The Effect of Alcohol Drink Consumption on Mice (*Mus muculus*) Liver Damage

Ersalina Nidianti [1]*, *Ary* Andini [1], *Mutiara Alifiansyah Kidang* Pinangkas [1], *Muhammad Afwan* Romdloni [1]

[1]Department of Medical Laboratory Technology, Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Jawa Timur, Indonesia
*Corresponding Author: E-mail: ersalinanidianti@unusa.ac.id, aryandini@unusa.ac.id, mutiaraalfiansyah061.nk20@student.unusa.ac.id m.afwanromdloni@unusa.ac.id

Introduction: Consuming alcoholic beverages can cause someone to become addicted to alcohol, because alcoholic beverages are addictive substances that have a negative impact on health if consumed regularly. Excessive consumption of alcoholic beverages can cause liver damage, heart damage, stroke, high blood pressure, digestive tract cancer, memory loss and confusion. This study aims to determine the effect of alcoholic beverages on liver damage in mice. **Methods**: The Type of research is experimental with a post-test only control group design. This study used 30 mice divided into 6 groups. Group 1 as a negative control (non treatment), group 2 as a positive control (70% ethanol), group 3 as a standard (CCl_4), group 4 was given 0.3 ml of alcohol drink code 1 treatment, group 5 was given 0.5 ml of alcohol drink code 1 treatment, and group 6 was given 0.7 ml of alcohol drink code 1 treatment orally for 14 days. The 15th day continued with surgery and blood sampling through the heart, then AST and ALT levels were measured. **Results**: The average AST results per group, in the negative control group were 78.52 U/L, the positive control group were 98.73 U/L, the standard group were 107.75 U/L, treatment group 1 were 80.21 U/L, treatment group 2 were 89.54 U/L, and treatment group 3 were 109.84 U/L. While the average ALT per group, in the negative control group were 88.66 U/L, the positive control group were 115.18 U/L, the standard group were 116.56 U/L, treatment group 1 were 91.41 U/L, treatment group 2 were 101.4 U/L and treatment group 3 were 130.2 U/L. **Conclusion**: There was no significant effect of consuming alcoholic beverages on liver damage as measured by AST and ALT levels.

Keywords: Alcoholic beverages; Liver damage; AST levels, ALT levels

1 Introduction

Alcohol consumption is increasing across the world and also in Indonesia. Between 1990 and 2017, the total amount of alcohol consumed grew from 21 billion litres to 35.7 billion litres. According to the 2018 Basic Health Research (Kemenkes, 2018), 3.3% of Indonesians consumed alcoholic beverages (Arfines, 2022). Excessive consumption of alcoholic beverages can lead to addiction, as they contain addictive substances. Beyond addiction, alcohol can also cause serious health issues such as liver damage, heart problems, stroke, high blood pressure, digestive tract cancer, memory loss, and confusion.

Liver damage is one of the most common diseases caused by excessive alcohol consumption. This is largely due to the production of Reactive Oxygen Species (ROS), which increase significantly with alcohol intake and are considered a major contributor to alcohol-induced liver injury. Alcohol disrupts the liver's normal function, leading to swelling caused by elevated levels of transaminase enzymes (Zhaou et al., 2017). The development of liver disease is also linked to fat accumulation in the liver, which results from a combination of impaired fat oxidation and increased fat production (lipogenesis). These changes are triggered by alterations in the liver's NADH/NAD^+ redox balance and by disrupted activity of transcription factors that regulate gene expression related to fat metabolism (Putra,2016).

Damage to the liver mechanism causes swelling due to an increase in the transaminase enzyme produced in the liver. To see an increase in the transaminase enzyme, use the Serum Glutamic Pyruvic Transaminase (SGPT) or commonly called Alkaline Phosphatase (ALP) and Serum Glutamic Oxaloacetic Transaminase (SGOT) examination, also known as Aspartate Transaminase (AST). ALP is a parameter for examining acute liver damage and is located in the liver, while AST is a parameter for examining chronic liver damage and is located in the heart, liver, skeletal muscle, kidneys, brain, pancreas, spleen and lungs (Widya Astuti, 2018).

