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Submission date: 28-Nov-2022 03:24PM (UTC+0800)
Submission ID: 1964980913
File name: Histopathology_after_mixed_liquor_consumption_in_Wistar_rat.pdf (974.83K)
Word count: 2928
Character count: 15421

GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

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GSC Biological and Pharmaceutical Sciences, 2022, 20(03), 324–329

Publication history: Received on 08 August 2022; revised on 18 September 2022; accepted on 20 September 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.20.3.0354

Abstract

In Indonesia illegal traditional liquor mostly contain ethanol and methanol some of illegal liquor Indonesia called cukrik (mixed liquor), Misuse of mixed liquor can lead to intoxication and may lead to death. Several studies have shown its effect on kidney damage. This study is to analyzing the effect of mixed liquor (ethanol and methanol) on the histopathological damage of male Wistar rat kidney. This was experimental laboratories using a post-test only control group design. We are using 28 wistar rats that were divided into four groups, each group consisting of 7 rats. Group control (C) was given 4 mL of distilled water; groups P1, P2, and P3 were given 1ml, 2 ml, and 4 ml cukrik in orally for 14 days. Mixed alcohol contains 4% methanol and 20% ethanol. Examination of histopathological features was carried out by identifying glomerular cells, tubular cells, and interstitial with HE (Hematoxylin and Eosin) stainning using EGTI (Endothelial, Glomerular, Tubular, and Interstitial) scoring. Data were analyze using kruskal wallis and Mann whitney test. With confidence level p<0,01. glomerular and tubular examination there were significant differences with p<0,01, in interstitial examination there was no significant damage found on the instrestitial with p<0,01. Consumption of cukrik produced glomerular and tubular damage on the histopathological image of male Wistar rats' kidneys, but there was no visible damage to the interstitials cell. With higher consumption of mixed liquor will make tubular and glomerular damage heavier.

Keywords: Liquor; Cukrik; Kidney; Tubular; Glomerular; Interstitial

1. Introduction

Liquor is a drink that contains ethanol with causes addiction [1]. Addiction is a complex state that affects brain function and behavior but is treatable [2]. Mixed liquor (cukrik) is a traditional drink that usually for the percentage content of alcohol is not too high, but the dangerous thing about cukrik is that it contains methanol. The market price of cukrik is lower compared to the cost of legal alcohol sold in the market therefore, a lot of people are poisoned by cukrik [1].

Ethanol is a compound that is colorless, has a distinctive odor, and usually ethanol is made for general anesthesia, but ethanol is also used to become alcoholic drinks that are legally sold in the market [3]. Methanol is known as a substance that is similar to ethanol but is more dangerous than ethanol. Methanol has several properties such as a colorless liquid with a strong odor which is usually used for extraction and is poisonous to the human body but many people abuse it to make drinks because the price is lower than legal drinks [4].

The danger of ethanol also causes cell damage caused by the metabolism in ethanol, ethanol will be metebaolism into fatty acid ethyl esters ,which will cause damage to tissues [5]. Methanol has the same as ethanol but is more dangerous,

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methanol will be metabolized by the body into formaldehyde which will then be converted into formic acid which ultimately results in oxidative stress and increases ROS(Reactive Oxidative Species) levels. High ROS can cause apoptosis in cells in the body [6]. This study aims to determine the effect of cukrik on tubular, glomerular, and interstitial damage.

2. Material and methods

This study is an experimental study using a post-test only control group design. This research was conducted in October 2019 at Department of Pharmacology and Department of Pathology Anatomy, Faculty of Medicine, Universitas Airlangga. This study used 28 male wistar rats which were divided into four groups. The treatment for male wistar rats was giving cukrik to wisrar rat for 14 days with feeding tube. This study used 28 rats which were divided into 4 groups, namely the control group and 3 treatment groups. Treatment group 1 (P1), Treatment Group 2 (P2), and Group 3 (P3) were given a mixture of methanol and ethanol respectively for 14 days. The total volume of the liquor mixture to be prepared for 14 days is 14 x [7 (1 + 2 + 4)] = 686 ml. 1000 ml mixed liquor is designed to simplify counting and anticipate spills. The drink mixture was made by mixing 208.3 ml 96% ethanol, 40.81 ml 98% methanol, and 750.89 ml distilled water.

