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# Effect of Tuak Dayak on Testicular Organ Performance: In Vivo Study

**Usu Sius[1*], Elisebath Wahyu Savitri[1], Rizkie Woro Hastuti[2]**

[1]Dharma Nursing Academy, Pontianak, Indonesia

[2]Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia

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***Corresponding author:**
Usu Sius

**E-mail address:**
*ususius@akperdharmainsan.ac.id*

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**A B S T R A C T**

Tuak Dayak is an alcoholic drink that is often consumed by the people of West Kalimantan. Infertility is related to ROS levels as a result of alcohol oxidative stress. This study aims to determine the effect of tuak dayak on the 8-OHdG levels of semen and testicular weight. This research is an experimental study using experimental methods with a post-test-only randomized control group design. The research subjects were 25 rats, randomly divided into 5 groups. Group K0 was only given 3.6 mL/kgBW/day of distilled water, groups K1 and K2 were given Tuak Aren at doses of 1.8 and 3.6 mL/kgBW/day, and groups K3 and K4 were given Tuak Dayak at doses of 1.8 and 3.6 mL/kgBW/day. Treatment for 4 weeks. On day 29, semen and testes were taken. The testes are weighed with an electric weigher. Analysis of semen in the testes was performed to check the levels of 8-OHdG. The results of the Mann-Whitney test on K4 and K3 had no effect on the 8-OHdG content of cement. The average result in K4 was the number of Leydig cells 3.68±0.18, the thickness of the tubular tissue was 2.13±0.14µg, and the testicular weight was 0.91±0.09 g, the lowest. The average 8-OHdG content of cement was 0.22±0.01 ng/mL, the lowest was K2, and the highest was at K0 1.14±0.05 g/mL. In conclusion, Tuak Dayak had the effect of lowering testicular weight but did not show any effect on the activity of 8-hydroxy-2'deoxyguanosine (8-OHdG) in the semen of rats.

## 1. Introduction

Alcoholic beverages are one of the main sources of health problems in Indonesia. Alcoholic beverages are consumed by nearly two million Indonesians, with a dependence of nearly 1.1 million people.[1] West Kalimantan is one of the regions where the consumption of alcoholic beverages exceeds the national average.[2] Tuak Dayak is a traditional alcoholic drink formed from the fermentation process of black or white glutinous rice. This Tuak Dayak is believed to have benefits for the body, but the alcohol contained in liquor has been proven to be the cause of various diseases, disabilities, and death and even has an impact on the socio-economic life of the community.[3]

A study states that ethanol can cause a decrease in the weight of the testes of rats.[4] This shows that testicular cells are very reactive to the presence of alcohol and aldehydes in the body. About 75% of alcoholics will experience testicular atrophy. This atrophy is believed to have a direct effect on the testes. The accumulation of alcohol interferes with the synthesis of the hormone testosterone, which worsens the occurrence of liver cell damage, where liver cells play a role in the induction of the aromatase enzyme to form the hormone estradiol and the hormone estrone. Furthermore, the inhibition of synthesis also occurs in the 5 α-reductase enzyme, where this

enzyme plays a role in the conversion of 5 α-dihydrotestosterone into the hormone testosterone. The hormone testosterone plays an important role in the development and maturation of spermatozoa.[5,6]

Other studies have also shown that acetaldehyde, the product of alcohol metabolism, is more toxic and can damage cells. The presence of alcohol can also increase free radicals. Reactive oxygen species (ROS).[7] ROS has been shown to cause 45-50% of infertility and correlates with sperm deoxyribonucleic acid (DNA) damage through alcohol-induced oxidative stress induction, resulting in a decrease in sperm membrane glutathione. Then if the free radical hydroxyl group (OH-) interacts with cell membranes, it will produce malonaldehyde (MDA). Then if it interacts with purine bases of nuclear DNA or mtDNA, it will form 8-hydroxy-2'deoxyguanosine (8-OHdG). As a result of the above series, the integrity of the sperm DNA nucleus is difficult to maintain. Then, there are modifications to the nucleotide bases of DNA, single and double breaks of DNA strands, and chromatin cross-linking of DNA. This series of damage will activate tumor necrosis factor- (TNF-α) cytokines and phagocytes in the program of necrosis and apoptosis of testicular cells, which then leads to cell death.[7-9] This study aimed to assess the effect of Tuak Dayak on the testes in vivo. Evaluation of testicular weight and levels of 8-hydroxy-2'deoxyguanosine (8-OHdG) was the test parameters assessed in this study.