Research by Sijid et al. (2020) found that consuming "Tuak" (a traditional alcoholic beverage) for 30 consecutive days can cause liver histopathological changes, including inflammation, sinusoidal dilation, hydropic degeneration, congestion, and necrosis, morphological signs of liver cell or tissue death. Similarly, a study by Hayatillah (2022) reported that drinking "Beer" for 14 days affected liver morphology, the hepatosomatic index, and the overall physiological balance in mice (*Mus musculus*) (Nidianti, 2023). Based on these findings, this study aims to investigate the effect of alcohol consumption on liver damage in mice, using AST and ALP levels as an indicator. The research is expected to provide insight into how excessive alcohol intake impacts liver function by examining AST and ALP levels in blood serum.

2 Method

This research has received ethical approval from the Ethics Committee of Nahdlatul Ulama University Surabaya, as stated in the ethics approval letter No.
0098/EC/KEPK/UNUSA/2024.

2.1 Tools and Materials

The tools used in this study included a photometer, dry cotton, pipettes, test tubes, serology tubes, tube racks, non-coagulant blood tubes, tissue paper, gloves, a feeding sonde, 1 cc syringes, micropipettes with yellow and blue tips, measuring cups, a pycnometer, a rotary evaporator, mouse cages, and an analytical balance. The materials used in this study were mice, distilled water, alcoholic beverages, mouse feed, and ALP and AST reagents.

2.2 Alcohol Content Measurement Distillation Method

Alcoholic beverages that have been obtained from online stores with 5 different types are pipetted as much as 200 ml, put into a distillation flask then put into a rotary evaporator for ± 2 hours. The distillation results are collected in a 100 ml measuring flask. Weigh the empty weight of the pycnometer and record the results. Then the results of the distillation are put into the pycnometer at a temperature of 20 ° C, then weighed and recorded the results. Weigh the pycnometer containing distilled water to the limit mark, then record the results, and then enter into the formula, and the results are matched in the pharmacological alcohol content table (Primadevi et al., 2016):

$$BJ\ Sample = \frac{weight\ of\ distillate\ pycnometer}{weight\ of\ empty\ pycnometer}$$

2.3 Experimental Animals

The animals used in this study were male mice (*Mus musculus*), aged 2–3 months and weighing between 27–30 gram. The mice were housed in the Experimental Animal Laboratory of the Faculty of Pharmacy, Airlangga University, Surabaya. They were provided with standard pellet feed and water *ad libitum*. The cage environment was maintained in a dry condition, with a controlled temperature range of 28–32°C.

2.4 Induction of Alcohol Dependence in Mice

The mice were divided into six groups: a negative control group (no treatment), a positive control group (0.3 mL of 70% v/v ethanol), a standard group (0.3 mL of carbon tetrachloride/CCl_4) (Dewi, 2021), and three treatment groups (P1~P3). Treatment Groups received high doses of alcoholic beverages made from a mixture of five different commercial brands. Specifically, Group P1 was given 0.3 mL/day/mice, Group P2 received 0.5 mL/day/ mice, and Group P3 received 0.7 mL/day/ mice. These treatments were administered daily for 14 consecutive days. On the 15th day, all mice were anaesthetized, and blood samples were collected via cardiac puncture for the assay of the biochemical markers of liver damage (Sijid et al. 2020).

2.5 AST and ALT Level Measurement

The collected blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum. The resulting serum was transferred into Eppendorf tubes for analysis. AST and ALT levels were measured using a commercial enzymatic test kit from Dsi-Germany according to the standard protocol. For each sample (Standard, Positive Control, Negative Control, Treatment Groups 1–3), 200 µL of R1 reagent and 800 µL of R2 reagent were mixed, followed by the addition of 100 µL of serum. The mixture was incubated at 37°C for 1 minute. Absorbance was then measured using a photometer at a wavelength of 340 nm.