In this study, the assessment used for reading tubular, glomerular, and interstitial histopathies was using EGTI (Endothelial, Gromerular, Tubular, and Interstitial) scoring system [7]. Data analysis of this research uses the application of statistical software product and service solution 22 for Windows (SPSS 22)(Statistical Product and Service Solution)

3. Results

The samples of this study were 28 adult male wistar rats with 7 rats in each group. Observation of glomerular cells, tubular epithelial cells, and interstitial cells were made on both kidney. The analysis was performed on 56 data. The data of EGTI score was an ordinal data, so non-parametric test (Kruskal-Wallis) was chosen. The result showed that the data of EGTI score had significant difference (p<0,01). Therefore, the analysis was continued with *Mann-Whitney* test.

Table 1 Glomerular comparison using EGTI scoring

Group	N	Median ± SD	Minimum	Maximum
Control	14	0.00 ± 0.53	0	0.2
Treatment P1	14	0.40 ± 0.22	0	0.8
Treatment P2	14	0.60 ± 0.19	0.2	1
Treatment P3	14	0.60 ± 0.26	0.4	1.2

Based on table 1, the results of this study are median results obtained from the assessment of EGTI scoring in glomerular histology showed differences in damage to the treatments P1, P2, and P3. In this observation, the largest median value was seen in treatment P3.

Table 2 Tubular comparison using EGTI scoring

Group	N	Median ± SD	Minimum	Maximum
Control	14	1.00 ± 0.27	0	1
Treatment P1	14	1.00 ± 0.00	1	1
Treatment P2	14	2.00 ± 0.00	2	2
Treatment P3	14	2.00 ± 0.00	2	2

Based on table 2, the results obtained from the assessment of EGTI scoring in kidney tubular histopathology showed an increase in the median value of tubular damage compared to the control group. But in treatments group P2 and P3, the median value is the same. The greatest damage was seen in the treatment groups P2 and P3.

