandung *Tribulus terrestris* dan *Panax ginseng* terhadap peningkatan libido mencit jantan. Penelitian ini menggunakan mencit yang terbagi menjadi 2 grup yaitu kelompok 7 dan 14 hari. Masing-masing grup dibagi menjadi 4 kelompok yaitu kelompok kontrol (pelarut), pembanding (Vitan), uji 1 (obat herbal 10 mg) dan uji 2 (obat herbal 20 mg). Parameter yang diamati adalah perilaku seksual mencit (*introduction, climbing, coitus*), konsentrasi sperma, motilitas sperma, bobot testis. Parameter bobot testis, konsentrasi sperma, motilitas sperma, perilaku *introduction* dan *climbing* menunjukkan tidak ada perbedaan bermakna antar kelompok pada perlakuan selama 7 hari kecuali perilaku coitus. Pada perlakuan selama 14 hari terdapat perbedaan bermakna perilaku *introduction* dan *climbing* antara kelompok kontrol dan uji 2, sedangkan parameter yang lain tidak ada perbedaan bermakna. Perbandingan perlakuan antara 7 hari dan 14 hari pada obat herbal 1x dosis tidak terdapat perbedaan bermakna pada setiap parameter libido mencit, sedangkan pada obat herbal 2x dosis hanya perilaku *introduction* yang menunjukkan perbedaan bermakna. Berdasarkan hasil penelitian, dapat disimpulkan bahwa pemberian obat herbal 2x dosis dapat meningkatkan frekuensi *introduction* pada perlakuan selama 14 hari.

Kata kunci : Libido, mencit, *Panax ginseng*, *Tribulus terrestris.*

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INTRODUCTION

LIBIDO was the biological desire for sexual intercourse. When a man cannot maintain or achieve a penile erection within the time required during sex, this was called erectile dysfunction. This situation was usually caused by low libido and testosterone. Low testosterone levels can cause a decrease in endothelial nitric oxide synthetase (eNOS) activity, which leads to a decrease in cyclic guanosine monophosphate (cGMP) levels in the penis. Decreased cGMP levels prevent the blood vessels in the penis from relaxing, making it difficult to get an erection. Testosterone also has the potential to influence the number of sperm produced[1].

The quality of life of a man with erectile dysfunction can be negatively affected because this condition can cause men to lose self-confidence, have character changes, and experience depression[2]. Middle-aged and elderly men often experience erectile dysfunction or impotence, which increases with age[3]. Diabetes, hypertension, and heart disease can cause complications in the form of erectile dysfunction. Diabetes will occur in 15% of men with erectile dysfunction, and 50% of diabetic sufferers will experience symptoms of erectile dysfunction (ED) after 5 years of suffering from diabetes[4]. The number of Indonesian people suffering from diabetes was estimated to reach 21.3 million in 2030[5]. This shows that the possibility of suffering from erectile dysfunction will be relatively high, both due to complications from chronic diseases and as a result of ageing.

Treatment for erectile dysfunction can be done by taking drugs that can increase cGMP levels, such as sildenafil, or herbal plants, which were useful as aphrodisiacs[6]. *Tribulus terrestris* and *Panax ginseng* were plants that have properties as aphrodisiacs. *Tribulus terrestris* contains the compound protodioscin (PTN), which was a steroid saponin. A study on 40 castrated mice showed that administration of *Tribulus terrestris* and testosterone could increase the sexual behaviour of mice in the test group compared to the control group[7]. The main compound that acts as an aphrodisiac in Panax ginseng was ginsenoside. The way ginsenoside treats erectile dysfunction was by helping the endothelium release nitric oxide (NO)[8]. A minimum dose of 250 mg/kg body weight of *Panax ginseng* extract in 12-week-old male mice weighing 350–400 grammes can increase the mice's libido, as indicated by the frequency of male mice riding female mice[9]. The diversity of treatment options for erectile dysfunction will help patients obtain the appropriate treatment so that they can improve their quality of life more easily.