## 2. Methods

This study is an experimental study with a post-test-only design with a control group design. A total of 25 male rats aged 9-12 weeks with a body weight of 200-250 grams were used in this study. Rats were randomly divided into 5 study groups consisting of 1 normal group, 2 control groups, and 2 treatment groups. Group K0: given 3.6 mL/day of distilled water orally; K1: given Tuak Aren 1.8 mL/day + aquadest 1.8 mL/day orally; K2: given Tuak Aren 3.6 mL/day orally; K3: given Tuak Dayak 1.8 mL/day + aquadest mL/day orally, K4: given Tuak Dayak 3.6 mL orally. Adaptation of rats was carried out for 1 week with treatment for 4 weeks. The rats were fed pellets with the AD II brand produced by PT. Japfa Comfeed Indonesia, Tbk and given mineral water to drink. On the 29$^{th}$ day, semen was taken to check the 8-OHdG levels, and the testes were weighed using an analytical scale to calculate the average weight of the testes.

Data analysis was carried out with the help of SPSS version 25 software, where first, the normality test using the Shapiro Wilk test was said to be normal if ($p>0.05$), and the homogeneity test using the Levene test was said to be homogeneous if ($p>0.05$). Normal and homogeneous data used parametric test with one way ANOVA test to find out if there was a significant difference ($p < 0.05$), and to find out whether there was a significant difference between groups, a post hoc LSD test was carried out with a significance level ($p < 0.05$). For data that is not normal and or not homogeneous, the non-parametric test is used, namely the Kruskal Wallis test with a significance level ($p <0.05$). There was a significant difference in the data followed by the Mann-Whitney test with a significance level ($p<0.05$).

## 3. Results and Discussion

The 8-OHdG levels of cement in each group in this study showed the highest mean (1.14±0.05 ng/mL) in group K0, while the lowest average was found in group K2, namely (0.22± 0.01 ng/mL). Based on the results of the analysis of the normality of the distribution of data using the Shapiro Wilk test, it showed that almost all groups of 8-OHdG cement content had a normal distribution ($p>0.05$), except for K3 $p=0.025$ ($p<0.05$). The results of the analysis of the homogeneity of the data variance were analyzed by the Levene test, which resulted in a p-value of 0.003 ($p <0.05$), which means that the variance of the 8-OHdG cement content in the five groups was not homogeneous.

