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ORIGINAL ARTICLE

Histopathological Changes in the Right, Median, and Left Liver Lobes of Wistar Rats: Effects of Chronic Binge Ethanol Exposure and Diet

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Abstract

Objectives: To examine the effect of alcohol exposure and diet between male and female rats, considering tissue damage in the right, median, and left liver lobes in different observation periods. **Methods:** The study used the protocol established by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) model with modification to determine the severity of damage in the three (right, median, and left) lobes of the liver of male and female Wistar rats under prolonged chronic-binge ethanol feeding using liquid and solid diets in various observation periods. **Findings**: Results show an evident histopathological alteration in the three liver lobes. The damage severity scoring index was categorized into mild, moderate, and severe. Using advanced Python programming and data visualization, the finding shows that early hepatological damage is defined by microvesicular and macrovesicular steatosis and hypertrophy formation, showing a damage score ranging from 1 to 2. Moderate to severe damage in the liver is defined by an increasing damage score with the addition of inflammation. The study also revealed that female rats are more susceptible to the effects of alcohol at early stages, with severe liver damage than males. Furthermore, the study found that alcohol damage in both liquid and solid meals cause significant damage to the liver's median lobe, followed by the right and left lobes. **Novelty:** Female rats are more susceptible to alcohol-induced liver injury than male rats. The median lobe was first severely affected by alcohol. Tissue visualization is improved by computational analysis.

Keywords: Chronic binge ethanol exposure; Liver lobe pathology; Wistar rats; Solid diet; Liquid diet

1 Introduction

Liver disease caused by alcohol is one of the worldwide health problems and continues to be the common reason for both morbidity and mortality issues [1], [2]. The majority of Filipinos are alcohol consumers in Southeast Asia, resulting in alcoholism as one of the major concerns in the culture and social life of Filipinos. Data on the cases of the degree of alcohol addiction and abuse are limited, even with the apparent negative impact of alcohol in the community. The detrimental health consequences of alcoholism are a neglected medical problem in the country. Filipinos with chronic conditions caused by alcoholism are not subject to medical issues [3].

The synergistic action of chronic ethanol consumption and binge in a mouse model induces liver damage leading to cirrhosis and hepatocellular carcinoma, which is the leading cause of mortality among alcoholic patients [4], [5].

Various studies were conducted to simulate drinking patterns and mimic the spectrum of pathological damage in the liver and determine the underlying mechanism and impact of liver injury caused by binge ethanol feeding [6], [7]. Experimentally, researchers made use of various animal models and utilized multiple methods to understand the pathological features of liver damage caused by alcohol exposure, such as single or multiple ethanol binge administration, alcoholic liver disease model, *ad libitum* drinking water with ethanol, the Lieber-DeCarli liquid diet feeding model, the intragastric infusion model, the Chronic-Plus-Binge NIAAA Model, and the Gao-binge model [1], [4], [8]. These models show various liver pathological damages, such as drinking water with ethanol, which shows mild steatosis and hepatocyte injury. In contrast, a chronic ethanol liquid diet produces hepatic steatosis and mild hepatocyte injury [1]. Lieber-DeCarli liquid diet containing ethanol for 4-6 weeks shows a slight elevation of serum ALT, mild steatosis, and minimal or no inflammation [4], [8].

Among these models, the chronic-plus-binge model developed by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) has been recognized for its ability to replicate features of acute and chronic liver disease in human alcohol liver disease (ALD) [1], [4], [8]. However, detailed investigations into the lobe-specific histopathological effects of prolonged ethanol exposure in rats with consideration of the pattern of cellular morphology damage, sex, time of exposure, and diet are limited.

This study aims to modify and extend the NIAAA model by subjecting the Wistar rats to 90 days of chronic ethanol exposure with periodic binge administration. Liver tissues from the right, median, and left lobes were collected at 11-day intervals to assess and compare the severity and progression of histopathological changes. The goal is to better understand the histopathological changes and liver damage progression of alcohol induced liver injury as influenced by sex, duration of alcohol exposure, and diet.