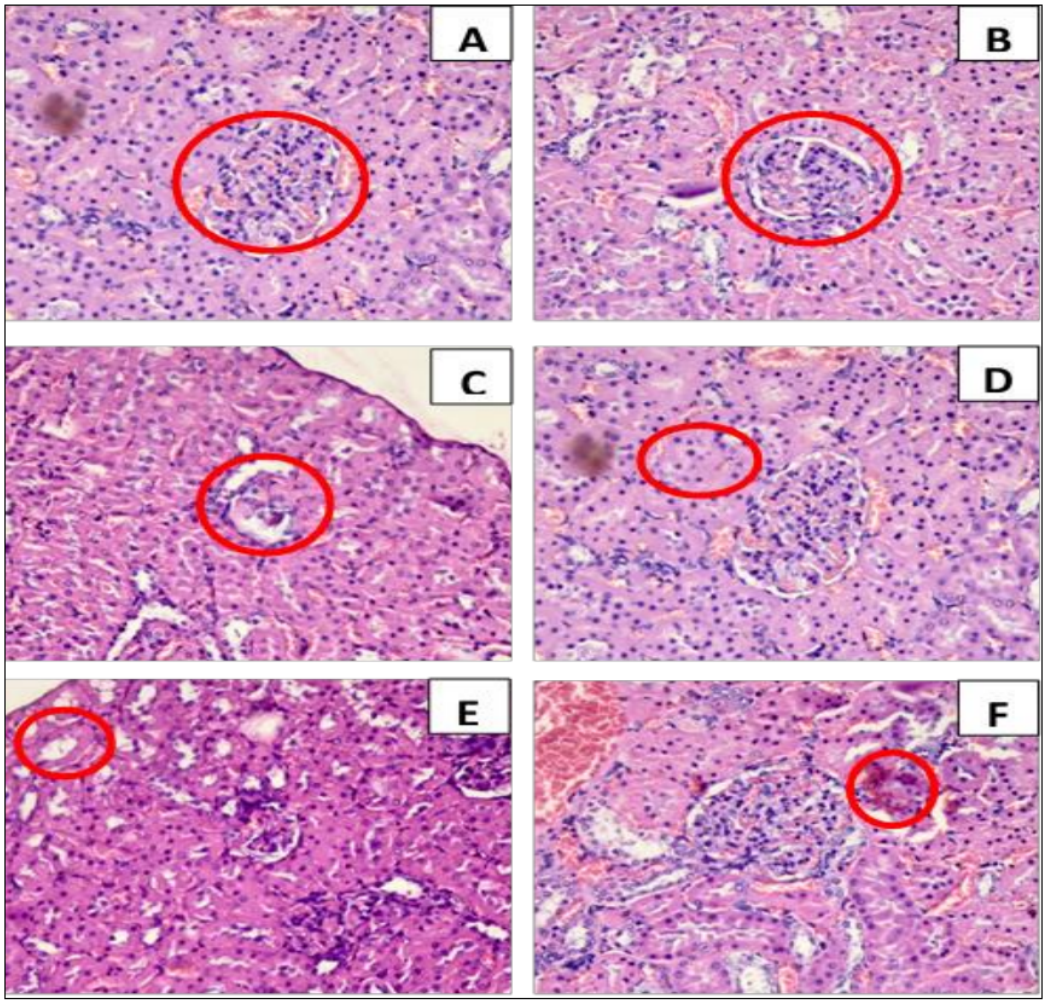

Figure 1 Histology of rats kidney with HE (Hematoxylin and Eosin) stain using 400x magnification. Normal gromerular (A), Thickening of bowman capsule in the glomerular of the kidney (B), retraction of glomerular tuft of the kidney (C), normal tubular (D), Tubular damage with loss brush border of the kidney (E), interstitial damage is indicated by bleeding in the interstitial (F)

Table 3 Insterstitial comparison using EGTI scoring

Group	N	Median ± SD	Minimum	Maximum
Control	14	0.00 ± 0.47	0	1
Treatment P1	14	0.00 ± 0.50	0	1
Treatment P2	14	1.00 ± 0.51	0	1
Treatment P3	14	0.00 ± 0.50	0	1

Based on table 3, the results obtained from the assessment of EGTI scoring on the interstitial increased damage compared to the control group. But in the treatment group of P1 and P3, the same median value was seen. The greatest damage was seen in treatment group P2.

Table 4 Mann Whitney non-parametric test for glomerular, tubular, and Interstitial histopathology features using EGTI Scoring

Comparison Between Groups	p-value		
	Glomerular	Tubular	Interstitial
C vs P1	0.000	0.317	0.691
C vs P2	0.000	0.000	0.134
C vs P3	0.000	0.000	0.691
P1 vs P2	0.006	0.000	0.264
P1 vs P3	0.007	0.000	1.000
P2 vs P3	0.809	1.000	1.000

The results of glomerular histopathological non-parametric *Mann-Whitney* test using EGTI scoring, found that there were significant differences between the control group with the treatment groups P1, P2 and P3 where $p < 0.01$. There was also a significant difference between the treatment group P1 and treatment groups P2 and P3 with $p < 0.01$. Likewise, there were also significant differences between the treatment groups P2 and P3 where $p < 0.01$.

Tubular histopathological non-parametric *Mann-Whitney* test results using EGTI scoring, found that there were significant differences between the control group with treatment groups P2, and P3 where $p < 0.01$. There was also a significant difference between the P1 treatment group and P2 and P3 treatment groups with $p < 0.01$. In the control treatment with P1 treatment group and P2 treatment group with P3 treatment group, there was no significant difference where $p > 0.01$.

The results of the nonparametric *Mann-Whitney* histopathology interstitial test using *EGTI scoring* found that there was a difference but not significant between the control group with the treatment groups P1, P2, and P3 where $p > 0.01$. There was also an insignificant difference between treatment group P1 and treatment groups P2 and P3 with $p > 0.01$. There was no significant difference between treatment groups P2 and P3 where $p > 0.01$.