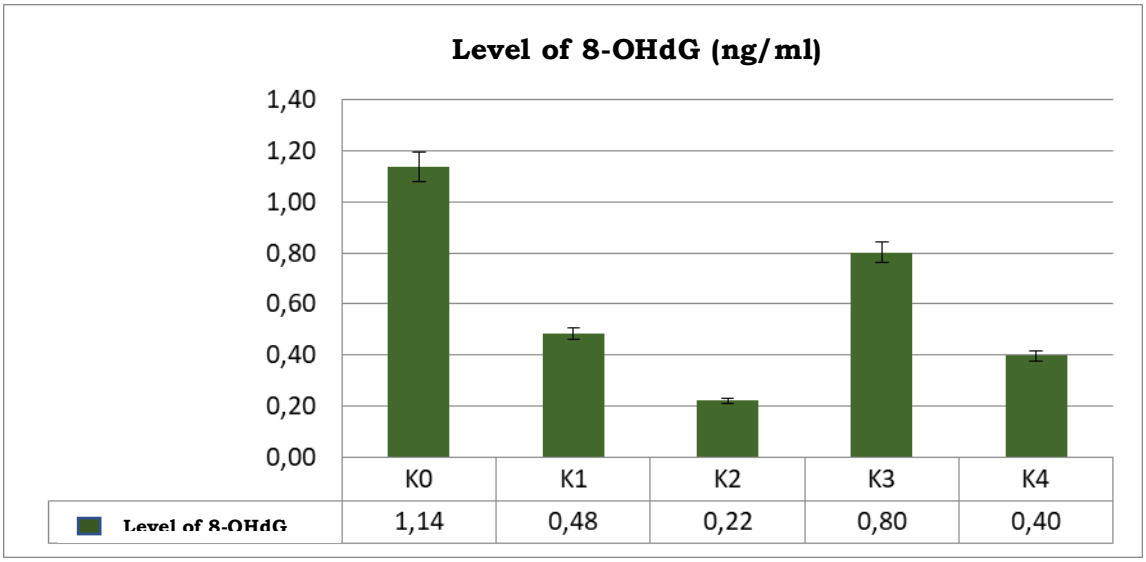


Figure 1. Graph of the average 8-OHdG content of cement between groups.

The results of the Mann-Whitney test showed that the comparison of the average 8-OHdG levels of cement between the two groups were all significant ($p<0.05$). Comparison of 8-OHdG levels of semen between the normal group (K0) and the treatment group (K3, K4) and the normal group (K0) and the control group (K1, K2) had a significant difference. Although there was a significant difference, Tuak Dayak given every day for 4 weeks had no effect on increasing the 8-OHdG levels of semen. Differences will be seen between the control group (K1, K2) and the treatment group (K3, K4), where Tuak Dayak has more effect than Tuak Aren on increasing the 8-OHdG content of cement.

Testicular weight in each group in this study showed that group K0 had the highest average testicular weight of 1.46±0.38, while group K4 had the lowest average testicular weight of 0.91±0.013. Based on the results of the analysis of the normality of the data distribution using the Shapiro Wilk test, it showed that almost all testicular weight groups had a normal distribution ($p>0.05$), except for K2 $p=0.011$ ($p<0.05$). The results of the analysis of the homogeneity of the data variance were analyzed by the Levene test resulting in a p-value of 0.057 ($p>0.05$), which means that the variance of the testicular weight data in the five groups was homogeneous. The requirements for the test for the mean difference of > 2 groups were not met parametrically (there was one group that was not normally distributed, although the data were all homogeneous), so the difference in the mean testicular weight in the five groups were analyzed non-parametrically with the Kruskal Wallis test. The significance of the difference in the mean testicular weight in the Kruskal Wallis test with p-value = 0.024 ($p <0.05$) was followed by a test of the mean testicular weight difference between the two groups using the Mann-Whitney test.

