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Administration of Creatine Monohydrate® increased the estrogen levels but have no effect on testosterone levels in male albino rats (*rattus norvegicus*) with moderate physical activity

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ABSTRACT

Background: Our laboratory assessment indicated that the Creatine Monohydrate® supplement contains phytoestrogen and phytoprogesteron. This study was aimed to prove that the administration of Creatine Monohydrate® supplement could increase the estrogen levels and decrease testosterone levels in male albino rats (*Rattus norvegicus*) with moderate physical activity given.

Methods: A true experimental with pre-test and post-test randomized control study was conducted using 14 albino male rats. The samples were divided into two groups; the control groups (P0) that received aquadest and moderate physical activity, and treatment group (P1) that received 0.4 mg Creatine Monohydrate® supplement twice a day and moderate physical activity given. Moderate physical activity (swimming) was given five times a week for 14 days. Estrogen and testosterone level were assessed before and after treatment was given.

Results: Before the treatment, the estrogen and testosterone levels between P0 group and P1 group were comparable. Post-test results showed a significant different in estrogen levels between the P0 and P1 group (23.17±2.86 and 27.82±3.06 ng/ml respectively; $p<0.05$) as well as testosterone levels (18.15±2.95 and 21.69±2.14 ng/ml respectively; $p<0.05$) after 14 days of treatment. A paired analysis showed an elevating of estrogen levels but no effect on testosterone levels in P1 group that treated with Creatine Monohydrate®

Conclusion: This study indicated that the administration of Creatine Monohydrate® increased the estrogen levels but have no effect on testosterone levels in male albino rats (*Rattus norvegicus*) with moderate physical activity.

Keywords: Creatine monohydrate®, estrogen, testosterone, moderate physical activity

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INTRODUCTION

Naturally, all human being will experience aging that caused by deterioration of body functions, including impaired hormone productions. Hormone disorder is one of the internal factors that affect aging (Pangkahila, 2011).

Nowadays, young men do exercises in the fitness center to build a muscular and fat-free body. They often consume supplements in order to accelerate their body building program, such as creatine monohydrate®, which often to be found in the fitness center. Many studies have showed the usefulness of creatine monohydrate® in improving physical ability, improving maximum strength, increasing fat-free muscle mass, decreases the build-up of lactic acid in blood, and rapidly regenerates ATP during high intensity interval training.[2,3,4,5] Creatine monohydrate® supplementation improves cellular energetic by improving ATP turnover via providing phosphocreatine in ADP and AMP precursors, thus increaseing the physical capacity of athletes.[6]

Estrogen is a hormone that mainly found in a woman. Other than estrogen, woman also has a testosterone hormone but, in much smaller amount. The estrogen can also be found in men, although in much fewer amount than the testosterone hormone. Hormonal changes such as increased estrogen levels (especially in young men) are considered as one of the factors of several mental and health issues including erectile dysfunction and feminization. This phenomenon is though to be precipitated by the conversion of testosterone into estrogen by the aromatherase.[1]

The results of phytochemical analysis of creatine monohydrate® at the Analytical Laboratory, Udayana University revealed that the creatine monohydrate®

contained 0.48 pg/g phytoestrogens and 0.29 pg/g fitoprogesterone, while no testosterone was found. Supplementation of estrogen-containing food could increase the levels of the estrogen in the blood, where it will suppress the production of hypothalamic GnRH and lower the release of LH and FSH. Decreased level of those hormones will eventually lead to suboptimal production of testicular testosterone.[7]

Physical activity or exercise as many as 3-4 times a week with moderate intensity could minimize the production of excessive free radicals and improved endogenous antioxidants status.[1] Mild to moderate physical activity could also trigger IGF-1 secretion locally in the contracting skeletal muscles, in which it will be released into the circulation gradually and affect other tissues. Leydig cells are one of the targets of IGF-1and increasing the IGF-1 in circulation during physical activity will trigger the proliferation and secretion of testosterone in Leydig cells.[8]