This study aimed to see the effect of giving herbal medicine containing *Tribulus terrestris* and *Panax ginseng* on increasing libido in male mice. Increased libido was measured using the parameters of testicular weight, sperm concentration, sperm motility, and mice sexual behaviour (introduction, climbing, and coitus)[10].

MATERIALS AND METHODS

MATERIALS. Herbal medicine containing Tribulus terrestris and Panax ginseng, Vitan medicine containing Tribulus terrestris 250 mg (PT Midix Graha Farma, Sukabumi, Indonesia), CMC Na, and NaCl 0.9%

Animals. The experimental animals used were mice (Mus musculus), consisting of 48 male mice and 96 female mice with a weight of ± 20 grammes and an age of around 10–12 weeks.

Equipments. CCTV camera (Xiaomi, Beijing, China), counting chambers improved neubauer hemocytometer (Assistent, Germany), leukocyte pipette (Assistent, Hesse, Germany), light microscope (Olympus, Tokyo, Japan).

METHODS. Preparation of Test, Comparator, and Control Solutions The test solution was made into a suspension by mixing herbal medicine containing *Tribulus terrestris* and *Panax ginseng* with CMC Na. The comparison solution was made by mixing the Vitan drug containing *Tribulus terrestris* 250 mg with CMC Na. The control solution only contained water and CMC Na.

Preparation of Test Animals. The number of male mice used was determined based on the Federer formula, namely $(n-1)(t-1) \geq 15$, with t being the number of groups and n being the number of samples. The number of groups in this study was 8, so the minimum number of male mice needed in one group was 4 male mice. In this study, 6 male mice were used in 1 group, with 2 mice as spare. In addition, female mice were needed to observe introduction, climbing, and coitus behavior. When observing sexual behaviour, in 1 cage, there was 1 male mouse paired with 2 female mice, which were in the estrus phase. A total of 48 male mice and 96 female mice were used. All mice were adapted for 7 days so that they could adapt to the research environmental conditions. Also, they were placed in cages made of husks, which were changed every 2–3 days with a room temperature of 25°C–30°C. The lighting in the cage was set to be bright for 12 hours and dark for 12 hours.

Treatment of Mice. The research was carried out using methods that met the requirements of the code of ethics issued by the University of Surabaya

Code of Ethics Committee No. 11/KE/1/2022. A total of 48 male mice were set into 2 large groups, namely groups treated for 7 days (24 mice) and 14 days (24 mice) randomly. Then, each group was randomly split into 4 groups of six mice each. The control group was given CMC Na suspension, the comparison group was given Vitan drug suspension, test group 1 was given 0.3 mL of herbal medicine once, and test group 2 was given 0.6 mL of herbal medicine twice. Next, the mating process of mice was observed by looking at the frequency of introduction, climbing, and coitus processes. On the 8th and 15th days, the testicular weights of male mice were weighed, sperm motility was observed, and sperm concentration was calculated. Sperm motility was observed on a convex glass object based on 4 categories, namely: (A) sperm that move straight and fast; (B) sperm that move not straight; (C) sperm that move in place; and (D) immotile sperm. The calculation of sperm concentration was done by counting the number of sperm contained in the five boxes on both sides of the hemocytometer and then calculating the average. The results of calculating the average sperm count were entered into the sperm concentration calculation formula[11].

Data Analysis. Data analysis used the SPSS programme with the one-way ANOVA method on data that was normally distributed and homogeneous. The Kruskal-Wallis method was used if the data was not normally distributed and not homogeneous. To compare treatment for 7 days and 14 days between test groups 1 and 2, T-test analysis was used if the data was normally distributed and homogeneous, while Mann-Whitney was used for data that was not normally distributed and not homogeneous.

RESULTS AND DISCUSSION

Libido Parameters of Male Mice in Treatment for 7 and 14 Days. Observation of libido parameters in treatment for 7 days gave results as shown in Table 1.