2 Methodology

2.1 Wistar Rat Model and Ethanol Feeding Protocol

Two-week-old rats were divided into two groups according to sex differences. The rats were subjected to chronic binge alcohol feeding using the National Institute on Alcohol Abuse and Alcoholism (NIAAA) Model with slight modification. The model comprised two groups of rats in a liquid diet (Lieber de Carli diet with ethanol) and two other groups in a solid diet (ordinary pellets for rats). Thus, the experiment was composed of four groups and was treated following the design in Figure 1. The experimental phase followed the process in the design (Figure 1) every 10 days and extended until 90 days. Two rats were sacrificed per observation period under the research design (Figure 1). Liver (Right, median, and left lobe) tissues were analyzed in terms of the severity of the damage. Observation and dissection were done every 11^{th}, 21^{st}, 31^{st}, 41^{st}, 51^{st}, 61^{st},71^{st}, 81^{st}, and 91^{st} day for each of the four groups of rats (male and female). Extending the observation period to 90 days provided the researchers with a comparative dataset for liver injury based on labeled and analyzed histopathological images.

2.2 Histological preparations.

The male and female Wistar rats were anesthetized, sacrificed, and euthanized through cervical dislocation. The liver was excised immediately, cut about 2-3mm on the ventral side, and fixed in 10% formaldehyde in phosphate-buffered saline overnight. After these, specimens were placed in appropriately labeled cassettes, and the tissue was immersed in a series of ethanol solutions for 15 minutes with increasing concentration (70%, 90%, 95%, 100% ethanol). Then, 30 minutes of 100% ethanol, followed by 45 minutes of 100% ethanol. After dehydration, tissues were immersed again with a clearing agent, xylene. With a clearing sequence of 20 minutes, 20 minutes, and 45 minutes, respectively. The dehydrated tissue was infiltrated in paraffin wax with an infiltration sequence of 30 minutes, followed by another 30 minutes, and lastly, 45 minutes, then embedded. The embedded tissue was sectioned 5mm thick and de-paraffinized using a digital magnetic stirrer hotplate (TT-DMSH-350S) and xylene. Deparaffinized tissue was soaked in 100%, 90%, and 80% ethyl alcohol, then immersed in distilled water. After this, the tissue slide was stained with eosin and hematoxylin. The tissue was immersed in distilled water, followed by an Eosin stain. The tissue was dehydrated

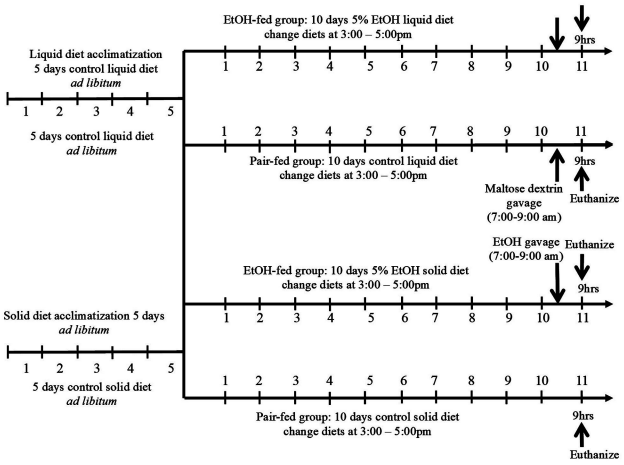

Fig 1. Test Animals (Wistar Rats) in Liquid and Solid Diets with Ethanol Feeding Protocol (based on National Institute on Alcohol Abuse and Alcoholism (NIAAA) Model) [4]

successively using 80%, 90%, and 100% isopropyl alcohol, and lastly with xylene. The slides were covered with coverslips using DPX and dried overnight. The slide was examined under the microscope if properly mounted, and photomicrographs were captured using Swift M10LB under 400x total magnification.

2.3 Scoring of Histopathology Slides for Liver Damage Index Scoring

The system to score histopathologic changes in the liver considered the Ishak Histology Activity Index and the histological activity from Liang 2014 [9] shown in Table 1 and Table 2 with modification in the interpretation of liver damage. Results of the histopathological condition of the liver were interpreted and validated by Anatomic and Clinical Board-Certified pathologists from the collaborating agency, Mariano Marcos Memorial Hospital and Medical Center (MMMH -MC), Department of Pathology and Laboratories at the City of Batac, Ilocos Norte.