2.6 Data Analysis

Statistical analysis of the effect of alcoholic beverages on AST and ALT levels was conducted using both parametric and non-parametric methods. The parametric analysis employed a one-way ANOVA test, while the non-parametric analysis used the Kruskal–Wallis test.

3 Results And Discussion

3.1 Alcohol Content Determination

Based on the results of the examination of alcohol content in various types of alcoholic beverages, the pure alcohol content in the sample code 1 was found to have the highest content of 44.5% as seen in table 1:

Table 1 Results of Alcohol Level Examination

Sample Code	Organoleptic	Ethanol Content %	Group
1	Form: Liquid, Color: Brown, Odor: Distinctive fragrance	44,50%	C
2	Form: Liquid, Color: Clear, Odor: Distinctive fragrance	40,50%	C
3	Form: Liquid, Color: Clear, Odor: Distinctive fragrance	39,40%	C
4	Form: Liquid, Color: Yellow, Odor: Distinctive fragrance	4,51%	A
5	Form: Liquid, Color: Red, Odor: Distinctive fragrance	0,98%	A

Based on the results of the alcohol content analysis, all tested alcoholic beverages were found to contain high levels of alcohol. Among the five beverage samples, three were classified as Class C alcoholic beverages: Sample 1 contained 44.5% alcohol, Sample 2 had 40.5%, and Sample 3 had 39.4%. The remaining two were classified as Class A alcoholic beverages, with Sample 4 containing 4.51% alcohol and Sample 5 containing 0.98%. The examination process began with an organoleptic assessment, evaluating each sample's appearance, color, and odor. Following this, a distillation test was conducted using a distillation apparatus. The principle behind distillation involves separating the components of a solution based on differences in their boiling points, allowing for the isolation of alcohol from the mixture (Primadevi, 2016).

Before measuring the specific gravity of alcoholic beverages, they are distilled for 1 hour to separate ethanol from water and other components to obtain pure ethanol. By taking 200 ml of alcoholic beverages and then putting them into a distillation flask, carry out the distillation process until approximately 2 ml of distillate is obtained from the volume of the liquid being tested (Butterworths, 1968). After distillation, weigh the empty pycnometer while weighing the pycnometer containing distilled water at a temperature of 20°C and weighing the pycnometer containing distillate at a temperature of 15°C which indicates that the distillate temperature has dropped from 30°C. Weighing must be done quickly to avoid evaporation because ethanol evaporates easily. The alcohol content in the liquid shows a comparison with water. Alcohol has volatile properties because it has a range of carbon values from C1 to C5 and has a boiling point of 0°C-50°C (Primadevi, 2016).