4. Discussion

The results showed that giving cukrik could damage the rats' kidney glomerulus. In accordance with the results of giving alcohol to 20% ethanol and 4% methanol at a dose of 1 ml, 2ml, 4ml in each treatment group found significant differences in damage between groups. This is the same as the kidney tubular, there was significant different between the control group and the P2 and P3 treatment groups, while in the control group and the P1 group there was no damage.

In lfina study who researched Spraque Dawly rats which got the effect of giving a mixture of essential oil of tuber grass and ethanol 43%. In that study, there was a significant increase in glomerular and tubular damage along with an increase in the number of essential oils and ethanol [8]. This is also in line with Zeni Vania study on the effects of 40% ethanol administration orally in adult male Wistar rats, glomerular enlargement and glomerular atrophy [9].

This is also in line with Raju study which examined male rats by administering 10% ethanol and 20% at a dose of 1 ml/day for 48 days, found differences in the increasingly severe damage to the kidney tubular parts in line with alcohol concentration [10]. This also occurred in the study of Brzoska et al., 2012 in rats given ethanol at 10% levels for 12 weeks showing signs of hypertrophy damage and degeneration of the tubular epithelial kidney [11]. In the study of Latchoumycandane et al., 2020 did it in rats with 17% ethanol in the first 2 days and continued with 6.4% ethanol for 4 weeks. Histopathological examination also found damage to the kidney tubular [12].

In the study of Verhelst which examined patients with methanol poisoning through medical records recorded in 1987 - 2001. It was found that there were hydropic changes in tubular cells [6]. Unlike the case with Wibowo's study, 2012 rats given formalin were divided into 3 treatment groups with formaldehyde levels of 50, 100, 200 mg/kg BW for 3 months. The results obtained with histopathological features found the most severe tubular damage at a dose of 200 mg/kg body weight but no significant differences were found between each group [13].

This study is also similar to Kawiratha which examined male Wistar rats given 40% methanol and 20% ethanol with a dose of 1.2 ml ethanol and 0.2 ml methanol with differences in the duration of administration. In this study, it was found that the longer the administration, the more damage of glomerular dand tubular [14]. Comparing the research we made with lower methanol levels (4%) and the same ethanol 20% showed that a low dose of methanol showed significant glomerular kidney damage. This can explain how dangerous the use of mixed alcohol ethanol and methanol.

Different from the others the interstitial results showed that the administration of cukrik did not find significant damage to the kidney interstitials, there was no significant difference between the control group and the treatment group. In the Fruchter study, 2011 it was found that in 17-year-old men who were exposed to excessive alcohol (ethanol) intoxication coupled with NSAID (Non-steroidal anti-inflammatory drugs) administration can cause (Acute Interstitial Nephritis). But in this study, there are data about the increase in damage with the addition of consumption [15].

In this study, it was obtained by administration of mixed alcohol with levels of 20% ethanol and 4% methanol with differences in the dose of 1 ml, 2 ml, and 4 ml, it was found that the damage was more influential on the existing glomerular and tubular damage which can be seen from the results of statistical tests and previous literature. However, in the interstitial difference, the statistical test found no significant difference between the control group and the treatment group and a lack of literature that discusses damage of interstitial that occurs with alcohol intoxication. Damage between 4% of methanol and 20% of ethanol occurred in the glomerular and tubular kidneys. Further research is needed to see the amount and level of alcohol which can also damage the interstitial kidneys.

There are some limitations on this research, which is the difficulty of the researcher to examine glomerular histology because the stain researcher use is HE (Hematoxylin and Eosin) staining. the recommend examination a histopathological image of the kidney using special stain, because special stain is useful for the detailed analysis of the glomerular structure.