Table 1. Rubric for Liver Damage (Severity Index)

Liver Damage Indicators	1	2	3	4	Total Score
Microvesicular Steatosis	<5%	5-33%	33-66%	>66%	4
Macrovesicular Steatosis	<5%	5-33%	33-66%	>66%	4
Number of Inflammatory foci/field	<0.5	0.5-1.0	1.0-2.0	>2.0	4
Hypertrophy	<5%	5-33%	33-66%	>66%	4
				Total	**16**

Table 2. General Scale on the Classification of Liver Damage

Damage Score	Classification
12-16	Severe
8-11	Moderate
4-7	Mild

2.4 Data Processing and Analysis

Considering the right, median, and left lobes of each rat, liver organs were histologically prepared in three sections per observation period to attain more details in data collection on the possible damage in the three lobes of the liver from male and female groups. The certified pathologist's numerical data scoring was analyzed using computer software packages (free online OpenCV, Seaborn, and Python software).

2.5 Ethical approval

All protocols for handling test animals were approved by the Institutional Animal Care and Use Committee (IACUC) of the Mariano Marcos State University (Animal Use Protocol No.: 2018- 025). It was also approved by the Bureau of Animal Industry (BAI) of the Philippines (Animal Research Permit Ref. No.: AR-2020-099). All animal procedures and management followed the Rules and Regulations on Scientific Procedures Using Animals.

3 Results and Discussion

3.1 Anatomy of Liver Tissue Damage in Wistar Rats

The study used Wistar rats fed with alcohol in the Lieber-DeCarli liquid diet and pair-fed with the control Lieber-DeCarli liquid diet. In addition, solid diet with alcohol and solid diet without alcohol were also used. Liver organs were harvested, and tissues were prepared from two treatment and two control groups for male and female rats at different observation periods. The severity index determined the reading of the pathological changes in the rat's liver following histological criteria as an indicator for liver damage, such as microvesicular and macrovesicular steatosis, inflammation, and hypertrophy. This result is shown in the histological condition of the liver, as observed in detail in Figure 2. The figure summarizes the histological changes observed in the Wistar rat liver based on the readings of registered pathologists. **Fig. 2A** shows normal liver tissue from the control group with no noticeable histological and morphological abnormalities. One of the damage indicators for liver caused by alcohol is the presence of microvesicular steatosis (**Fig. 2B**). Numerous small vesicles of fat characterize this but do not displace the nucleus. Microvesicular steatosis appeared to develop in the left and middle lobes of the liver from the early to the latter stages of the observation period. There is no shown pattern of damage severity, but such conditions occur in all liver lobes. Another histopathological scoring index to determine liver damage is the presence of macrovesicular steatosis (**Fig. 2C**). It is histologically characterized by hepatocytes containing a single vacuole of fat filling up the hepatocytes and displacing the nucleus to the cell's periphery. Macrovesicular steatosis appeared to develop from the early stages of the observation period to the latter observation period in the left and middle lobes of the liver. However, in the right lobe of the liver, there is an absence of increasing severity of damage for both male and female rats.

Furthermore, inflammation is another damage indicator manifested by the excessive amount of scar tissue that builds up in the organ (**Fig. 2D**). Generally, inflammation appeared to develop from the early stages of the observation period and progressed during the 7^{th} observation period in the left and middle lobes of the liver. Hypertrophy is another manifestation of liver damage characterized by an increase in the size of an organ or individual cell (**Fig. 2E**) that may occur for many reasons. Hepatocellular hypertrophy refers to an increased hepatocyte, but not an increased number of hepatocytes. The occurrence of hypertrophy appeared to develop from the early stages of observation periods to the latter observation periods in the left and middle lobes of the liver. Also, no pattern is shown regarding the severity of damage, but such conditions occur in all lobes of the liver.

Microvesicular and macrovesicular steatosis, inflammation, and hypertrophy in the liver as damage indices were analyzed using all the representative animal samples across treatments in various observation periods, comparing male and female rats fed with chronic binge ethanol feeding. The large amount of data gathered on the scoring indices was subjected to a computer-assisted analysis of the scoring index dataset, done to precisely compare the damage in the liver lobes (right, middle, and left) as affected by ethanol exposure and diet between male and female groups in different observation periods. The designed program was utilized to analyze the degree of damage to the liver caused by alcohol in liquid and solid diets.

