The Effect of Mixed Liquor Administration on The Johnsen's Score and The Number of Sertoli Cells and Leydig Cells on The Wistar Strain White Rats (*Rattus norvegicus*)

Aqidah Khariri[1], Anny Setijo Rahaju[2,4], Arifa Mustika[3]

[1]Student, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [2]Lecturer, Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga Surabaya, Indonesia, [3]Lecturer, Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Airlangga Surabaya, Indonesia, [4]Lecturer, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

To analyze the effect of mixed liquor administration on the Johnsen's score and the number of Sertoli cells and Leydig cells on the Wistar strain white rats (*Rattus norvegicus*). A total of 28 rats were divided into 4 groups: C, P1, P2, and P3. Rats were given mixed liquor with different dose, namely P1 (1 ml), P2 (2 ml), and P3 (4 ml) each day for 14 days, then compared to C group which was given 4 ml of distilled water using an oral gavage. Mixed liquor consists of 20% ethanol and 4% methanol. The histopathological features were evaluated by the Johnsen's score, the number of Sertoli cells, and Leydig cells in cross-sectional preparation of rat testicular tissue with 400× magnification. Datas were analyzed using the Kruskal-Wallis test and One-Way ANOVA test with a confidence level of $p < 0.05$. The P3 group had the lowest Johnsen's score and the number of Sertoli cells, 6.442±0.293 and 5.942±0.674, respectively. A significant decrease in increased dose occurred in the Sertoli cell count but not in the Johnsen's score. Group P2 had the lowest number of Leydig cells, 6.421±0.360. The administration of mixed liquor caused a decrease in Johnsen's score and the number of Sertoli cells and Leydig cells on the Wistar strain white rat (*Rattus norvegicus*).

Keywords: *mixed liquor, spermatogenic cells, Sertoli cells, Leydig cells.*

Introduction

Alcoholic drinks or liquor are drinks that contain ethyl alcohol or ethanol (C_2H_5OH) which are known to cause addiction.[1] Addiction that is satisfied continuously will cause a tolerance effect, that is a desire to increase the dose in order to get the same effect.[1,2] This type of mixed alcoholic drink is often called mixed liquor or *miras oplosan* in Indonesian. Ethanol, which is commonly consumed has negative effect on the male reproductive organs and is associated with several incidence of infertility.[3,4] A study by Fauziah proves that *arak bali*, Balinese wine can reduce the number of spermatogenic cells and the size of the seminiferous tubules of mice.[5] In another study by Antari, *arak bali* also had an impact on lowering the quality of spermatozoa and testosterone levels.[6]

Methanol is one of the substances that is often added because it is effective to increase the effect of drunk.[7] Mixed liquor containing methanol is also reported to cause some death in several areas in Indonesia.[8,9] Ethanol is known to have an effect on male infertility, so the addition of methanol to mixed liquor may have more impact on male reproductive organs.[3,4,10]

Apart from the substance themselves, cell damage is also caused by metabolites of these substances.[11-14] The transformation of ethanol into acetaldehyde and free radicals is known to occur directly in the testes.[15,16] Cell damage by ethanol and methanol occurs due to increased levels of ROS, decreased GSH levels, and

Corresponding author:
Anny Setijo Rahaju
Email: anny_sr@fk.unair.ac.id

decreased ATP synthesis.[11-13] Therefore, this study aims to analyze the effect of mixed liquor administration on the Johnsen's score and the number of Sertoli cells and Leydig cells on the Wistar strain white rats (*Rattus novegicus*).

Materials and Methods

This study is an experimental laboratory study with post-test only control group design. This study used 28 male Wistar rats (*Rattus norvegicus*) which were divided into four groups. The treatment given was administration of mixed liquor with an oral gavage for 14 days to determine its effect on the histopathological features of rats' testes.

Mixed liquor was given at different doses in each group, namely 1 ml (P1), 2 ml (P2), and 4 ml (P3). The composition of mixed liquor was 20% ethanol and 4% methanol. The composition was determined based on the results of gas chromatography tests of mixed liquor samples.[17] This study used 28 rats in each group, so the total volume of mixed liquor to be prepared for 14 days was 14×[7(1+2+4)] = 686 ml. 1000 ml of mixed liquor was made to simplify calculations and anticipate spills. The mixed liquor was made by mixing 208.3 ml of 96% ethanol, 40.81 ml of 98% methanol, and 750.89 ml of distilled water. Assessment of the histopathological features was carried out by identifying spermatogenic cells, Sertoli cells, and Leydig cells referring to Pintus et al.[18] The data was analyzed by statistical software product and service solution 20 for Windows (SPSS 20).

Results and Discussion

The samples of this study were 28 adult male rats with 7 rats in each group. Observation of spermatogenic cells, Sertoli cells, and Leydig cells were made on both testes, so that each rat produced two data which were then averaged. The analysis was performed on 28 datas. The data of Johnnsen's score was an ordinal data, so non-parametric test (Kruskal-Wallis) was chosen. The result showed that the data of Johnnsen's score had significant difference (p=0.000). Therefore, the analysis was continued with Mann-Whitney test.