Table 1. Data on libido parameters of male mice in treatment for 7 days.

Test Parameters	Groups	N	Average	SD	Normality Shapiro-Wilk	Homogeneity	P
Testicular Weight	Control	6	0.20	0.03	0.384	0.844	0.051 (ANOVA)
	Comparator	6	0.24	0.03	0.201		
	Test 1	6	0.21	0.06	0.066		
	Test 2	6	0.27	0.05	0.785		
Sperm Concentration	Control	6	26.17	5.19	0.764	0.004	0.468 (Kruskal-Wallis)
	Comparator	6	63.50	54.31	0.190		
	Test 1	6	103.33	85.28	0.098		
	Test 2	6	74.67	87.89	0.018		
Sperm Motility	Control	6	62.14	18.20	0.090	0.472	0.674 (ANOVA)
	Comparator	6	59.43	17.36	0.413		
	Test 1	6	69.62	10.34	0.147		
	Test 2	6	66.74	14.97	0.522		
Introduction Behavior	Control	6	48.17	9.70	0.834	0.024	0.113 (Kruskal-Wallis)
	Comparator	6	44.50	8.69	0.963		
	Test 1	6	89.33	51.15	0.500		
	Test 2	6	47.83	25.11	0.716		
Climbing Behavior	Control	6	11.83	6.31	0.372	0.335	0.676 (ANOVA)
	Comparator	6	16.00	6.32	0.565		
	Test 1	6	13.00	8.15	0.680		
	Test 2	6	17.33	12.56	0.884		
Coitus Behavior	Control	6	2.00	0.89	0.167	0.121	0.043 (ANOVA)
	Comparator	6	5.83	2.48	0.794		
	Test 1	6	2.50	2.59	0.272		
	Test 2	6	3.00	2.83	0.538		

Based on the data in Table 1, it shows that there were no significant differences between groups in the parameters of testicular weight, sperm concentration, sperm motility, and sexual behaviour (introduction and climbing), while coitus behaviour shows significant differences between groups. Data from observations of libido parameters in treatment for 14 days, as stated in Table 2, shows that there were no significant differences between groups in the parameters of testicular weight, sperm concentration, sperm motility, and coitus behaviour, while introduction and climbing behaviour show significant differences between the control group and test group 2.

Table 2. Data on libido parameters of male mice in treatment for 14 days.

Test Parameters	Groups	N	Average	SD	Normality Shapiro-Wilk	Homogeneity	P
Testicular Weight	Control	6	0.23	0.04	0.740	0.884	0.953 (ANOVA)
	Comparator	6	0.23	0.02	0.739		
	Test 1	4	0.24	0.03	0.161		
	Test 2	4	0.23	0.03	0.117		
Sperm Concentration	Control	6	61.17	32.78	0.464	0.224	0.214 (Kruskal-Wallis)
	Comparator	6	78.33	40.45	0.176		
	Test 1	4	110.25	28.98	0.213		
	Test 2	4	98.75	68.98	0.046		
Sperm Motility	Control	6	58.69	6.47	0.012	0.935	0.306 (Kruskal-Wallis)
	Comparator	6	62.58	5.58	0.658		
	Test 1	4	67.08	7.44	0.387		
	Test 2	4	58.85	7.70	0.470		
Introduction Behavior	Control	6	55.33	14.94	0.151	0.218	0.027 (ANOVA)
	Comparator	6	78.17	33.02	0.605		
	Test 1	4	92.75	8.27	0.810		
	Test 2	4	99.50	17.86	0.920		
Climbing Behavior	Control	6	8.17	4.36	0.968	0.250	0.038 (ANOVA)
	Comparator	6	16.33	14.65	0.126		
	Test 1	4	11.00	5.35	0.361		
	Test 2	4	26.50	4.93	0.808		
Coitus Behavior	Control	6	2.17	1.17	0.421	0.054	0.398 (Kruskal-Wallis)
	Comparator	6	5.00	6.87	0.045		
	Test 1	4	4.25	2.22	0.798		
	Test 2	4	4.50	2.65	0.689		