To determine which of the damage indicators first manifested during liver damage caused by alcohol exposure, the scoring index datasets were analyzed using the computer software Seaborn and Python. The various damage parameters used in the computation of severity index on how they are distributed on each of the severity classes are shown in Figure 3. The chart on liver severity indicator shows that tissue samples categorized in mild class are defined by the presence of microvesicular, macrovesicular, and hypertrophy conditions with damage levels ranging from 2 to 4.0. Moderate liver tissue damage is defined by a higher level of microvesicular steatosis (2.0-4.0), and hypertrophy damage (2.5-4.0) coupled with increased macrovesicular steatosis and inflammatory damage. Severe liver tissue samples were observed with high damage involving the four parameters, with hypertrophy consistently gaining a score of 4 in all samples.

3.2 Liver Damage as Affected by Chronic-Binge Alcohol Exposure and Type of Diet

The feeding protocol was one of the variables considered in the study. The study was composed of two treatment groups and two control groups. Each treatment was exposed to the same amount and concentration of alcohol intake and water source. The experimental setup in the two groups was provided with a Lieber-DeCarli liquid diet and a Solid pellet diet, and they were given the same amount of alcohol intake for the whole study duration; these are treatment one and treatment three, respectively. The

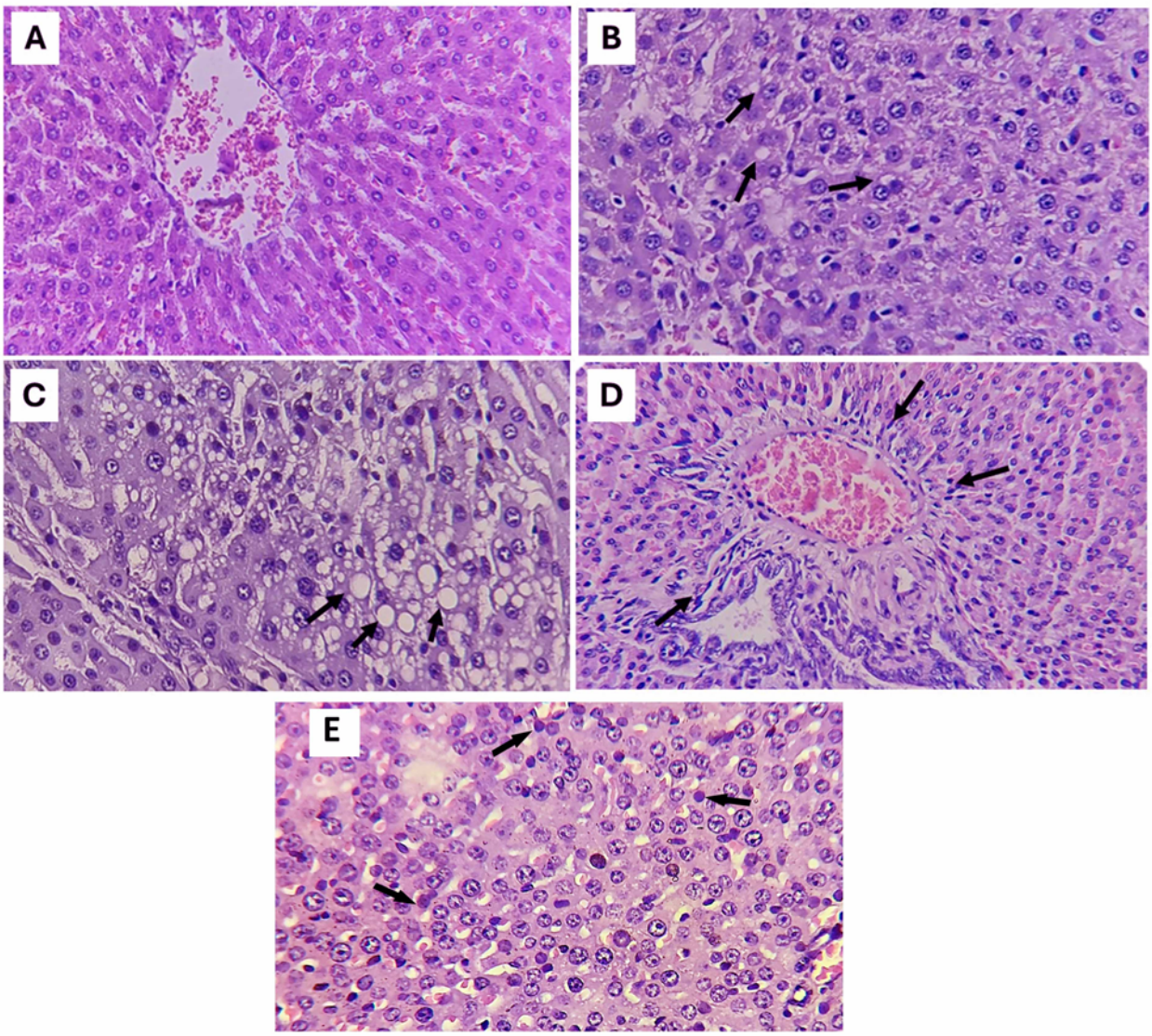