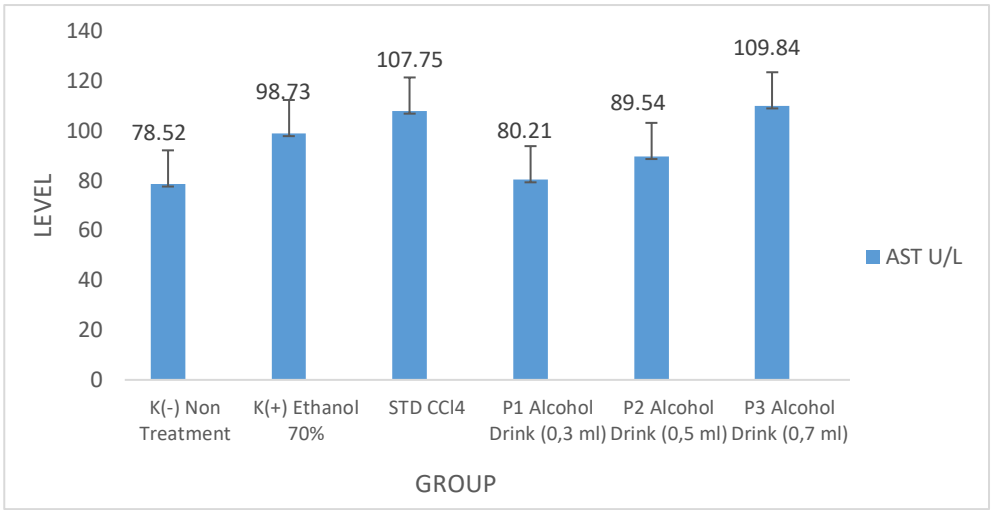

Fig 1. AST Levels (U/L)

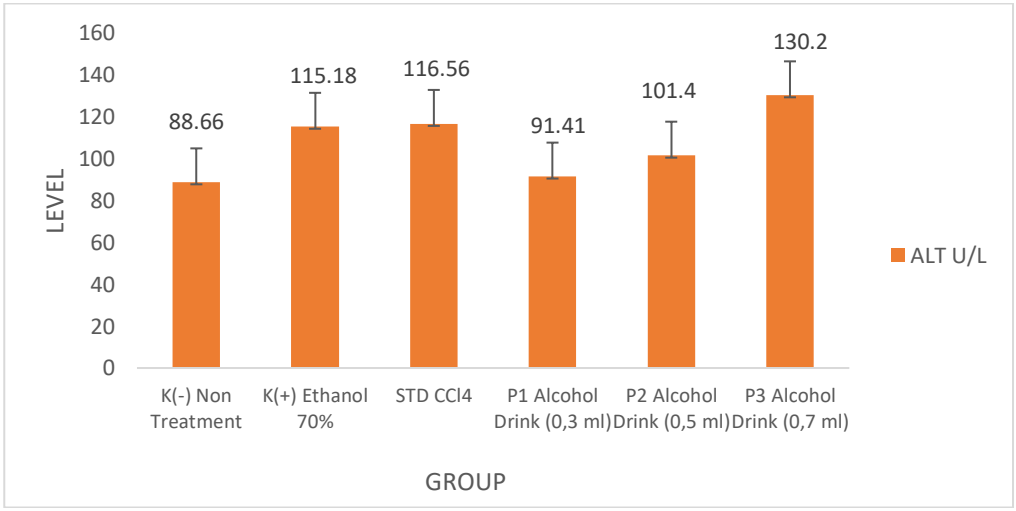

Fig 2. ALT Levels (U/L)

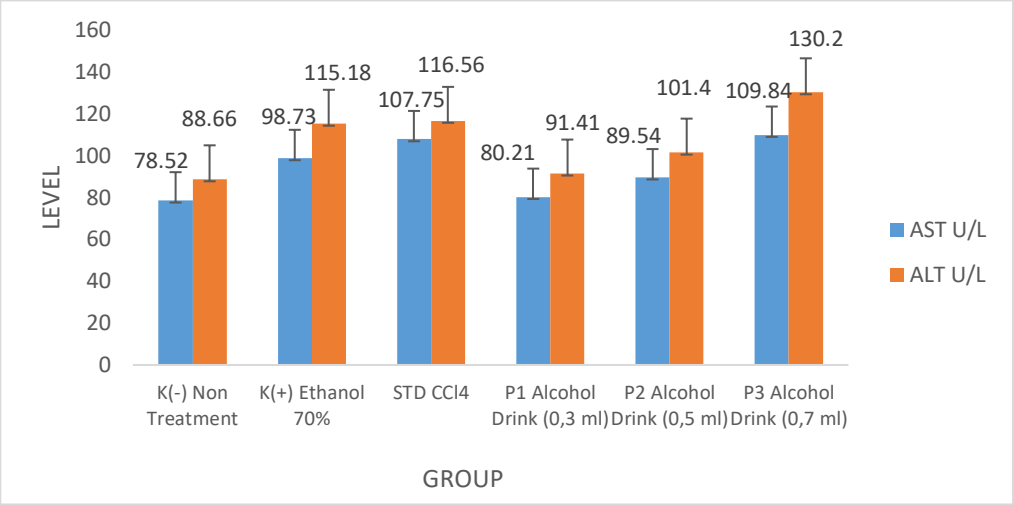

Fig 3. Combination AST-ALT Levels (U/L)