Table 1. Comparison of the Johnsen's score in each group.

Group	N	Mean±SD	*p*-Value
C	7	8.350±0.165	0.000*
P1	7	7.550±0.104	
P2	7	7.478±0.152	
P3	7	6.442±0.293	

*p <0.05, significantly different by statistic

Table 2. Mann-Whitney test analysis for the comparison of the Johnsen's score and the number of Leydig cells.

Comparison between Groups	C vs P1	C vs P2	C vs P3	P1 vs P2	P1 vs P3	P2 vs P3
p-value of Johnsen's score	0.002*	0.002*	0.002*	0.301	0.002*	0.002*
p-value of the number of Leydig cells	0.002*	0.002*	0.002*	0.083	0.442	0.025*

*p <0.05, significantly different by statistic

Table 3. Comparison of the number of Sertoli cells in each group.

Group	N	Mean ± SD	Normality	p-Value	Homogeneity
C	7	11.542 ± 0.401	0.380	0.000*	0.291
P1	7	9.657 ± 0.504	0.552		
P2	7	7.557 ± 0.53	0.793		
P3	7	5.942 ± 0.674	0.361		

Table 4. Post-hoc analysis for the comparison of the number of Sertoli cells.

Comparison Between Groups	Mean Difference	CI95%		*p*-Value
		Lower Bound	Upper Bound	
C vs P1	1.885	1.094	2.677	0.000*
C vs P2	3.985	3.194	4.777	0.000*
C vs P3	5.600	4.808	6.391	0.000*
P1 vs P2	2.100	1.308	2.891	0.000*
P1 vs P3	3.714	2.922	4.505	0.000*
P2 vs P3	1.614	0.822	2.405	0.000*

*p <0.05, significantly different by statistic

Table 5. Comparison of the number of Leydig cells in each group.

Group	N	Mean±SD	Normality	p-Value
C	7	11.378 ± 0.297	0.006	0.000*
P1	7	6.907 ± 0.511	0.806	
P2	7	6.421 ± 0.360	0.608	
P3	7	7.150 ± 0.621	0.304	

*p <0.05, significantly different by statistic

The Mann-Whitney test showed that the Johnsen's score of the control group (C) was significantly higher than the group receiving mixed liquor with different doses: 1 ml (P1), 2 ml (P2), and 4 ml (P3) with the value of p=0.002 on each compared group. So, all of the treatment groups (P1, P2, and P3) had significant reduction in Johnsen's score compared to the control group. Besides, the significant reduction was also found in the increasing dose from P1 group to P3 group (7.550 vs 6.442; 0.002); and from P2 group to P3 group (7.478 vs 6.442; 0.002), but there was no significant reduction from the P1 group to P2 group (7.550 vs 7.478; 0.301).

The data normality of the number of Sertoli cells was tested by Shapiro-Wilk test; the result showed that each group was normally distributed (p >0.05). The analysis was continued by the One-Way ANOVA test and showed a significant difference in the number of Sertoli cells (p=0.000). The homogeneity test was also conducted to determine the selection of the post-hoc test method; the result showed that the data was homogeneous. Therefore, the analysis was continued with the post-hoc test.

The post-hoc test showed that the number of Sertoli cells of the control group (C) was significantly higher than the group receiving mixed liquor with different doses with the value of p=0.000 on each compared group. So, all of the treatment groups (P1,P2, and P3) had significant reduction in the number of Sertoli cells compared to the control group (C). Besides, the significant reduction was also found in the increasing dose from P1 group to P2 group (9.657 vs 7.557; 0.000); from P1 group to P3 group (9.657 vs 5.942; 0.000); and from P2 group to P3 group (7.557 vs 5.942; 0.000).

The data normality of the number of Leydig cells was tested by Shapiro-Wilk test; the result showed that the control group (C) was not normally distributed (p=0.006) and the remaining groups were normally distributed (p >0.05). The analysis was continued by the non-parametric test (Kruskal-Wallis) and showed a significant difference in the number of Leydig cells (p <0.05). Therefore, the analysis was continued with the Mann-Whitney test.

The Mann-Whitney test showed that the number of Leydig cells of the control group (C) was significantly higher than the group receiving mixed liquor with different doses with the value of p=0.002 on each compared group. So, all of the treatment groups (P1,P2, and P3) had significant reduction in the number of Leydig cells compared to the control group (C). But, there were no significant difference in the increasing dose between P1 group and P2 group (6.907 vs 6.421; 0.083); and between P1 group and P3 group (6.907 vs 7.150; 0.442). There was significant difference between P2 group and P3 group (6.421 vs 7.150; 0.025) but P3 group was higher than P2 group and also P1 group.