Fig 2. Histological photomicrograph of a Wistar rat liver stained with hematoxylin-eosin, captured using a Swift M10LB microscope under 400x total magnification. The figure shows normal liver histology in panel (A), the presence of microvesicular steatosis in panel (B), the presence of macrovesicular steatosis in panel (C), inflammation in panel (D), and hepatocyte hypertrophy in panel (E), these histopathological changes were observed across treatment group, with varying degrees of severity as indicated by the scoring index

control group was provided as a basis for comparison of the possible effects of food diets. Thus, a group of rats was also fed with a formulation of Lieber-DeCarli liquid diet control provided by the supplier without alcohol intake, and a pair-fed group for the solid diet, labeled as treatment two and treatment four, respectively.

The effect of the various treatments in the progression of liver damage at different observation periods (Figure 4) shows that the progression of alcoholic liver disease is faster in treatment three (solid diet and alcohol intake). This is manifested by the apparent damage in the liver at the early alcohol exposure, showing a high frequency of the population exhibiting moderate damage in the liver as early as the first week (seven days) of alcohol intake, and severe damage was recorded on the 3rd week of exposure. The severity of damage progressed throughout the study. In contrast, most of the livers treated with a liquid diet and ethanol exhibited mild damage during the first three observation periods, with moderate damage occurring. The severity of damage started at the 6th observation period. This result is an indication that drinking, coupled with food intake, accelerates the metabolic process of alcohol, leading to an apparent progression of liver damage.

