Oral administration of Bali Robusta coffee (*Coffea canephora*) extract prevented the reduction of Leydig cells and testosterone levels in male Wistar rats (*Rattus norvegicus*) with excessive physical training

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Abstract

Background: Excessive exercise cause a deterioration of organ function and structure. Overtraining will cause reduce antioxidant reserve and excess free radical production. In this condition, there will be a decrease in the number of Leydig cells in the testis and followed by a decrease in testosterone level. Bali robusta coffee extract contains active compounds such as alkaloids, saponins, flavonoids, tannins and polyphenols. This study aims to prove whether Bali robusta coffee extract (*Coffea canephora*) can prevent a decrease in the number of Leydig cells and testosterone levels in male Wistar rats (*Rattus norvegicus*) with excessive physical training.

Methods: Thirty-six rats were randomly divided into 2 groups: the control group (placebo aquabidest 2 ml) and the treatment group (Bali robusta coffee extract of 1 gram/kg BW). Both groups received excessive physical training in the form of swimming until the subjects experienced fatigue for 21-days.

Results: The results showed that the mean of Leydig cell number in the treatment group was significantly higher, which was 3.70 ± 0.89 cell/field of view, compared to the control group, which was 2.92 ± 0.65 cell/field of view ($p = 0.005$). The mean testosterone level in the treatment group was also significantly higher, which was 6.32 ± 0.21 nmol/ml, compared to the control group, which was 1.95 ± 0.32 nmol/ml ($p < 0.001$).

Conclusion: The administration of Bali robusta coffee extract (*Coffea canephora*) prevented a decrease in the number of Leydig cells and testosterone levels in male Wistar rats (*Rattus norvegicus*) with excessive physical training.

Keywords: Bali Robusta coffee extract, Leydig cells, Testosterone, Overtraining

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Introduction

Aging is a time-dependent adaptation that ultimately becomes maladapted (dysregulated), no longer obeying the principle of hormesis and leading to self-elimination and death. However,

along with the development of science, especially in the field of Medicine, aging is considered as a disease that can be avoided, prevented and slowed down with various efforts based on evidence-based medicine. The aging process that is left untreated or an attempt to restore it to its original function can reduce the quality of life so that it can accelerate the aging process.¹

One of the factors that cause aging is the accumulation of oxidative damage caused by high levels of free radicals in the body. Free radicals can be triggered by stress or excessive physical training.^{2,3} The underlying mechanism is the excessive physical activity can increase oxygen consumption by 100-200 times.⁴ Physiologically, 2-5% of the oxygen used during the metabolic process will be converted to superoxide, which will trigger oxidative stress and can damage all cells in the body including Leydig cells.⁵

Leydig cell apoptosis due to overtraining is mediated by the free radicals-induced oxidative damage. The association between apoptosis and Leydig cells has been previously reported in experimental animals.⁶ The induction of apoptosis in Leydig cells due to free radicals is mediated by the activation of the intrinsic pathway of apoptosis⁷, the p53-independent pathway, the induction of autophagy⁶, and endoplasmic reticulum stress.⁸ Because Leydig cells produce testosterone through a process known as steroidogenesis⁹, the death of Leydig cells due to excessive physical training can cause a decrease in testosterone levels which leads to aging and infertility. Previous studies reported that 21 days of overtraining in 24-year-old men can reduce testosterone levels.¹⁰ Testosterone is one of the essential hormones whose levels decrease due to aging, and vice versa, decreased testosterone levels can accelerate the aging process.¹ Hence, efforts to prevent a decrease in the number of Leydig cells and a decrease in testosterone levels due to excessive physical training are important Anti-Aging Medicine steps.¹¹

Because the pathogenesis of overtraining is mediated by free radicals, antioxidants administration may serve as a step to prevent the pathogenesis of overtraining.¹² Coffee is a fairly high source of polyphenols. The polyphenol content in coffee includes caffeine, chlorogenic acid, coumaric acid, ferulic acid, and synapic acid.¹³ In addition, the phytochemical analysis showed that the Bali Robusta coffee extract used in this study contains a flavonoid of 9,203.34 mg/100g, total phenols of 79,325.95 mg/100g, tannin of 27,532.06 mg/100g, and IC50 of 98.42 ppm (unpublished data).

Previous research has also shown excellent antioxidant activity, where Bali Robusta coffee a dose of 0.5 mg/200g BW for 21 days can significantly prevent the elevation of malondialdehyde (MDA) levels

of hyperuricemic rats.¹⁴ Other studies have also shown that the antioxidant activity of Bali Robusta coffee extract increased the total antioxidant status in metabolic syndrome rats.¹⁵ This study aimed to prove whether Bali Robusta coffee extract (*Coffea canephora*) can prevent a decrease in the number of Leydig cells and testosterone levels in male Wistar rats (*Rattus norvegicus*) with excessive physical training.

Methods

This research was an experimental study, using the posttest-only control group design. The subjects of this study were healthy male rats (*Rattus norvegicus*), 6 months old, and 200 grams in weight. Thirty-six rats were randomly divided into 2 groups. The control group was the male Wistar rat group given a placebo aquabidest 2 ml once a day. The treatment group was the male Wistar rat group given Bali Robusta coffee extract at a dose of 1 gram/kg BW/day dissolved in 2 ml aquabidest. Both groups received excessive physical training in the form of swimming until the subjects experienced fatigue. After 21 days of treatment, blood samples were taken for testing of testosterone and testis was taken for examination of the number of Leydig cells using by evaluation of histologic slides stained with Hematoxylin Eosin. The mean difference of Leydig cell number and testosterone level between groups was tested using an independent T-test with 0.05 significance.