Libido Parameters of Male Mice Between 7 Days and 14 Days of Treatment in Test Groups 1 and 2. The results of the analysis of the libido parameters of male mice between treatment for 7 days and 14 days in test group 1 (mice that received 1x dose of herbal medicine), as shown in Table 3 and shown in Figures 1–6, show that all libido parameters, namely testicular weight, sperm concentration, sperm motility, and sexual behavior (introduction, climbing, and coitus), were not significantly different. This can happen because mice treated for 14 days had already ejaculated first, so on the 15th day, the testicles were taken and sperm concentration and motility were measured; the results were not significantly different from the group treated for 7 days.

The results of the analysis of mice's libido parameters, as listed in Table 4 and shown in figures 1–6, show that male mice given a 2x dose of herbal medicine had significant differences between treatment for 7 days and 14 days in the parameters of introduction behavior. Mice given a double dose of herbal medicine had a higher introduction frequency when given for 14 days compared to 7 days.

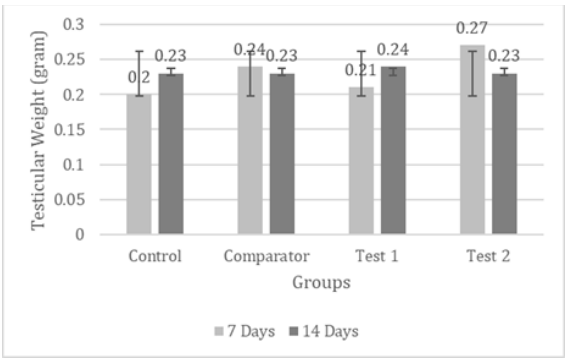

Figure 1. The average of testicular weight during 7 days and 14 days of treatment in each group.

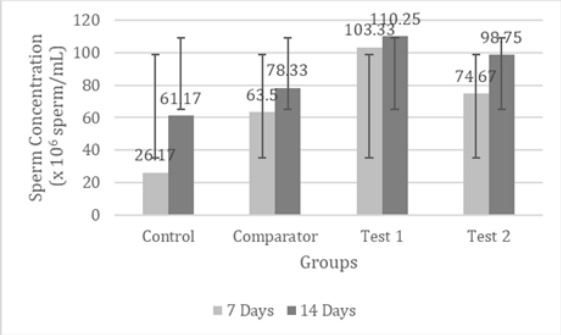

Figure 2. The average of sperm concentration during 7 days and 14 days of treatment in each group.

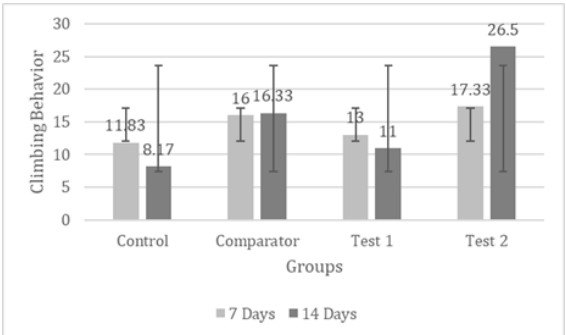

Figure 5. The average of climbing behavior during 7 days and 14 days of treatment in each group.

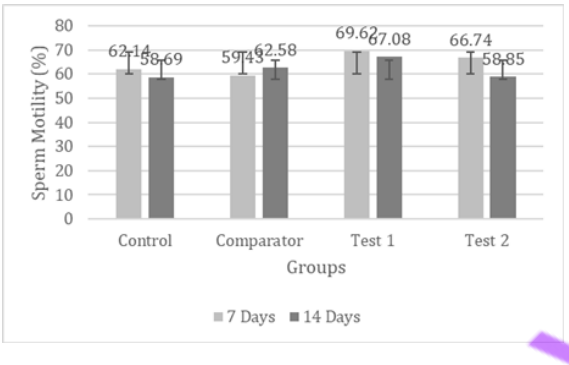

Figure 3. The average of sperm motility during 7 days and 14 days of treatment in each group.