In various animal studies, high concentrations of alcohol are commonly used to induce specific physiological and behavioral effects associated with alcohol consumption and dependence. These concentrations are often applied in models of binge drinking or chronic alcohol exposure to replicate the patterns of excessive alcohol intake observed in humans. The primary objective is to simulate human-like alcohol use behaviors and investigate the biological mechanisms underlying alcohol-related disorders (Augier et al., 2023).

Liver damage caused by alcoholic beverages can be assessed by measuring the levels of AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase). As shown in Figure 1, the average AST level in male mice from the negative control group (K−) was 78.52 U/L, which remains within the normal physiological range. This is expected, as the group did not receive any treatment. According to Almonte (2009), the normal AST level in mice is approximately 99 U/L. In contrast, the positive control group (K+), which was administered 70% ethanol, exhibited an elevated average AST level of 98.73 U/L, indicating mild liver stress or damage due to ethanol exposure. The standard group, which received carbon tetrachloride (CCl_4), showed a higher average AST level of 107.75 U/L, suggesting that CCl_4 induces more severe hepatotoxicity compared to ethanol. Among the treatment groups, Groups P1 and P2 had average AST levels of 80.21 U/L and 89.54 U/L, respectively—both still within the normal threshold. However, Group P3, which received the highest dose of alcoholic beverages, showed the highest average AST level at 109.84 U/L, indicating significant liver damage at higher exposure levels. Alcoholic beverages in Indonesia are divided into three groups based on their ethanol content: Group A (ethanol content up to 5%), Group B (ethanol content greater than 5% - 20%), and Group C (ethanol content greater than 20% - 55%). The positive control used 70% v/v ethanol, while the treatment groups P1, P2, and P3 used the highest alcohol content, 44.5% (Group C) sample code 1. We tested five brands of alcoholic beverages purchased online, and the highest ethanol content was found to be (tabel 1). Based on research conducted by Sijid et al. (2020) using male *Mus musculus* mice, four treatments were carried out: P0 = 0 mL/day/mouse; P1 = 0.1 mL/day/mouse; P2 = 0.2 mL/day/mouse; and P3 = 0.3 mL/day/mouse. Giving tuak (a traditional alcoholic beverage) to mice at different doses affected the histopathological appearance of the mice's liver, with the most influential dose being 0.3 mL/day/mouse.

The increase in AST levels in this study was a mild type, which is often seen in cirrhosis, neonatal hepatitis, fatty liver and drug toxicity which only increased 1-3 times from normal values. Because the AST enzyme is not an enzyme that is specific for signs of liver disease

because this enzyme is also produced in the heart and muscles. And AST levels only increased 1-3 times from normal values, it is possible that alcohol-induced mice were experiencing disorders in other organs besides the liver, such as the heart muscle, kidneys and skeletal muscles (Amri et al., 2020).

The average ALT (Alanine Aminotransferase) level in male mice from the negative control group (K−), which received no treatment, was 88.66 U/L within the normal range, indicating healthy liver function. In the positive control group (K+), which was treated with 70% ethanol, the ALT level increased to 115.18 U/L, reflecting liver stress due to alcohol exposure. Similarly, the standard group (STD), which received carbon tetrachloride (CCl_4), showed an average ALT level of 116.56 U/L, slightly higher than the ethanol group, confirming the known hepatotoxic effects of CCl_4. In the treatment groups, ALT levels increased in a dose-dependent manner. Group P1 recorded an ALT level of 91.41 U/L, still close to the normal range. Group P2 had a higher level of 101.4 U/L, while Group P3, which received the highest dose of alcoholic beverages, showed the most elevated ALT level at 130.2 U/L. These findings suggest that increasing alcohol intake leads to greater liver damage, as evidenced by elevated ALT levels.

