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Administration of Deer Placenta Supplement Orally Increased the Testosterone Hormone Levels in Young Adult Female Wistar Rats (*Rattus norvegicus*)

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Abstrak: Proses penuaan pada wanita mengakibatkan berbagai gangguan yang tidak menyenangkan. Saat ini banyak beredar suplemen yang diproduksi dengan tujuan mengatasi hal tersebut, salah satunya ialah *deer placenta frozen age*. Berdasarkan uji laboratorium, setiap kapsul suplemen tersebut mengandung testosteron sebesar 3,08 ng/g yang tidak dicantumkan dalam komposisinya. Kadar testosteron berlebihan pada wanita dapat menyebabkan efek negatif. Penelitian ini bertujuan untuk membuktikan bahwa pemberian suplemen *deer placenta* secara oral pada tikus Wistar betina dewasa muda dapat meningkatkan kadar testosteronnya. Jenis penelitian ialah *true experimental* menggunakan *randomized pretest-posttest control group design*. Sampel penelitian ialah 36 ekor tikus Wistar betina dewasa muda dibagi atas dua kelompok. Suplemen *deer placenta* diberikan sebesar 56,16 mg secara oral dengan *force feeding* selama 21 hari kepada kelompok perlakuan, dan plasebo pada kelompok kontrol. Darah tikus diambil di awal dan akhir penelitian untuk diperiksa kadar testosteronnya dengan metode ELISA. Dengan uji *t-independent* dilakukan analisis komparabilitas peningkatan rerata kadar testosteron antara sebelum dengan sesudah intervensi pada kedua kelompok, dan didapatkan perbedaan secara bermakna ($p<0,05$). Simpulan penelitian ini ialah pemberian suplemen *deer placenta* secara oral efektif meningkatkan kadar hormon testosteron dalam darah tikus wistar betina muda. Perlu dilakukan penelitian lebih lanjut dengan mempertimbangkan siklus *estrous* tikus betina.
Kata kunci: *deer placenta*, *antiaging*, testosteron, tikus Wistar betina dewasa muda

Abstract: Aging process in women results in various unpleasant disorders. Therefore, currently there are many supplements produced to overcome this problem. One of them is deer placenta frozen age supplement. Based on laboratory analysis, each capsule of this supplement contained 3.08 ng/g testosterone, which was not included in the ingredients. High testosterone in women can cause devastating negative effects. Therefore, this study was aimed to prove that adminis-tration of deer placenta supplement orally could increase testosterone levels in young adult female Wistar rats. This was a true experimental study using a randomized pretest-posttest control group design. Samples were 36 young female Wistar rats divided into two groups. Deer placenta supplement was given 56.16 mg orally by force feeding for 21 days to the treatment group and placebo to the control group. The rat blood was taken at the beginning and at the end of this study to examine the level of testosterone using ELISA method. The T-independent test was used to compare the increase of the mean testosterone level before and after treatment of the two groups. The result showed that there was a significant difference between the two groups ($p<0.05$). In conclusion, administration of deer placenta supplement orally is effective in increasing the blood testosterone level in young adult female Wistar rats. Further study is needed by considering the estrous cycle of female rats.
Keywords: deer placenta, anti-aging, testosterone, young adult female Wistar rats

INTRODUCTION

According to the American Academy of Anti-Aging Medicine (A4M), the definition of aging is physical and mental impairment due to normal process caused by physiological dysfunction, inter alia, hormonal change which in many cases can be altered with appropriate medical intervention.[1] One of the most important homones in human body is testosterone, which is the main androgen hormone in our blood circulation.[2] Testosterone hormone level will decrease due to aging, causing many unpleasant dysfunction and complaints. Therefore nowadays, there are so many studies using hormones as therapy to slow down the aging process and resolve the age related dysfunctions and complaints, and also to stay healthy in our midlife.[3]

Nowadays we often see many beauty clinics and medical practices offering many kinds of therapy and medication to delay the effect of aging process, especially in women. One of the most popular treatment products is deer placenta.[4] According to their websites, deer placenta supplement trade mark by frozen age is a supplement extracted from New Zealand deer's placenta and packed in a capsulated form.[5] This product is sold with their main benefit is to delay aging process.