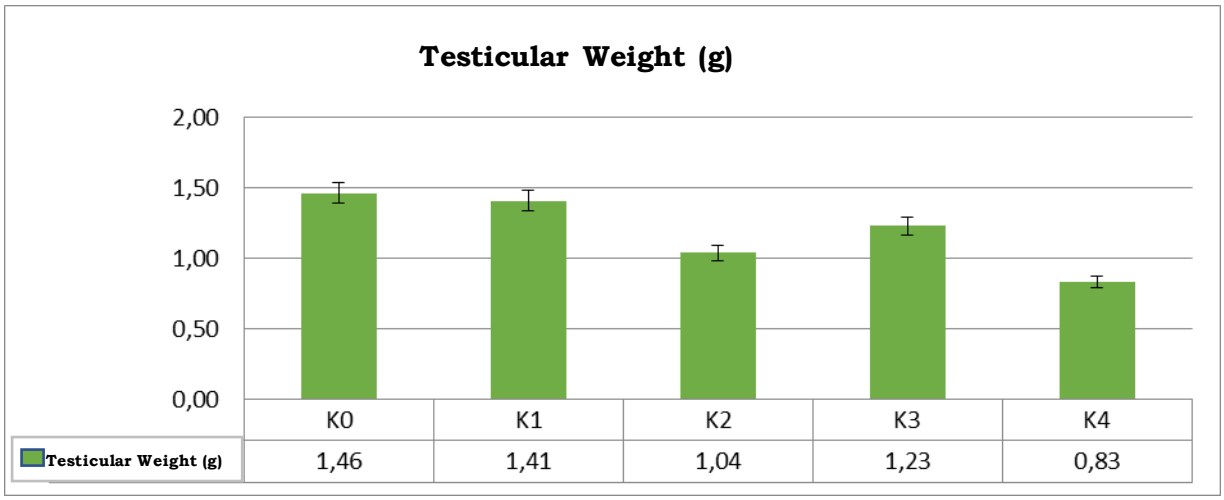


Figure 2. Graph of mean testicular weight.

The results of the Mann-Whitney test showed that the comparison of the average testicular weight between the two groups was five, which had significance ($p < 0.05$). Meanwhile, the comparison of the average testicular weight between the non-significant ones, namely K0 vs. K1, K0 vs. K2, K0 vs. K3, K2 vs. K3, K2 vs. K4 ($p > 0.05$). The comparison of testicular weight between K0 and K4 showed that the administration of tuak Dayak with a maximum consumption dose of 3.6 mL/kgBW/day for 4 weeks significantly reduced testicular weight. In comparison, the four groups that have an effect on lowering testicular weight sequentially start from K2, K3, K1, and K0.

The increase in ROS is related to the accumulation of alcohol in the body. If the presence of ROS due to oxidative stress, alcohol is not balanced with the number of antioxidants, it can disrupt cell integrity. Cell instability can be in the form of proteins, cell membranes, nuclear DNA, or mitochondrial DNA. Because 8-OHdG is a product of the oxidation of the hydroxyl group (OH) with a guanine base. Based on other studies, alcohol has a correlation between oxidative stress and DNA damage to cells in the testes that have an impact on impaired spermatogenesis and infertility. Therefore, to evaluate oxidative status, antioxidant defense system and DNA damage need to be carried out simultaneously with sperm parameters. Alcohol has also been reported to decrease the capacity of mitochondria to synthesize mitochondrial proteins due to changes in mitochondrial ribosomes that make them less functional. This leads to polypeptide depression associated with oxidative phosphorylation leading to enzyme inactivation. Ultimately, this results in numerous changes in mitochondria that can promote apoptotic and necrotic cell death, thereby contributing to the development of alcohol-induced testicular damage. In addition, reactive oxygen species (ROS) promote the activation of mitochondrial permeability transitions leading the cells to a pro-apoptotic pathway. Therefore, increased ethanol-induced cell apoptosis along with necrosis and suppression of cell proliferation may lead to testicular atrophy. Caspase-3 activation is one of the key points in the transmission of apoptotic signals because caspase-3 cleavage activity results in various morphological and biochemical types of apoptosis. When caspase-3 is active, it stimulates the release of PARP. The release of this PARP will degranulate cells and eventually be recognized by macrophages and phagocytosed. Therefore, to determine the presence of cell death, it is necessary to check the levels of 8-OHdG in other types of samples, namely urine. However, it is necessary to conduct further research on testicular cell death due to autophagy-mediated apoptosis and autophagy.[10-11]

The weight of the testes is the totality of the testicular organs. If there is a reduction in the number

of cells in the testes themselves, the weight of the testes will decrease automatically. The decrease in testicular weight as a result of this study was due to a reduction in the number of Leydig cells and thinning of the thickness of the seminiferous tubule tissue. Several previous studies have stated that testicular weight is an indicator of male fertility. However, this needs to be investigated further because the factors that can affect the results can be said to be less valid due to the way the testicles are taken, the presence of fat tissue, or the method of weighing/tools used. Therefore, to prove it is necessary to add other variables, namely sperm quality, and testosterone, as an indicator of cell damage in the testes. To prove that the testicular weight decreased, it was seen in the treatment group, especially the highest dose group (K4), where there was damage to Leydig cells and seminiferous tubule tissue. If the Leydig cells are damaged, the biosynthesis of the hormone testosterone decreases. The seminiferous tubules as the site of spermatogenesis will affect the spermatozoa produced.[12-15]