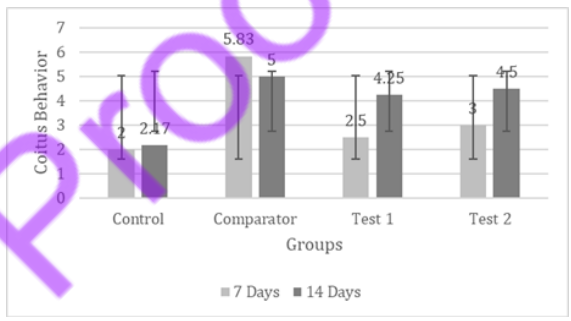

Figure 6. The average of coitus behavior during 7 days and 14 days of treatment in each group.

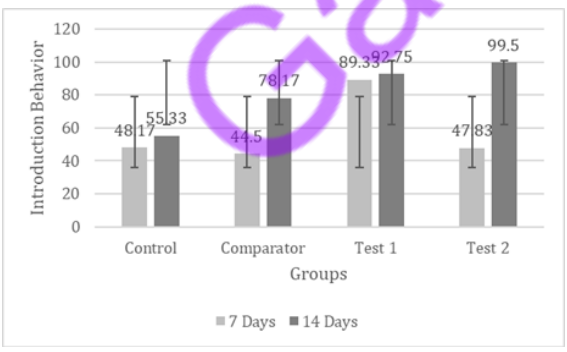

Figure 4. The average of introduction behavior during 7 days and 14 days of treatment in each group.

Based on research conducted by Singh *et al*., 2012[12]. *Tribulus terrestris* extract gives better results when given regularly for 13 days compared to only being given once. The significant difference in introduction frequency indicates that administering two doses for 14 days can provide better results than 7 days. The introduction process occurs when male mice were able to recognise pheromones from female mice. This behaviour was what underlies the processes of climbing and coitus. The more introduction processes occur, the greater the possibility of climbing and coitus[12]. Based on research conducted by Akram, *et al*., 2011[13], the use of *Tribulus terrestris* (750 mg–1250 mg/day) can increase sexual desire. In mice with a body weight of 0.03 kg, 4.62–7.71 mg of *Tribulus terrestris* was needed to get the maximum aphrodisiac effect. In this study, there was an increase in introduction behaviour by administering herbal medicine for 14 days, but this was not followed by climbing or coitus behavior. This could possibly happen because female mice have missed their estrus phase, so their acceptance of male mice was reduced.

Table 3. Data on libido parameters of male mice between 7 days and 14 days of treatment in test group 1.

Test Parameters	Treatment Duration	N	Average	SD	Normality Shapiro-Wilk	Homogeneity	P
Testicular	7 days	6	0.21	0.06	0.066	0.433	0.354
Weight	14 days	4	0.24	0.03	0.161		(T-Test)
Sperm	7 days	6	103.33	85.28	0.098	0.001	1.000
Concentration	14 days	4	110.25	28.98	0.213		(Mann-Whitney)
Sperm Motility	7 days	6	69.62	10.34	0.147	0.103	0.685
	14 days	4	67.08	7.44	0.387		(T-Test)
Introduction	7 days	6	89.33	51.15	0.500	0.098	0.900
Behavior	14 days	4	92.75	8.26	0.810		(T-Test)
Climbing	7 days	6	13.00	8.15	0.680	0.567	0.679
Behavior	14 days	4	11.00	5.35	0.361		(T-Test)
Coitus	7 days	6	2.50	2.59	0.272	0.760	0.302
Behavior	14 days	4	4.25	2.22	0.798		(T-Test)

Table 4. Data on Libido Parameters of Male Mice Between 7 Days and 14 Days of Treatment in Test Group 2.