Many of the benefits of the products are similar to the benefit of testosterone as a hormone replacement therapy, therefore, a laboratorium test was carried out to determine if the product contained testosterone hormones. According to the laboratorium test by Chemical Analytic Laboratorium Medical Faculty of Udayana University, the product contains significant level of testosterone hormone as much as 3.08 ng/g each capsule, albeit, it is not stated in their ingredients. To date, consuming product containing testosterone, specially for women, can cause undesire side effects, such as *polycystic ovary syndrome* (PCOS).[6] Other side effects of high testosterone level in women are acne, alopecia, menstrual disturbance, infertility, hirsutism, insulin resistance, glucose intolerance, obesity, dyslipidemia, virilization, vocal changes, and hypertension.[7] Testosterone therapy in women is still relatively new in medical practice. High level of testosterone hormone in women can cause various physical and psychological disorders, especially reproductive disorders from mild to severe.

Therefore, this study was conducted to prove the effect of using deer placenta supplement product, whether it can increase blood testosterone level if given to young adult female Wistar rats (*Rattus norvegicus*) as well as an additional reference that can be used by the community, especially women, before consuming these products.

MATERIALS AND METHODS

This study was conducted at the Integrated Biomedical Laboratory in the Department of Pharmacology of the Medical Faculty of Udayana University, Denpasar, Bali. This was a true experimental study using a randomized pretest-posttest control group design.[8] In the group of subjects, a random sample allocation was carried out in order to obtain two groups. One group as a control group that was given a placebo and the other group as the treatment group that was given oral deer placenta supplement. Population in this study was young adult female Wistar rats (*Rattus norvegicus*). Samples were 36 young adult female Wistar rats, that met the inclusion criteria for body weight of ±200 grams and age of 63-98 days, which was equivalent to 18-24 years of age in humans.[9]

This product recommended dose for human consumption is two capsules per day, each containing 1,560 mg of the supplement extract. According to the conversion table by Laurence, the conversion factor from human to mouse dose is 0.018, so the dose of deer placenta supplement given to the rats in this study was 56.16 mg/rat/day.[10]

The procedure was, as follows: 36 rats were adapted for seven days with two to three rats in one cage, given 20 grams of food a day which was replaced every morning and water ad libitum. Rats randomly divided into two groups, 18 rats for the control group and 18 rats for the treatment group. Before the experiment started, all the

rats had one milliliter of their blood drawn from the medial part of the orbital sinus of the right eye to examine the pretest testosterone levels by using ELISA method.

For 21 days, the treatment group was given deer placenta supplement diluted with glycerin at a dose of 56.16 mg, orally by force feeding. Meanwhile, the control group was given placebo, also by force feeding. At the end of the experiment, the two groups had one milliliter of their blood drawn from the medial part of the orbital sinus of the right eye to examine the posttest testosterone levels, by using ELISA method. Data were analyzed to compare the levels of the testosterone hormone between the two groups.

This study was carried out based on the feasibility of research ethics from the Ethics Commission for the Use of Animals in Research and Education at the Faculty of Veterinary Medicine, Udayana University No. 363/KE-PH-Lit-2/III/2018.

RESULTS

The results of the testosterone levels were presented in the form of a descriptive analysis including mean, standard deviation, median, minimum, and maximum values. Table 1 showed that the mean pretest testosterone level of the control group was 3.92±0.68 mmol/L, with minimum value of 3.21 mmol/L, maximum value of 5.15 mmol/L, and median of 3.54 mmol/L. The mean pretest testosterone levels of the treatment group was 4.29±0.62 mmol/L, with minimum value of 3.41 mmol/L, maximum value of 5.39 mmol/L, and median of 4.31 mmol/L. Meanwhile, after the intervention or post-test, the mean testosterone level of the control group was 3.90±0.68 mmol/L, with minimum value of 3.27 mmol/L, maximum value of 5.13 mmol/L, and median of 3.51 mmol/L, meanwhile the mean testosterone level of the treatment group was 4.35±0.70 mmol/L with minimum value of 3.34 mmol/L, maximum value of 5.57 mmol/L, and median of 4.26 mmol/L.