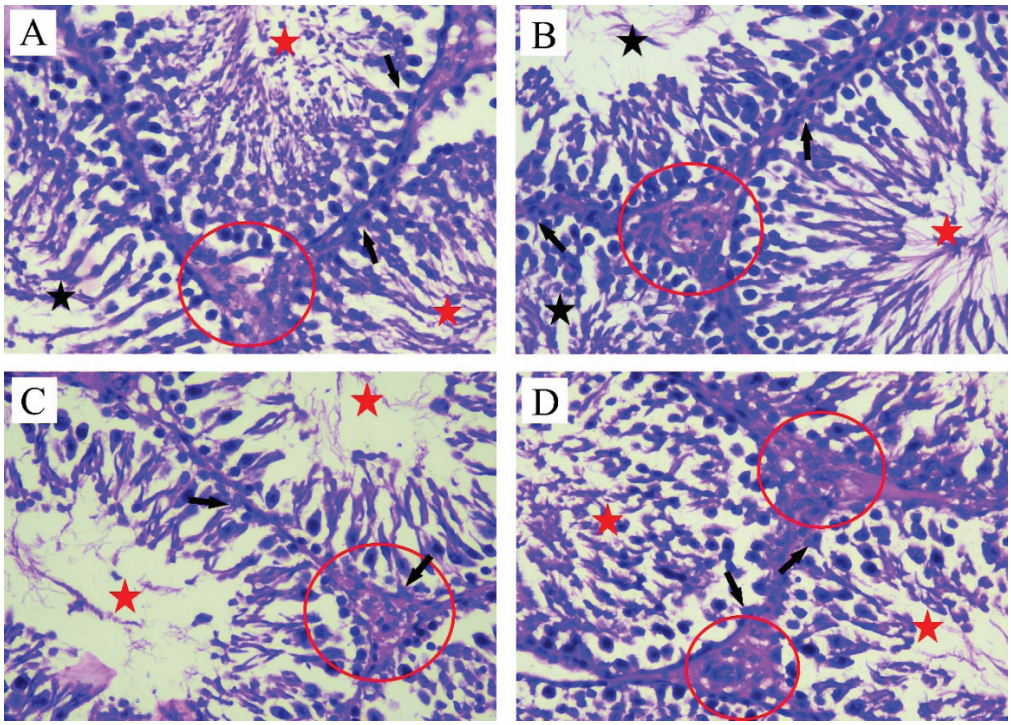

Figure 1. Histopathology of the cross-section of seminiferous tubules. A: C (control) group; B: P1 group; C: P2 group; D: P3 group at 400× magnification. The Johnsen's score for each tubule was dominated by: A. Score of 9 (red star) and 8 (black star); B. Score of 8 (red star) and 7 (black star); C. Score of 7 (red star); D. Score of 7 (red star). Black arrow: Sertoli cells. Red circle: Leydig cells on intertubular region.

The study showed that there was a significant decrease of the Johnsen's score in the treatment group (P1, P2, and P3) compared to the control group (C) (p <0.05). It means that mixed liquor administration can reduce spermatogenesis activity and the number of spermatogenic cells, as evidenced by a decrease in the Johnsen's score at the increasing doses.[19] Increasing dose of mixed liquor can increase the testicular damage of rats.[20] This study found that there was no significant decrease in the Johnsen's score from the P1 to P2 group. In addition, the spermatogenic cells were still not protected by antioxidants from the toxic effects of ethanol.[21]

The decrease in the number of Sertoli cells per tubule in the increasing of dose was found to be significant (p <0.05). These result is in line with a study by Figueiro et al. that there was a significant decrease in the number of Sertoli cells in all treatment groups of mice that were given ethanol.[22] The Sertoli cells are part of the testicular blood barrier (BTB), which separate the basal compartment and the adluminal compartment.[23] BTB is a collection of protein structures consisting of several types of cellular junctions.[23] The testes are organs that are susceptible to alcohol because alcohol can penetrate the BTB.[24] This can occur because the structural proteins that make up the tight junction are interfered due to alcohol.[25]

This study found a significant decrease in the number of Leydig cells on the treatment group (P1, P2, and P3) against the control group (C) (p <0.05), while there was no significant decrease (p >0.05) between treatment groups (P1, P2, and P3) as the dose increased. Several studies showed that there was a decrease in testosterone levels in the ethanol-treated group.[20,21] A decrease in testosterone levels was followed by an increase in LH levels after ethanol administration for 4 weeks as a compensatory feedback.[21,26] In this study, there was a decrease in the number of Leydig cells from P1 to P2 group. Moreover, the P3 group had a higher number of Leydig cells than the P1 group. It might be due to increase in LH level that stimulates Leydig cell progenitor proliferation.[27]

Conclusion

The administration of mixed liquor caused a decrease in Johnsen's score and the number of Sertoli cells and Leydig cells of the Wistar strain white rat (*Rattus norvegicus*).

Conflict of Interest: The authors declare that they have no conflict of interest.

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