**4. Conclusion**

Tuak Dayak had an effect in lowering testicular weight but does not show an effect on the activity of 8-hydroxy-2'deoxyguanosine (8-OHdG) in the semen of rats.

**5. References**


1. Pribadi ET. Alcohol abuse in Indonesia: Determinant, SWOT and CARAT analysis. J Health Science Prev. 2017; 1(1).
2. Hanafi E, Siste K, Limawan AP, Sen LT, Christian H, et al. Alcohol and cigarette use related behaviors during quarantine and physical distancing amid COVID-19 in Indonesia. Front Psychiatry. 2021.
3. Sius U, Savitri EW, Sicily. Differences the effects of Tuak Dayak and Tuak Aren on the number of Leydig cells and seminiferous tubule thickness. J Phys: Conf Series. 2021: 1764.
4. Albano, E. Alcohol, oxidative stress, and free radical damage. Proc Nutrition Soc. 2006; 65(3):278-90.
5. Suwadi A, Agus D, Lembar A. Alcohol addiction and cognitive function among café visitors in South Jakarta. Althea Med J. 2019; 6(1).
6. Isaac U, Akpuaka F, Ndukwe G. The effect of intake of palm wine on body weight and testicular microarchitecture in adult wistar rats. J Exp Clin Anat. 2017; 16(1).
7. Perez-Gallardo RV, Briones LS, Diaz-Perez AL, Gutierrez S, Rodriguez-Zavala JS, et al. Reactive oxygen species production induced by ethanol in *Saccharomyces cerevisiae* increases because of a dysfunctional mitochondrial iron-sulfur cluster assembly system. FEMS Yeast Res. 2013;13(8): 804-19.
8. Yang CF, Zhong YJ, Ma Z, Li L, Shi L, et al. NOX4/ROS mediate ethanol-induced apoptosis via MAPK signal pathway in L-02 cells. Int J Mol Med. 2018; 2984.
9. Hosen MB, Islam MR, Begum F, Kabir Y, Howlader MZH. Oxidative stress-induced sperm DNA damage, a possible reason for male infertility. Iran J Reprod Med. 2015; 13(9):525-32.
10. Sun Q, Zhang W, Zhong W, Sun X, Zhou Z. Pharmacological inhibition of NOX4 ameliorates alcohol-induced liver injury in mice through improving oxidative stress and mitochondrial function. Biochim Biophys Acta. 2017; 1861:2912-21.
11. Sinha K, Das J, Pal PB, Sil PC. Oxidative stress: The mitochondria-dependent and mitochondria-independent pathways of apoptosis. Arch Toxicol. 2013; 87:1157-80.
12. Hoyt LR, Randall MJ, Ather JL, DePuccio DP, Landry CC, et al. Mitochondrial ROS induced by chronic ethanol exposure promote hyper-activation of the NLRP3 inflammasome. Redox Biol. 2017; 12:883-96.

13. Finelli R, Mottola F, Agarwal A. Impact of alcohol consumption on male fertility potential: a narrative review. Int J Environ Res Public Health. 2022; 19(1):328.
14. Vignera SL, Condorelli RA, Balercia G, Vicari E, Calogero AE. Does alcohol have any effect on male reproductive function? A review of literature. Asian J Androl. 2013; 15(2):221-5.
15. Bai S, Wan Y, Zong L, Li W, Xu W, et al. Association of alcohol intake and semen parameters in men with primary and secondary infertility: A cross-sectional study. Front Physiol. 2020: 566625.