Abbreviations

- ROS: Reactive Oxidative Species
- EGTI: Endothelial, Glomerular, Tubular, and Interstitial
- HE: Hematoxylin and eosin
- SPSS: Statistical Product and Service Solution
- NSAID: Non-steroidal anti-inflammatory drugs

5. Conclusion

The consumption of cukrik resulted in glomerular and tubular damage in the histopathological features of the kidneys of male wistar rats, but no visible damage to interstitial cells. Higher consumption of cukrik will exacerbate tubular and glomerular damage.

Compliance with ethical standards

Acknowledgments

We would like to express our sincere gratitude to all doctors and staff in Department of Pharmacology and Department of Pathology Anatomy, Faculty of Medicine, Universitas Airlangga. who help made this research complete.

Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

The animal studies followed an approved procedure with the number: 183/EC/KEPK/FKUA/2020 by Health Research Ethics Committee Universitas Airlangga School of Medicine, Surabaya, Indonesia.

References

[1] Mulyadi M. Darurat Miras Oplosan. Pusat Penelitian Badan Keahlian DPR RI. 2014, 6(24): 9-12.

[2] [Internet]. 2020 [cited 22 January 2020]. Available from: https://www.psychiatry.org/patients-families/addiction/what-is-addiction

[3] Shakhashiri. Ethanol [Internet]. Scifun.org. 2009 [cited 22 January 2020]. Available from: http://www.scifun.org/chemweek/pdf/ethanol.pdf

[4] Methanol [Internet]. Pubchem.ncbi.nlm.nih.gov. 2019 [cited 4 June 2019]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Methanol

[5] Zakhari S. Overview: How Is Alcohol Metabolized by the Body? [Internet]. PubMed Central (PMC). 2022 [cited 28 March 2020]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6527027/

[6] Verhelst, D., Moulin, P., Haufroid, V., Wittebole, X., Jadoul, M. and Hantson, P., Acute Renal Injury Following Methanol Poisoning: Analysis of a Case Series. International Journal of Toxicology, 2004, 23(4), pp.267-273.

[7] Khalid U, Pino-Chavez G, Nesargikar P, Jenkins R, Bowen T, Fraser D et al. Kidney ischaemia reperfusion injury in the rat: the EGTI scoring system as a valid and reliable tool for histological assessment. 2019.

[8] Ulfina, N. and Wulan, J., Effects of Administration of Essential Oil from Teki Grass Bulbs (Cyperus rotundus L.) on Histopathological Features of Ethanol-induced White Rat (Rattus norvegicus) Sprague dawley strain. Majority, 2018, 7(3), pp.99-105.

[9] Vania Z. 2016. The Effect Of 40% Alcohol Administration Orally Towards Kidney Histopathology Imaging In Male Wistar Mice. Universitas Kristen Maranatha

[10] Raju, D. A Study of Lipid Profile and Lipid Peroxidation in Chronic Kidney Disease with Special Reference to Hemodialysis,. Journal of Clinical Research & Bioethics, 2013, 04(01).

[11] Brzoska, M., Moniuszko-Jakoniuk, J., Pilat-Marcinkiewicz, B. and Sawicki, B., Liver And Kidney Function And Histology In Rats Exposed To Cadmium And Ethanol. Alcohol and Alcoholism, 2003, 38(1), pp.2-10.

[12] Latchoumycandane C, Nagy L, McIntyre T. Chronic ethanol ingestion induces oxidative kidney injury through taurine-inhibitable inflammation. Free Radical Biology and Medicine. 2014;69:403-416.

[13] Wibowo M, Suharto G, Megawati A. The Effect of Oral Formalin in Staged Doses for 12 Weeks on Histopathological Appearance of Wistar Rat Kidneys. Diponegoro Medical Journal, 2012.

[14] Kawiartha, M., Normasari, R. and Hermansyah, B., Effects of Oplosan Alcohol on Histopathological Changes in Wistar Rat Renal Organs Jantan. e-Jurnal Pustaka Kesehatan, 2018, 6(3), pp.393-397.

[15] Fruchter, L., Alexopoulou, I. and Lau, K., Acute interstitial nephritis with acetaminophen and alcohol intoxication. Italian Journal of Pediatrics, 2011, 37(1), p.17.

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