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Effect of White Turmeric Rhizome Extract (*Curcuma zedoaria*) on Testis Histology of Male Wistar Rat

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Abstract

Traditional medicine is an alternative in medicine because of the minimal side effects and low cost. One of the spices that are widely grown in Indonesia and used for traditional medicine is white turmeric rhizome. White turmeric rhizome has a chemical content such as curcumin which can be used as an anticancer. The function of white turmeric rhizome as an anticancer is not selective and may interfere with the normal cell function that actively performs mutation such as seminiferous tubule cells can cause antifertility effect. To find out the antifertility effects of white turmeric rhizomes in the testis, a rat was examined by looking at the histology of testis due to the effects of white turmeric rhizome. The study was conducted for two months from April 2019 until June 2019. All experimental procedures were performed in accordance with the Medical Research Ethics Committee Faculty of Medicine University of Prima Indonesia. Research of the animals were carried out in experimental cages at the Pharmacology and Toxicology Laboratory, Faculty of Pharmacy, University of North Sumatera Medan, while testis histopathology was made in Department of Histology, Faculty of Medicine, University of North Sumatera Medan. This research used 24 white male Wistar rats (Rattus noverticus), with ages 6-8 weeks and weighing 160-200 grams. Samples were grouped randomly into 4 groups, each group consisting of 6 rats. Each group then was given ethanol extract of white turmeric rhizome obtained from UPT Materia Medica Batu, East Java, which was determined based on the method of calculating conversion factors with doses of ethanol extract of white turmeric rhizome 20, 40 and 60 mg / 200 gram rats and control groups. All data obtained then tested using the normality test and Shapiro-Wilk and the result is normal distributed data ($P > 0.05$), then continued with the variance analysis test (ANOVA) obtained p value <0.05. The results showed that there was a significant difference between the decrease in the number of spermatogenic cell layers and mitosis count due to the effects of white turmeric rhizome.

Keywords: white turmeric rhizome; curcuma zedoaria; curcumin; histology of testis.

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1. Introduction

Indonesia is a tropical country that is rich in medicinal plants. Traditional medicine is an alternative in medicine because of the minimal side effects and low cost. One of the spices that are widely grown in Indonesia and used for traditional medicine is white turmeric rhizome. White turmeric rhizome has a chemical content such as essential oils, curcumin, gum, resin, starch and tannins and can be used as anticancer, antibacterial, antithrombic, antifungal, antioxidant, hepatoprotective [1]. As the toxic effects of curcumin in-vivo is antifertility [2]. The function of white turmeric rhizome as an anticancer is not selective and may interfere with the normal cell function that actively performs mutation such as seminiferous tubule cells can cause antifertility effect [3]. To find out the antifertility effects of white turmeric rhizome on the testis, this study was conducted on mice [2]. This study was conducted to see the testis histology due to the effect of white turmeric rhizome [4].

Curcumin contained in the white turmeric rhizome has many functions including anticancer, anti-inflammatory, antihypertensive, antidiabetic, antimicrobial, immunodilator, antifungal, antiproliferation and nephroprotective [5]. Through various mechanisms, the role of curcumin with its chemoprotective and chemopreventive effects can stimulate antioxidant enzymes in various cell lines. Some in vitro studies have been conducted to determine the antioxidant content in turmeric rhizome, such as curcumin and its derivatives, demethoxycurcumin and bisdemethoxycurcumin [7,8,9]. Curcumin, which is the main element of turmeric, has an aspirin-like component. Curcumin is metabolized by conjugation and reduction pathways, with different results. What will be done in this study is the administration of white turmeric rhizome extract 10mg / 200gram orally [10,11]. On the basis of the research conducted by Siswanti T. et.al. and Handajani N.S., the researchers wanted to find out whether the white turmeric rhizome (Curcuma zedoaria) had an antifertility effect on seminiferous tubule cells in rat testis. By knowing the antifertility effects of white turmeric rhizome extract, it is expected to be able to think of the side effects [3].