Aizawa *et al*. reported that treadmill exercise, 30 m/min for 30 minutes in mice was able to increase the testosterone and 3β-HSD/17βHSD concentrations in the skeletal muscles.[9] Increasing the local testosterone in the skeletal muscles will influence the total testosterone levels in blood, but these hormonal changes vary by individual influenced by the type of physical exercise, duration, and intensity.[9,10]

Recently, the number of creatine monohydrate® supplement user is increasing and many those are sold with suspicious ingredients. A long-term use and excessive dose of creatine monohydrate® could lead to hormonal disorders. Therefore, a study to prove that the administration of Creatine Monohydrate® supplement might increase the estrogen levels and decrease testosterone levels with moderate physical activity is required.

METHODS

This study was conducted using true experimental with pre-test and post-test randomized control design. The subjects were 14 albino male Wistar rats, 2-3 month old, weight 150-200 gram, and obtained from *"LBT Divisi Pengembangan Obat dan Hewan Coba Bagian Farmakologi Fakultas Kedokteran Universitas Udayana"*. All the experimental protocols and treatments were approved by the ethical committee of animal experiments in Faculty of Veterinary Medicine, Udayana University (No. 404/KE/PH-Lit-2/VI/2018).

The samples were divided into two groups; the control groups (P0): treated by aquadest and moderate physical activity, and treatment group (P1): treated by Creatine Monohydrate® supplement and moderate physical activity. The Creatine Monohydrate® supplement was given twice a day (0.4 mg each) and the moderate physical activity (swimming) was given five times a week for 14 days. Before and after treatment, blood was collected from the medial canthus sinus orbitalis. Estrogen and testosterone levels were then measured by using ELISA method in accordance with manufacturer's instruction. Data were presented in mean ± SD. Independent data were analyzed using student *t*-test and paired data were analyzed using paired *t*-test.

RESULTS

According to the results of this study, it appear that the estrogen levels of the P0 group and P1 group were not different before the treatment commenced (control and treatment: 24.18±5.03 and 26.76±3.29 ng/L respectively; $p>0.05$). The same result also found in testosterone levels also were not different before the treatment (control and treatment: 19.29±4.32 and 23.06±6.49 ng/L respectively; $p>0.05$). However, the post-test results showed a significant different in estrogen levels between P0 and P1 group (23.17±2.86 and 27.82±3.06 ng/ml respectively; $p<0.05$). The testosterone levels were also significantly different between P0 and P1 group after 14 days of treatment (18.15±2.95 and 21.69±2.14 ng/ml respectively; $p<0.05$). A paired analysis showed an increased of estrogen levels but no effect toward testosterone levels in P1 group treated with Creatine Monohydrate®.

DISCUSSION

The elevating of estrogen levels in the P1 group were possibly due to phytoestrogens and phytoprogesterone in creatine monohydrate®. The supplementation of estrogen-containing food will increase the endogenous levels of estrogen in blood, where the increase in estrogen will suppress the production of hypothalamic GnRH so that the release of LH and FSH will also decrease. Decreasing the anterior pituitary hormone will suppress the production of testosterone.[7] High levels of estrogen in men (normal blood values of 2-180 pg/ml) could affect the reproductive health.[11] In men, high estrogen levels can cause gynaecomastia, breast enlargement, prostate cancer, low sperm levels in semen, infertility and erectile dysfunction. [11] Nevertheless, creatine monohydrate® supplementation is not recommended for men's health, especially in long-term application.