The Shapiro-Wilk test was used as the normality test, and the results showed that the data of the treatment group, pretest and post-test, was normally distributed ($p>0.05$), meanwhile the data of the control group, pretest and post-test, were not normally distributed ($p <0.05$). Furthermore, the data of control group were transformed using the logarithmic function, however, the results were still not normally distributed. Since the control group data was still not normally distributed, data of the increases of testosterone levels between pretest and post-test were made and tested, and the result was normally distributed ($p>0.05$).[11]

Testosterone level data were tested for homogeneity using the Levene's test. The results of the analysis showed that all data were homogeneous ($p>0.05$). Although the data had been transformed using the logarithmic function, they were still not normally distributed, therefore, the comparability analysis was carried out with the aim to evaluate the comparison of the median values of testosterone levels between the control group and the treatment group before and after treatment. The analysis was conducted by using the Mann-Whitney test, the Wilcoxon test, and the t-paired test, presented in Table 2.

Table 2 showed that the testosterone median level of the control group before treatment was 3.54 (3.39-4.74) mmol/L and of the other group before treatment was 4.31 (3.71-4.83) mmol/L. Meanwhile after treatment, the testosterone median level of the control group was 3.51 (3.35-4.74) mmol/L and of the treatment group was 4.26 (3.79-4.99) mmol/L.

Moreover, the Mann-Whitney test showed that there was no significant difference in testosterone levels between the control group and the treatment group, both before and after treatment ($p>0.05$). The Wilcoxon test and the t-paired test also showed that there was no significant difference in testosterone levels between before and after treatment, both of the control group and the treatment group ($p>0.05$). This p-value comparison was shown in Figure 1.

Table 1. Results of descriptive analysis of testosterone levels

Variable	Group	n	Mean	Standard Deviation	Median	Min	Max
Testosteron levels pretest	Control	18	3.92	0.68	3.54	3.21	5.15
	Treatment	18	4.29	0.62	4.31	3.41	5.39
Testosteron levels post-test	Control	18	3.90	0.68	3.51	3.27	5.13
	Treatment	18	4.35	0.70	4.26	3.34	5.57

Table 2. Differences in median of the testosterone levels between groups before and after treatment

Group	Before treatment median (Q1-Q3)	After treatment median (Q1-Q3)	p	Increases of testosteron levels
Control	3.54 (3.39-4.74)	3.51 (3.35-4.74)	0.206***	-0.02±.,07
Treatment	4.31 (3.71-4.83)	4.26 (3.79-4.99)	0.108****	0.05±0.13
p	0.074*	0.055*		0.043**

Explanation: *Mann-Whitney Test; **T-independent Test; ***Wilcoxon Test; ****T-paired Test

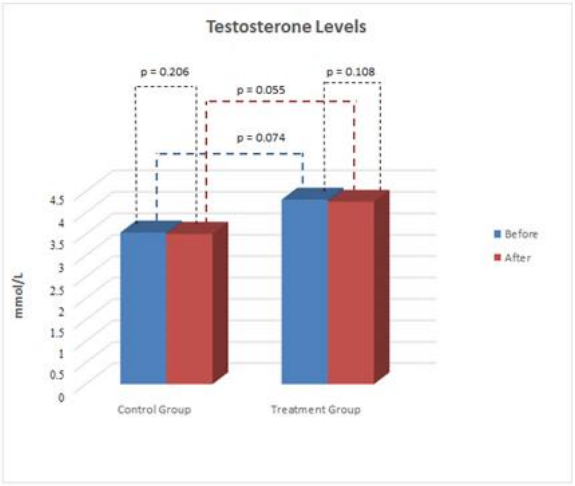

Figure 1. Comparability analysis of the median value of testosterone levels between the control and treatment groups

Further analysis was carried out on the the increased mean levels of testosterone between before and after treatment in each group.[12] In the treatment group, there were an increases in mean levels of testosterone between before and after the intervention for about 0.05 mmol/L, while in the control group it was -0.02 mmol/L, as shown by Figure 2. Comparability analysis was conducted with the independent t-test to compare the increases of the mean testosterone level before and after treatment between the two groups, and it was found that there was a significant difference between them (p <0.05).