2. Method and Material

This study was conducted for two months from April 2019 until June 2019. All experimental procedures were performed in accordance with the Medical Research Ethics Committee Faculty of Medicine University of Prima Indonesia. Research of the animals were carried out in experimental cages at the Pharmacology and Toxicology Laboratory, Faculty of Pharmacy University of North Sumatera Medan, while testis histopathology was made in Department of Histology, Faculty of Medicine University of North Sumatera Medan. This research used 24 white male Wistar rats (Rattus noverticus) with ages 6-8 weeks and weighing 160-200 grams. Samples were grouped randomly into 4 groups, each group consisting of 6 rats. Each group then was given ethanol extract of white turmeric rhizome obtained from UPT Materia Medica Batu, East Java, which was determined based on the method of calculating conversion factors with doses of ethanol extract of white turmeric rhizome 20, 40 and 60 mg / 200 gram rats and control groups. After research was done on the rats, euthanasia was done using ether in the fourth week. Surgery is done to take the testis organs. The testis that have been taken then were put into pots containing 10% formalin. After that, the renal histopathology preparations using Hematoxylin Eosin (HE) staining was carried out in the Department of Histology University of North Sumatra. From each organ, the testis tissue preparations were made and each of the preparations was observed under a light microscope at five

different views with 400x magnification. In each field of view, the damage to the seminiferous tubules were seen in the decrease of mitosis count and spermatogenic cell layers [5]. All data results were tested for normality test and using Shapiro-Wilk. If the data were normally distributed (P> 0.05), then followed by variance analysis (ANOVA) test to determine the effect of white turmeric rhizome extract on variations in dosage against histopathological changes in rat testis. If the variance analysis showed the significantly different results (P <0.05), then to find out the average difference in the results of observations in each treatment group compared using the Post-Hoc LSD test performed using SPSS (Statistical Package for the Social Sciences) 25.00 program for Windows [12,13].

3. Result and Discussion

The results of observations of rat testis spermatogenic cell layers can be seen in Figure 1. The rat testis spermatogenic cell layer in group C was 6.83 ± 0.307. In treatment groups by administering white turmeric rhizome doses of 20 mg / 200 grams, 40 mg / 200 grams of rats and 60 mg / 200 grams of rats (T1, T2 and T3) each showed a decrease in the spermatogenic cell layer to 5.83 ± 0.307, 4.83 ± 0.307 and 4.00 ± 0.365.