Table 1. Comparison Analysis

Group	Pre-test (Mean±SB)	Post-test (Mean±SB)	Different (Δ)	*p**
Estrogen (ng/L)				
Control (P0)	24.18 ± 5.03	23.17 ± 2.86	-1.01 ± 6.32	0.686
Treatment (P1)	26.76 ± 3.29	27.82 ± 3.06	1.06 ± 2.78	0.351
*p***	0.279	0.012	0.933	
Testosterone (nmol/L)				
Control (P0)	19.29 ± 4.32	18.15 ± 2.95	-1.14 ± 2.88	0.335
Treatment (P1)	23.06 ± 6.49	21.69 ± 2.14	-1.36 ± 6.05	0.574
*p***	0.225	0.024	0.441	

***p**= Significance for paired *t*-test, ***p**= Significance for independent *t*-test

Moderate physical activity can reduce the estrogen levels. According to the previous research, physical activity alone could not increase the estrogen levels; instead, it caused the reduction in estrogen level.[12] Other studies also suggested that physical activity could reduce the estrogen levels.[13] This condition is caused by a disruption of LH secretion, which also affects the release of GnRH from the hypothalamus. After 14 days of treatment, the testosterone levels were increased in the P0 group due to moderate physical activity. This phenomenon is related to the theory increased testosterone production in response to physical activity, both mild and severe with sufficient duration.[14] This is also related to the increase in lactate accumulation after exercise.[10] Other studies suggested that increased blood flow and blood vessels dilatation induced by exercise were associated with the release of nitric oxide which can increase testosterone secretion in addition to an increase in sympathetic nerve activity.[15] Regular physical activity can improve the function of homeostasis in accordance with the body needs; it is including hormonal balance and the associated more organized metabolism which includes improvement in endocrine function such as an increased of total testosterone levels.[9]

A study showed that both mild and moderate physical activities can trigger the IGF-1 secretion in contracting skeletal muscles. It will be released into the blood circulation gradually and affect the expression in other tissues, one of which is Leydig cells; resulting in increased of IGF-1 in circulation during physical activity. It will trigger the proliferation and secretion of testosterone in Leydig cells.[8] During physical activity, hormonal response is influenced by the intensity and frequency of the exercise, time intervals, and volume. Testosterone does not increase in the excessive training, but cortisol level does increase. Overtraining causes catabolic hormones elevation, a decrease in testosterone and decrease the level of protein synthesis.[16]

The testosterone response is also different between tonic and aerobic exercise. Tonic training stimulates testosterone greater than aerobic exercise due to the anaerobic glycolytic pathway.[17] Other researchers showed that treadmill exercise, 30 m/min for 30 minutes in mice was able to increase the testosterone and 3β-HSD/17βHSD concentrations in skeletal muscles. Increasing the local testosterone in skeletal muscles will influence the total testosterone levels in blood, but this hormonal change varies among individual, influenced by the type of physical exercise, duration, and intensity.[10]

Moderate physical activity reduces estrogen levels and testosterone levels, even though they were statistically insignificant. Creatine monohydrate® and physical activity increased the estrogen levels and decreased testosterone levels. In this study, creatine monohydrate®, which contains phytoestrogens and the phytoprogesterone, did not significantly reduce the testosterone levels. Theoretically, estrogen is a negative feedback and lower GnRH secretion will reduce the testosterone levels. From this study, it can be concluded that the content of estrogen in creatine monohydrate® supplement and physical activity did not act as a negative regulator thus it did not decrease the testosterone levels. High levels of estrogen should reduce the testosterone levels.[7] An increase of estrogen levels may also cause by an increase in testosterone which converted to estrogen by the aromatherase process.[1]

CONCLUSION

This study indicated that the administration of creatine monohydrate ® increased the estrogen level failed to induce a decrease in testosterone level in male albino Wistar rats (*Rattus norvegicus*) with moderate physical activity. Further observational study on humans, using both men and women with different age, body weight, exercise intensity, exercise duration and length of usage of creatine monohydrate® supplementation are required to provide more reliable data on the effect of creatine monohydrate®.

CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication

AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

FUNDING

This study was self-funded without any contribution from third party.

ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Udayana University with approval letter number 404/KE-PH-Lit-2/VII/2018

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