Administration Of 45% Robusta (Coffea canephora) Green Coffee Bean Ethanol Extract Solution Could Not Increased Hair Growth But Inhibited The Increase Of 5α-Reductase Type 1 Levels In Male Wistar Rats (Rattus norvegicus) Exposed To Topical Testosterone

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Abstract

Background: Hair loss can occur due to disturbances in the follicular cycle. The most common type of hair loss is androgenetic alopecia, which occurs as a result of androgen hormone stimulation of the hair follicles, causing a transition from a long to a short anagen phase and a short to a long telogen phase, then causes miniaturization of the hair follicle and falls out. Robusta green coffee beans contain many compounds, which contain polyphenol (chlorogenic acid) and alkaloid (caffeine) predominantly. These compound has a 5α-reductase inhibitory action, thereby prolonging the anagen phase, promoting hair growth and reducing hair loss. This research was conducted to prove the effectiveness of 45% green robusta coffee beans given topically in increasing hair length growth and inhibiting the increase of 5α-reductase type 1 levels.
Methods: This research is an experimental study with a randomized posttest only control group design. In this study, 30 male wistar rats, 9 weeks old, were divided into 3 groups namely, the control group (K) which was exposed to topical testosterone and ethanol 75% as a negative control, group P1 which was exposed to topical testosterone and given the basic ingredients of solution, and group P2 which was exposed to topical testosterone and given solution contain 45% robusta green coffee bean ethanol extract for 21 days. After the treatment was finished, the length of the hair was measured using caliper, and the level of 5α-reductase type 1 was examined using ELISA test.
Results: The results showed that the mean hair length in group P2 was 8.24 ± 0,899%, not significantly different from group K 8,645 ± 2,311% and group P1 8,98 ± 1,885%. The mean level of 5α-reductase type 1 in group P2 467,61 ± 101,82% was significantly different from group K 700,95 ± 197,67% and not significantly different from group P1 579,39 ± 165,76%. Comparative analysis using the One Way Anova test showed that the mean hair length was not significantly different between groups, but the level of 5α-reductase type 1 indicating a significant difference between groups (p value <0,05).

Conclusion: Conclusion for this experiment is that the administration of 45% robusta green coffee bean extract solution did not significantly increase hair growth, but proved effective in inhibiting the increase of 5α-reductase type 1 levels in male wistar rats exposed to topical testosterone.

Keywords: Robusta green coffee bean extract; Coffea canephora; hair length; 5α-reductase type 1

1. Introduction

One of the visible aging processes is thinning hair, due to several structural changes in the hair, so that hair is more brittle and falls out easily. Hair itself has an important role as a skin protector from exposure to pollution and maintaining appearance in both men and women. Damage that occurs to hair, such as hair loss, can cause a variety of psychological problems, including loss of self-confidence, and affects social communication, thus having a negative impact on quality of life (Truong et al., 2017; Völker et al. al, 2020).

Hair loss can occur due to disturbances in the follicular cycle. The most common hair loss is androgenetic alopecia, which is characterized by abnormal changes in the hair cycle and hair follicle structure. In androgenetic alopecia, the conversion of testosterone to dihydrotestosterone (DHT) by 5α-reductase enzymes in the dermal papillae plays a major role (Azzouni et al, 2012; Ceruti et al, 2017). DHT will bind to androgenic receptors to form hormone-receptor complexes and then induce the related target genes, so that the anagen phase becomes short and the telogen phase which should be short becomes long. This causes a progressive decrease in hair bulb size and hair thickness, which causes miniaturization of hair follicles, and hair loss (Orăsan et al, 2017; Truong et al, 2017; Völker et al, 2020). Previous studies showed that 5α-reductase was significant in many androgen metabolic disorder-related diseases such as androgenetic alopecia (Lao et al.,2021).

Green coffee beans are coffee beans that have not been roasted. Robusta green coffee beans contain many chemical constituents that play an important role in influencing the quality and the characterization of coffee, such as carbohydrates, nitrogen compounds (proteins, free amino acids, caffeine, trigonellin), fats (coffee oil, diterpenes), minerals, and polyphenols (chlorogenic acid) (Farah, 2012; Sualeh et al, 2020). Robusta green coffee beans predominantly contain chlorogenic acid, which is the main polyphenol, and caffeine, which is an alkaloid (Sualeh et al, 2020; Völker et al, 2020). In previous study said that chlorogenic acid and caffeine has an inhibitory action against 5α-reductase, thereby prolonging the anagen phase, reducing hair loss and promoting hair growth. (Dhurat et al, 2017; Saewan et al., 2022).

2. Materials and methods

2.1 Materials

The basic ingredients of the solution consist of 96% ethanol, distilled water, menthol, propyl paraben, methyl paraben and propylene glycol. The testosterone was obtained from Sigma-Aldrich, with a preparation of 100 µL of 0.05% testosterone solution (dissolved in 75% ethanol). SRD5A1 Elisa kit was bought from Bioassay Technology Laboratory.

2.2 Methods

A randomized post-test only control group design was conducted among 30 male wistar rats for 21

consecutive days at the Laboratory Animal Unit, Department of Pharmacology, Faculty of Medicine, Udayana University. The wistar rats were divided into 3 groups, namely, control group (K) that is exposed to topical testosterone and ethanol 75%, group P1 that is exposed to topical testosterone and solution base, and group P2 that is exposed to topical testosterone and 45% Robusta green coffee bean ethanol extract solution.