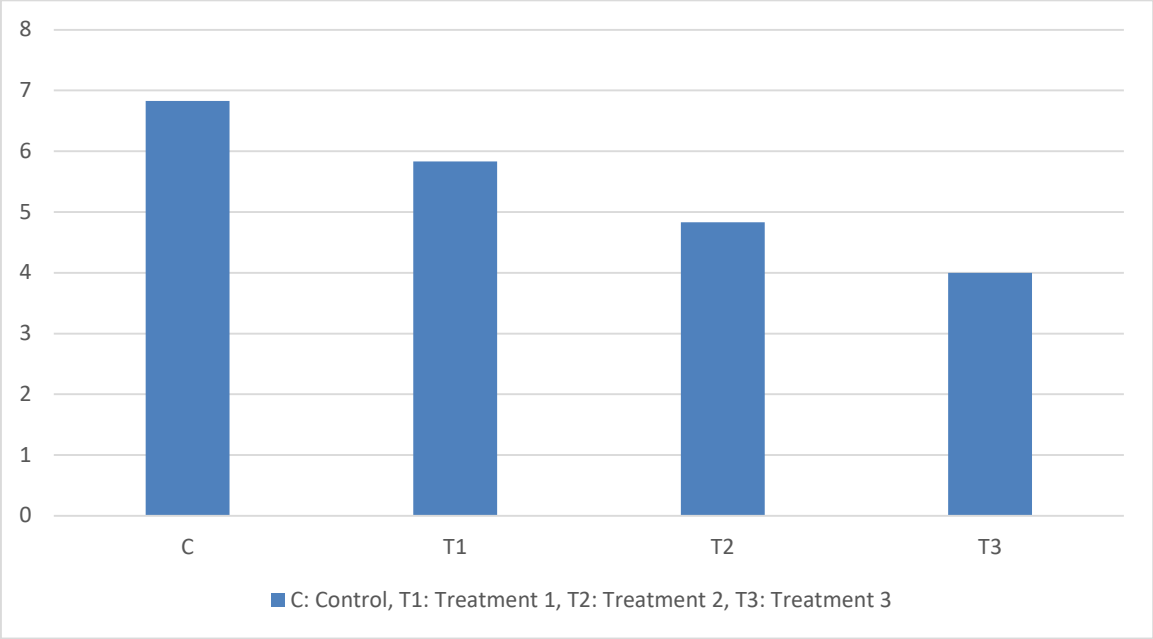

Figure 1: Number of spermatogenic cell layers of rat testis in each treatment

The results of the observation of mouse testis mitosis count can be seen in Figure 2. The mitosis count in the testis of rats in group C was 12.33 ± 0.955. In treatment groups by administering white turmeric rhizome doses of 20 mg / 200 grams, 40 mg / 200 grams of rats and 60 mg / 200 grams of rats (T1, T2 and T3) each showed the decreased mitosis count to 9.00 ± 0.516, 8.00 ± 0.447 and 6.50 ± 0.563.

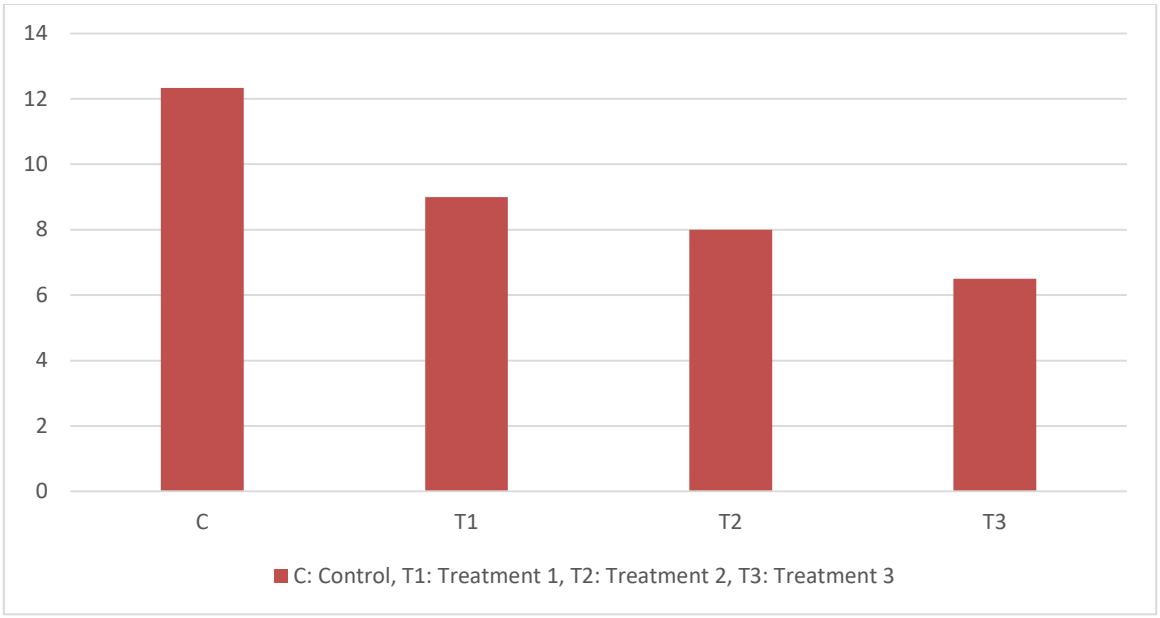

Figure 2: Number of mouse testis mitosis in each treatment

Based on the results of the research analysis, we found a decrease in the number of spermatogenic cell layers in groups T1, T2 and T3 compared to controls. This is consistent with the research conducted by Handajani (2003), Siswanti and his colleagues (2003) and Murphy and his colleagues (2012) who found that there would be a decrease in the number of spermatogenic cell layers by administering white turmeric rhizome. (Figure 3)