AST and ALT are among the most sensitive biochemical markers for detecting liver cell damage. These enzymes function as endoenzymes within hepatocytes, where they play key roles in amino acid metabolism, including synthesis and catabolism. Under normal physiological conditions, AST and ALT are present in the bloodstream at very low levels, resulting in minimal serum activity. However, when liver tissue is damaged—often due to toxic exposure or disease—the permeability of the hepatocyte membrane increases, allowing these enzymes to leak into the bloodstream. This leads to a significant elevation in serum AST and ALT levels. As a result, increased concentrations of these enzymes in the blood are strong indicators of hepatocellular injury and can be used to assess the extent of liver damage (Liu et al., 2019).

Based on the results of the Kruskal–Wallis statistical test, the obtained p-value was 0.416 ($p > 0.05$), indicating that there was no statistically significant difference in AST and ALT levels among the treatment groups.

This is in accordance with Huda's research (2017) which stated that overall SGPT and SGOT values were still within the normal range of SGPT and SGOT values in healthy mice because SGOT and SGPT examinations were screening and non-specific and showed significant differences ($p < 0.05$) in the Mann-Whitney post hoc test. And in a different study by Wang et al., 2022, it was shown that continuous use of alcoholic beverages can cause liver damage as indicated by the SGOT value of mice in the treatment group which increased significantly by 19.35 ± 2.59 IU/L. These results indicate that the more alcoholic beverages consumed, the more liver damage will be seen. This is in accordance with the research of Hayatillah & Hapsari (2022) which states that the greater the concentration of alcohol given, the more liver damage will be seen.

4 CONLUSION

In conclusion, our study shows that there is no significant effect of consuming alcoholic beverages on liver damage as measured by AST and ALT levels.

References

1. Almonte, T.J., Barnes, J.H., Bautista, S.C., De La Cruz, B., Lili, L., Paciencia, A.P. 2009. Serum Transaminase in mice after Whole-body X-irradiation and the Effect