Test Parameters	Treatment Duration	N	Average	SD	Normality Shapiro-Wilk	Homogeneity	P
Testicular Weight	7 days	6	0.27	0.05	0.785	0.621	0.190
	14 days	4	0.23	0.03	0.117		(T-Test)
Sperm	7 days	6	74.67	87.89	0.018	0.744	0.285
Concentration	14 days	4	98.75	68.98	0.046		(Mann-Whitney)
Sperm Motility	7 days	6	66.74	14.97	0.522	0.126	0.115
	14 days	4	58.85	7.70	0.470		(T-Test)
Introduction	7 days	6	47.83	25.11	0.716	0.435	0.008
Behavior	14 days	4	99.50	17.86	0.920		(T-Test)
Climbing	7 days	6	17.33	12.56	0.884	0.163	0.209
Behavior	14 days	4	26.50	4.93	0.808		(T-Test)
Coitus	7 days	6	3.00	2.83	0.538	0.688	0.424
Behavior	14 days	4	4.50	2.65	0.689		(T-Test)

The active ingredient of *Panax ginseng*, namely ginsenoside, functions to increase libido and sperm quality. Ginsenoside Rg1 increases the release of NO (nitric oxide) and cGMP in the corpus carvenosa of mice, causing relaxation of blood vessels and increasing blood flow to the penis. *Panax ginseng* extract can increase spermatogenesis in mice because it increases the expression of GDNF (glial cell line-derived neurotrophic factor) in Sertoli cells and the activation of CREM (cAMP-Responsive Element Modulator). GDNF controls sperm cell survival, and CREM was essential for spermatid cell maturation[14]. According to Habeeb, Sawad, and Abbas[15], the use of *Panax ginseng* root can increase testicular weight compared to the control group. This condition was caused by increased testosterone levels, which have an impact on testicular spermatogenesis. Testicles were part of the reproductive organs that produce sperm. The quantity of spermatogenic tissue and the maximum rate of the spermatogenesis process can influence testicular weight[16]. The greater the weight of the testicles, the greater the quantity of sperm produced, so that the testicles as a place for sperm production will also have a higher weight. Research conducted by Qureshi, *et al*., 2014[17] and Ara, *et al*., 2023[18], states that *Tribulus terrestris* was not effective in increasing testosterone levels so that it has an impact on the spermatogenesis process, namely that sperm concentration does not increase so that testicular weight also does not increase. Based on this, it was possible that herbal medicines containing *Tribulus terrestris* and *Panax ginseng have a* mutually cancelling effect between the active ingredients inside, so that the appearing effect cannot increase testicular weight, sperm concentration, and sperm motility.

CONCLUSION

Giving herbal medicine containing *Tribulus terrestris* and *Panax ginseng* at 2x doses (20 mg) can increase the frequency of treatment for 14 days.

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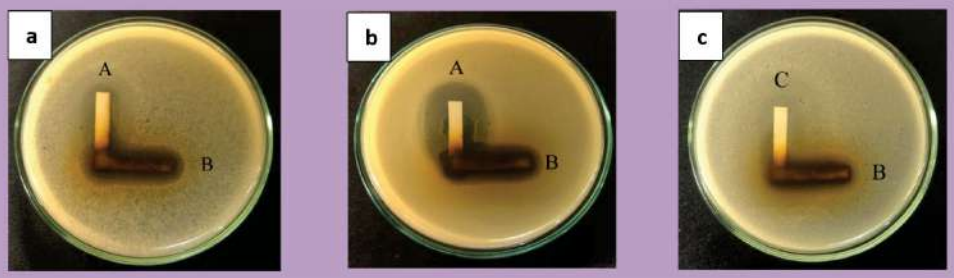

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