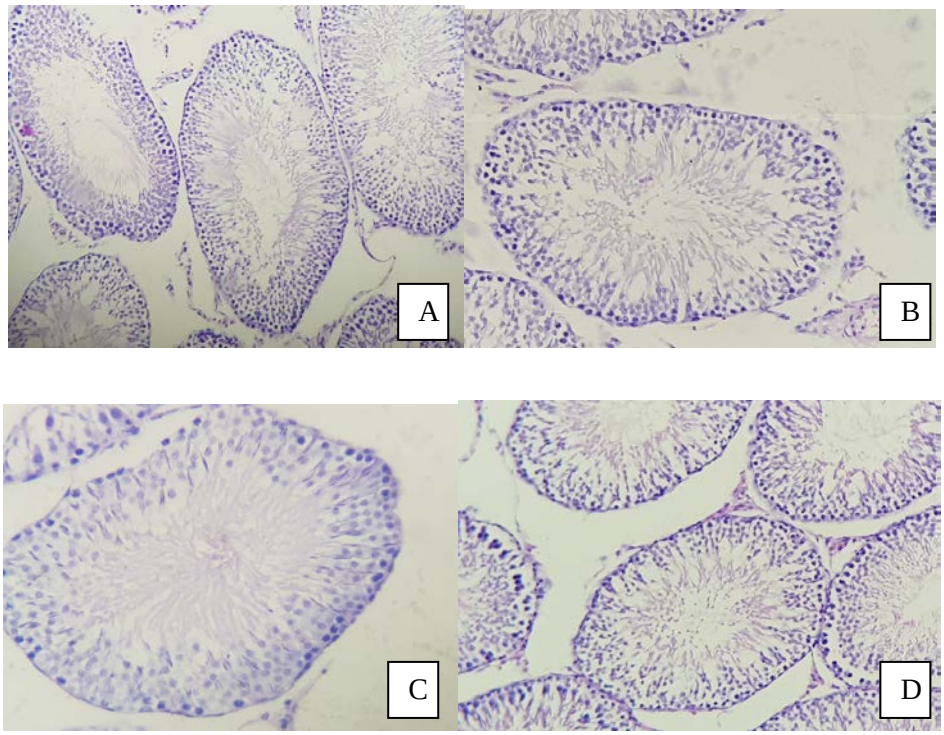

Figure 3: Cross section of the seminiferous tubules of the testes after administration of white turmeric rhizomes, HE staining, 400x magnification. A. Group C, B. Group T1, C. Group T2, D. Group T3.

Based on the results of the study analysis found a decrease in mitosis count in groups T1, T2 and T3 compared to controls. This is consistent with the research conducted by Handajani (2003) who found that there would be a decrease in mitosis count by administering white turmeric rhizome. The ANOVA result from the decrease in the number of spermatogenic cell layers and the decrease in mitosis count which gave a value of $p < 0.05$ showed that there was a significant difference between the decrease in the number of spermatogenic cell layers and a decrease in mitosis count with white turmeric rhizome. This is consistent with the research conducted by Handajani (2003), Siswanti and his colleagues (2003) and Murphy and his colleagues (2012) who found that there were significant differences in the decrease in the number of spermatogenic cell layers and / or a decrease in mitosis count by administering white turmeric rhizome. Post-Hoc LSD result from a decrease in the number of spermatogenic cell layers showed that group C had a significant difference with groups T1, T2 and T3 ($p < 0.05$), group T1 had significant differences with groups T2 and T3 ($p < 0.05$). Whereas in the LSD Post-Hoc test data decreased in mitosis count found that group C had a significant difference with groups T1, T2 and T3 ($p < 0.05$), group T1 had a significant difference with the T3 group ($p < 0.05$). In this analysis, it has not been compared because no results have been tested on the existing research.

4. Conclusion

Based on the results of the analysis and discussion of the study, it was concluded that there were significant differences between the decrease in the number of spermatogenic cell layers and mitoses count in the administration of white turmeric rhizome ($p < 0.05$). This shows that the administration of white turmeric rhizome (Curcuma zedoaria) containing curcumin has an antifertility effect on seminiferous tubule cells in testis of rats.

5. Suggestion

This study proved that the administration of white turmeric rhizome with a certain dose can cause a decrease in the spermatogenic cell layer and mitosis count, so it is expected that patient who will consume white turmeric rhizome as a herbal treatment must first consider this effect, especially for long-term consumption and further research is needed whether there are other effects of white turmeric rhizome on the testis or other organs.

Acknowledgements

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