of a Chemical Protector. Int. J. Rad. Biol. Related Study in Phys. Chem. Med., 9(1), 37-42.
2. Amri, I. A., Hendrasmara, M. F., Qosimah, D., Aeka, A., Rickyawan, N., Purwatiningsih, W., & Dameanti, F. N. A. E. P. (2020). Silver Nitrate (AgNO3) Solution Toxicity in Balb-c Mice Based on SGPT and SGOT Level. *Jurnal Medik Veteriner*, *3*(2), 251–257.
3. Arfines, P. P., Rachmalina, R., Mubasyiroh, R., Puspita, T., & Arfines, F. A. R. (2022). Prevalence of Indonesia'S Alcohol Consumption, Risk Factors, and Its Cluster Mapping: an Analysis of Indonesia Basic Health Survey 2018. *Gizi Indonesia*, *45*(2), 183–196.
4. Hayatillah, R., & Hapsari, W. K. (2022). Pengaruh Konsumsi Alkohol terhadap Subkronik Hepar dan Keseimbangan Tubuh pada Mencit (Mus musculus) Effect of Alcohol Consumption on Liver Subchronic and Body Balance in Mice (Mus musculus). *Jurnal Pendidikan Sains & Biologi*, *9*(2), 805–814.
5. Huda, M. N., Holidah, D., & Fajrin, F. A. (2017). Uji toksisitas subkronik jamu asam urat pada hati mencit galur balb-c (subchronic toxicity study of jamu asam urat in liver of balb-c mice). *E-Jurnal Pustaka Kesehatan*, *5*(1), 66.
6. Nidianti, E., Wulandari, D. D., & Azizah, C. N. (2023). Efek Pemberian Ekstrak Kacang Tanah (Arachis hypogaea L.) Terhadap Kadar Gula Darah pada Mencit (Mus musculus L). *Borneo Journal of Pharmascientech*, *7*(1), 6-11. https://doi.org/10.51817/bjp.v7i1.433
7. Putra AP, Suhartini, Widagdo H (2016). Hubungan Status Fungsi Hati Dengan Riwayat Pemakaian Alkohol pada Peminum Alkohol di Yogyakarta [skripsi]. Sumatra Utara: Pendidikan Dokter Universitas Gadjah Mada.
8. Sijid, S. A., Muthiadin, C., Zulkarnain, Z., & Hidayat, A. S. (2020). Pengaruh Pemberian Tuak Terhadap Gambaran Histopatologi Hati Mencit (Mus musculus) ICR Jantan. *Jurnal Pendidikan Matematika Dan IPA*, *11*(2), 193.
9. Wang, X., Yu, H., Xing, R., & Li, P. (2022). Hepatoprotective Effect of Oyster Peptide on Alcohol-Induced Liver Disease in Mice. *International Journal of Molecular Sciences*, *23*(15). https://doi.org/10.3390/ijms23158081
10. Zhou, T., Zhang, Y. J., Xu, D. P., Wang, F., Zhou, Y., Zheng, J., Li, Y., Zhang, J. J., & Li, H. Bin. (2017). Protective effects of lemon juice on alcohol-induced liver injury in mice. *BioMed Research International*, *2017*
11. Kemenkes, RI, (2018), Riskesdas Laporan Nasional 2018 : Konsumsi Minuman Beralkohol. Badan Penelitian Dan Pengembangan Kesehatan
12. Widya Astuti, N. P., Suaniti, N. M., & Mustika, I. G. (2018). Validasi Metode Dalam Penentuan Kadar Etanol Pada Arak Menggunakan Kromatografi Gas Detektor Ionisasi Nyala. *Jurnal Kimia*, 128. https://doi.org/10.24843/jchem.2018.v12.i02.p06
13. Dewi, N. D. M. A. (2021). *Gambaran histologi hati dan ginjal mencit (Mus musculus L.) yang diinduksi karbon tetraklorida (CCl4) setelah pemberian ekstrak daun sirsak (Annona muricata L.)*.
14. Primadevi, S., Kresnadipayana, D., D-Iii, J., Kesehatan, A., Kesehatan, I., Setia, U., Surakarta, B., & D-Iv, J. (2016). *Penetapan Kadar Etanol pada Minuman Beralkohol Berbagai Merk Melalui Pengukuran Berat Jenis*. www.biomedika.ac.id
15. A *Standardization* of *Methods* for *Determination* of the *Alcohol Content* of *Beverages* and *Distilled Potable*. *Spirits*," Pure and Applied Chemistry 17, 274 (1968).
16. Augier, G., Schwabl, V., Lguensat, A., Atudorei, M., Iyere, O. C., Solander, S. E., & Augier, E. (2023). Wistar rats choose alcohol over social interaction in a discrete-

choice model. *Neuropsychopharmacology*, *48*(7), 1098–1107. https://doi.org/10.1038/s41386-022-01526-8
17. Liu, B., Fang, Y., Yi, R., & Zhao, X. (2019). Preventive effect of blueberry extract on liver injury induced by carbon tetrachloride in mice. *Foods*, *8*(2). https://doi.org/10.3390/foods8020048
18. A *Standardization* of *Methods* for *Determination* of the *Alcohol Content* of *Beverages* and *Distilled Potable*. *Spirits*," Pure and Applied Chemistry 17, 274 (1968).
19. https://www.statista.com/chart/18009/annual-liters-of-alcohol-consumed-per-person-and-change-in-asia/