Results

Figure 1. Examination Leydig cell histology. (A) control group and (B) treatment group. The number of Leydig cells in the treatment group is greater than the control group

The number of Leydig cells was examined histologically on testicular tissue samples. The calculation was carried out by observing the number of Leydig cells in the right and left testicles in three fields of view in a zig-zag direction, with a magnification of 400 times then the results obtained were averaged.¹⁶ The histology of the testicular tissue after HE stains is shown in Figure 1. The results showed that the mean of Leydig cell number in the treatment group was significantly

higher, which was 3.70 ± 0.89 cell/field of view, compared to the control group, which was 2.92 ± 0.65 cell/field of view ($p = 0.005$). The mean testosterone level in the treatment group was also significantly higher, which was 6.32 ± 0.21 nmol/ml, compared to the control group, which was 1.95 ± 0.32 nmol/ml ($p < 0.001$).

Table 1. Comparison of Leydig cells number and testosterone levels

Variable	Group	n	Mean $\pm$ SD	P	Description
Leydig Cells (cells/field of view)	Control	18	2.92 ± 0.65	0.005	Significant
	Treatment	18	3.70 ± 0.89		
Testosterone (nmol/ml)	Control	18	1.95 ± 0.32	0.000	Significant
	Treatment	18	6.32 ± 0.21

Figure 2. Graphical comparison of Leydig cells number

Figure 3. Graphical comparison of testosterone levels

Discussion

The effect of Bali Robusta Coffee extract on the number of Leydig cells

The results of this study indicated that the Bali Robusta coffee extract can prevent declining Leydig cells in young Wistar rats treated with overtraining. The number of Leydig cells in the treatment group was higher than the control group because coffee contains active compounds such as alkaloids, saponins, flavonoids, and polyphenols.^{17,18} Likewise, a study showed that coffee contains tannins, alkaloids, flavonoids, coumarin, quinones, phenols and essential oils.¹⁹

Flavonoids are oxidized by radicals, resulting in more stable and less reactive radicals. Flavonoids consist of two aromatic rings attached to three carbons and are usually in the oxygenated heterocyclic form. Flavonoids will inhibit the aromatase enzyme, which is an enzyme that catalyzes the conversion of androgens to estrogen that will increase the testosterone and eventually the number of Leydig cells.²⁰

Polyphenol compounds are plant secondary metabolites derived from a biosynthetic pathway with precursors from the cyclic and/or acetate-malonate pathways. The function of these metabolites is to protect plants from biological and environmental stress. Therefore, these compounds are synthesized to respond to pathogens such as fungi or bacteria. Phenols can function as primary antioxidants because they can directly inhibit free radical reactions to lipid oxidation.²¹

Tannins have antioxidant activity, inhibit tumor growth, and inhibit enzymes such as reverse transcriptase and DNA topoisomerase. Tannins also induce the proliferation and division of Leydig cells and increase the number of Leydig cells in the testicular interstitial tissue.²²

The effect of Bali Robusta Coffee extract on testosterone levels

The results of this study showed that Bali coffee prevents the decrease in testosterone levels caused by overtraining. Leydig cells are the main testosterone-producing cells in men and male animals, so an increase in the number of Leydig cells has a direct impact on increasing testosterone levels.²³ Normal levels of testosterone in adult rats are 0.5-5.4 ng/ml²⁴, and in this study, the testosterone level were 1.95 ± 0.32 and 6.32 ± 0.21 nmol/L for the control and treatment group respectively. So that the two groups are still classified as having normal testosterone levels.

Flavonoids can increase testosterone levels by inhibiting the 5-alpha reductase enzyme, which converts testosterone to

dihydrotestosterone and increases testosterone levels.²⁵ Study reported that flavonoids are a class of compounds that function as antiandrogenic by blocking the work of the aromatase enzyme, where this enzyme serves to catalyze the conversion of androgens to estrogens resulting in the elevation of the testosterone hormone.²⁰ Flavonoids can also stabilize reactive oxygen compounds by reacting with the reactive arrangement of these radicals.²⁶

Tannins may increase testosterone levels through an increase in the number of Leydig cells due to MAPK activation.²⁷ To date, there have been no studies showing a direct effect of tannins on the activity of testosterone synthesis in Leydig cells or the adrenal cortex. Tannins affect biological activity that can coagulate protein, it is suspected that the enzyme protein (ATPase/dynein) is damaged, so that the energy release mechanism for spermatozoa motility will be disrupted.²⁸ So that if the tannin levels are sufficient, the amount of testosterone will also be well preserved and not decrease.

Relationship of Bali Robusta Coffee extract with anti-aging medicine

Excessive physical training is defined as excess exercise volume, intensity, duration, or burden.³ Many studies have shown that overtraining can lead to oxidative stress. Because Leydig cells are cells that produce testosterone⁹, the Leydig cells apoptosis cause a decrease in testosterone.¹⁰ Testosterone is an important hormone whose levels decrease due to aging and vice versa.¹ So efforts to prevent a decrease in the number of Leydig cells and a decrease in testosterone levels due to excessive physical training are important Anti-Aging Medicine steps, especially for community groups who are vulnerable to excessive physical activity such as athletes, police and soldiers.¹¹ The administration of Bali Robusta coffee extract ameliorated the overtraining-induced declining Leydig cells and testosterone, thus supporting the concept of anti-aging medicine.

Conclusion

The conclusion of this study was that the administration of Bali Robusta coffee extract (*Coffea canephora*) prevented a decrease in the number of Leydig cells and testosterone levels in male Wistar rats (*Rattus norvegicus*) with excessive physical training. It is necessary to compare the effectiveness of Bali Robusta coffee extract with other compounds to find out which natural ingredients are best in preventing the decrease in testosterone levels and Leydig cells. Additionally, further research to determine the potential toxicity of this coffee extract

is required.

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