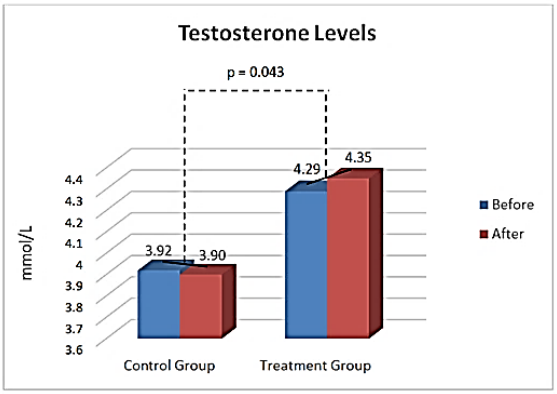

Figure 2. Comparability analysis of the increase of the mean testosterone levels between the control and treatment groups

DISCUSSION

This study showed that oral administration of deer placenta supplements could significantly increase testosterone levels in young adult female Wistar rats based on the independent t-test on the increases of the testosterone levels between before and after treatment in both groups, where in the treatment group there was an increase in testosterone level by 0.05 mmol/L.

These results supported a similar study

conducted on young adult male Wistar rats. The study concluded that there was a significant increase in the mean testosterone level of the treatment group given deer placenta supplement from 3.01867±0.282714 to 4.51656±0.796348 ($p<0.01$). Meanwhile, there was no increase in the mean testosterone level ($p>0.05$) in the control group given the placebo.[13]

Apart from its role in molecular transport between mother and fetus, the placenta is also one of the main endocrine organs during pregnancy. The placenta produces two kinds of steroid hormones, estrogen and progesterone, but not testosterone.[14] The other components in the product are also not compounds that contain natural testosterone. Therefore, the presence of testosterone in this supplement product can be said as an additional ingredient added into the product, but not listed in their ingredients data. This fact is certainly very unfavorable and can even endanger its users, especially women.

In this study, some data of the treatment group indicated a decrease of testosterone hormone level after treatment. This is most likely due to the different phases among the female estrous cycle of each rats. The estrous cycle is a reproductive cycle in female rats that resembles the menstrual cycle in women. This cycle is divided into four phases: proestrus, estrus, metestrus, and diestrus, which lasts for four to five days, therefore, the testosterone levels could change depending on the ongoing phase.[15]

One study mentioned that the best effect of testosterone administration in female rats depends on the presence or absence of corpus luteum at the first time giving the therapy. Therefore, it is possible to get two very different responses, depending on which phase of the estrous cycle the hormone testosterone is first given to the rats.[16] Another study examined sex steroid hormone levels based on the estrous cycle of female rats using the sensitive MS-based method. This study found that the levels of testosterone hormone in rats reached their highest level in the proestrus phase and the lowest level in the estrus phase of the estrous cycle.[17]

Therefore, it is necessary to learn more about the use of female rats for hormonal research as well as to increase knowledge about the hormonal signaling changes that occur and to explore possible interventions; these will help us to understand the ways to improve the quality of life and to prolong life.[18]

Based on the results of this study, although it has only been carried out on animals, it can be assumed that maybe the same results will occur if the product is given to women. While this product does not include testosterone in its ingredient composition (which is in fact in it), in the brochure, and at the product website, users are advised to take this supplement every day without any requirement to check their testosterone levels or do a medical consultation first. As a consequence of these reasons, the results can be used as a reference for consideration before consuming the product.

CONCLUSION

This study proves that oral administration of deer placenta supplement is effective in increasing the testosterone blood level in young adult female Wistar rats (*Rattus norvegicus*).

Conflict of interest

The authors affirm no conflict of interest in this study.

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