All wistar rats were adapted for 7 days before any treatment was started. On the seventh day, the rats were anesthetized then the dorsal hair (4cm x 4cm) of the rats was shaved using an electric hair clipper followed by applying Veet® hair removal cream. Then cleaned the dorsal area carefully to remove traces of the hair removal cream. On the eighth day, all groups are locally smeared with 100 µL of 0.05% testosterone solution (dissolved in 75% ethanol) on the depilated dorsal skin, once daily, for the next 21 days. After treatment with testosterone for 30 min, the depilated dorsal skin of three groups were smeared as follows: 100 µL of 75% ethanol solution as negative control, solution base (etanol 96%, aquades, mentol, propil paraben, metil paraben, dan propilen glikol), and 45% robusta green coffee bean ethanol extract solution, twice daily. Throughout the study period, all rats were given equally adequate amount of food and water. All the rats were euthanized at the end of the experiment, then 10 longest hairs were taken from each rat, and measured using a caliper. After that, reshaved the dorsal hair of the rats, and rats dorsal skins were removed, weighed, homogenized with a blender in cold PBS, and then centrifuged as before. 5a-Reductase type 1 concentrations in skin tissue were measured using standard diagnostic ELISA test kits, according to the product manual. The examination was done at the Department of Biochemical and Molecular Biology, Faculty of Medicine, Udayana University.

Acquired data were analyzed statistically using SPSS version 25.0 program. Results for mean, standard deviation, and significance test were shown. Statistically significant differences between the control and experimental groups were analyzed using One Way Anova. p Values less than 0.05 ($p<0,05$) as the threshold, were considered to be statistically significant.

3. Result

As shown in Table 1, showed that the mean value for the hair length was higher in group P1 (8,98±1,88) than group K (8,64±2,31) and group P2 (8,24±0,89). The higher mean value for 5α-reduktase type 1 was observed in group K (700,95±197,67) followed by group P1 (579,39±165,76) and group P2 (467,61±101,82).

Table 1. The Mean of hair length of Wistar rats in Group K, Group P1, Group P2

Variable	Group	N	Mean	SD	P
Hair Length	K	10	8,64	2,31	0,658
	P1	10	8,98	1,88	
	P2	10	8,24	0,89	
5α-reduktase type 1	K	10	700,95	197,67	0,011
	P1	10	579,39	165,76	
	P2	10	467,61	101,82	

Footnotes:
K : Control group (testosterone exposure + ethanol 75%)
P1 : Treatment group 1 (testosterone exposure + solution base)
P2 : Treatment group 2 (testosterone exposure + 45% Robusta green coffee bean ethanol extract solution)

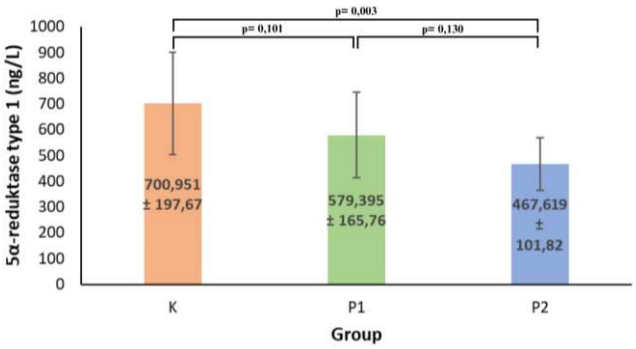

Figure 1. The mean of 5α-reductase type 1 levels in group K, P1, P2. Results were presented as mean±SD.

Data from the two variables were tested for normality and homogeneity with Saphiro-Wilk Test and Levene's Test. Both data were normally distributed with $p>0.05$, and both data were homogenous with $p>0.05$. Analysis of significant differences using the One Way Anova test (Table 1), was found that the mean value of hair length in the three groups after being given treatment was not significantly different ($p>0.05$), and the mean value of 5α-reductase type 1 levels in the three groups after being given treatment was significantly different ($p<0.05$). To find out which group is different from the control group, the result were analyzed using further test with the Least Significant Difference (LSD) test for the mean value of 5α-reductase type 1 levels. The column graph (Figure. 1) showed that 5α-reductase type 1 levels was significantly decreased in group P2, compared to group K ($p<0.05$).

4. Discussion

On exposure to topical testosterone, the conversion of testosterone to dihydrotestosterone (DHT) by 5α-reductase enzymes. In the presence of androgens, the anagen cycle of the affected hair follicle shortens and the short telogen phase becomes long, causing a progressive decrease in hair bulb size and hair thickness, resulting in hair shortening, decrease in hair diameter, miniaturization of hair follicles, and hair loss (Orăsan et al, 2017; Völker et al, 2020). In a study, it was found that the time for hair regrowth in the testosterone group was much longer than the control group (using 75% ethanol), indicating that giving testosterone can slow down hair growth. In addition, the expression of 5α-reductase also increased in the testosterone group, compared to the control group (Lao et al., 2021).

The result of hair length showed higher mean value in group P1 (8,98±1,88) compared to group K (8,64±2,31) and group P1 (8,24±0,89). The result of 5α-reductase type 1 levels was higher in group K (700,95±197,67) than in group P1 (579,39±165,76) and P2 (467,61±101,82). The data acquired for each variables were analyzed using One Way Anova test, and showed no significant difference ($p>0,05$) in hair length variable, but showed sginficant difference between group K and P2 ($p<0,05$) in 5α-reductase type 1 variable.

This study showed that 45% robusta green coffee bean extract solution was not proved to increase hair length in Wistar rats exposed to topical testosterone. However, administration of 45% robusta green coffee bean extract solution was shown to reduce levels of 5α-reductase type 1 in Wistar rats exposed to topical testosterone.

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