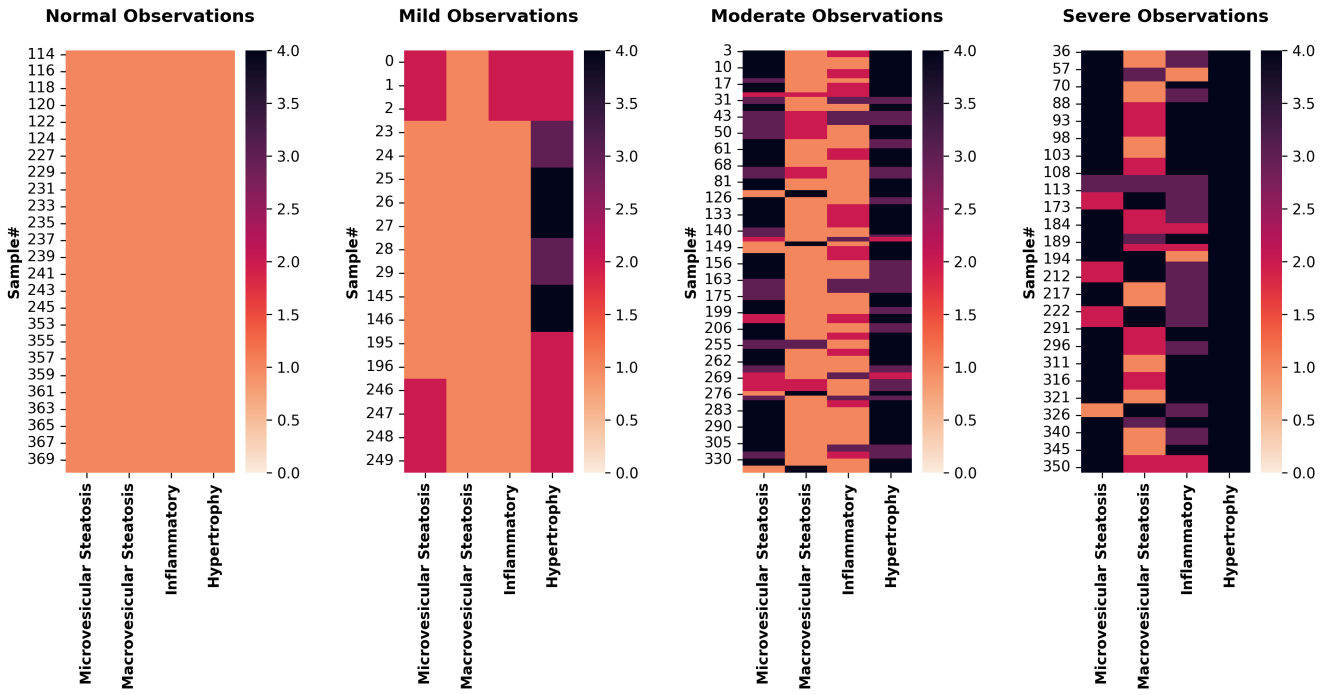

Fig 3. Damage severity in the liver of Wistar rats using Python and Seaborn

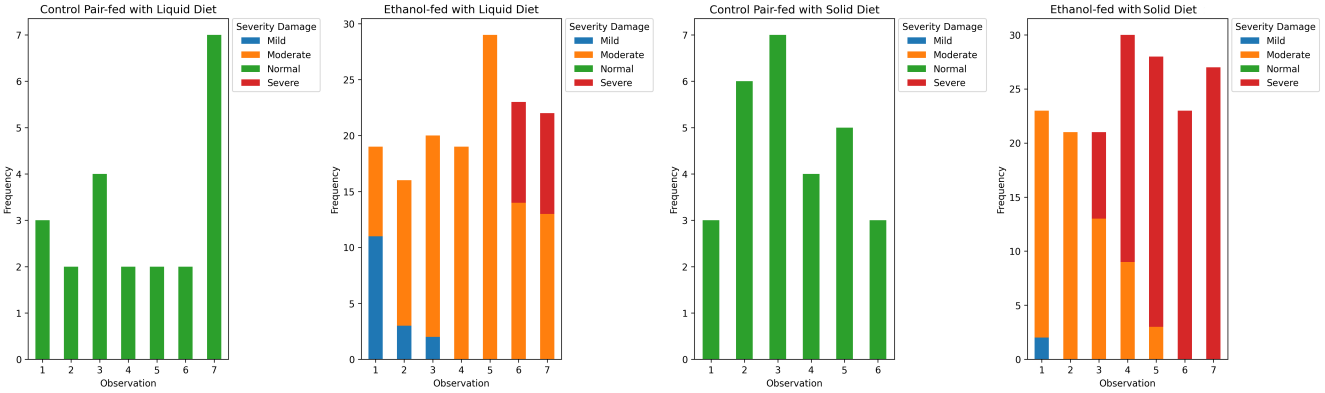

Fig 4. Effect of alcohol in liquid and solid diet in different observation periods

3.3 Severity of Damage in Specific Lobes of the Liver in Wistar Rats

The liver has been noted to be the common organ responsible for the detoxification of alcohol. Several studies have also shown how alcohol is metabolized in the liver, and metabolic pathways are being explained. However, it is very interesting to note which lobe of the liver is severely affected by alcohol. Pathophysiology and biochemical pathway of alcohol metabolism in the liver are very well studied. Still, based on literature searches, there is no noted explanation of which part of the liver is severely affected by alcohol intake. This study collected and analyzed various liver lobes of Wistar rats, including the left lobe, median lobe, and right lobe, at different observation periods. Tissue damage severity index score datasets were analyzed using advanced Python programming to determine liver damage points. The result of the study shown in Figure 5 indicates that in mild and moderate cases of liver damage, the median lobe of the liver is the first affected, and damage is indicated by a high occurrence of liver scoring index observed in every observation period fed with liquid and solid diet. It is also apparent that in moderate and severe cases, most of the liver sections were damaged.

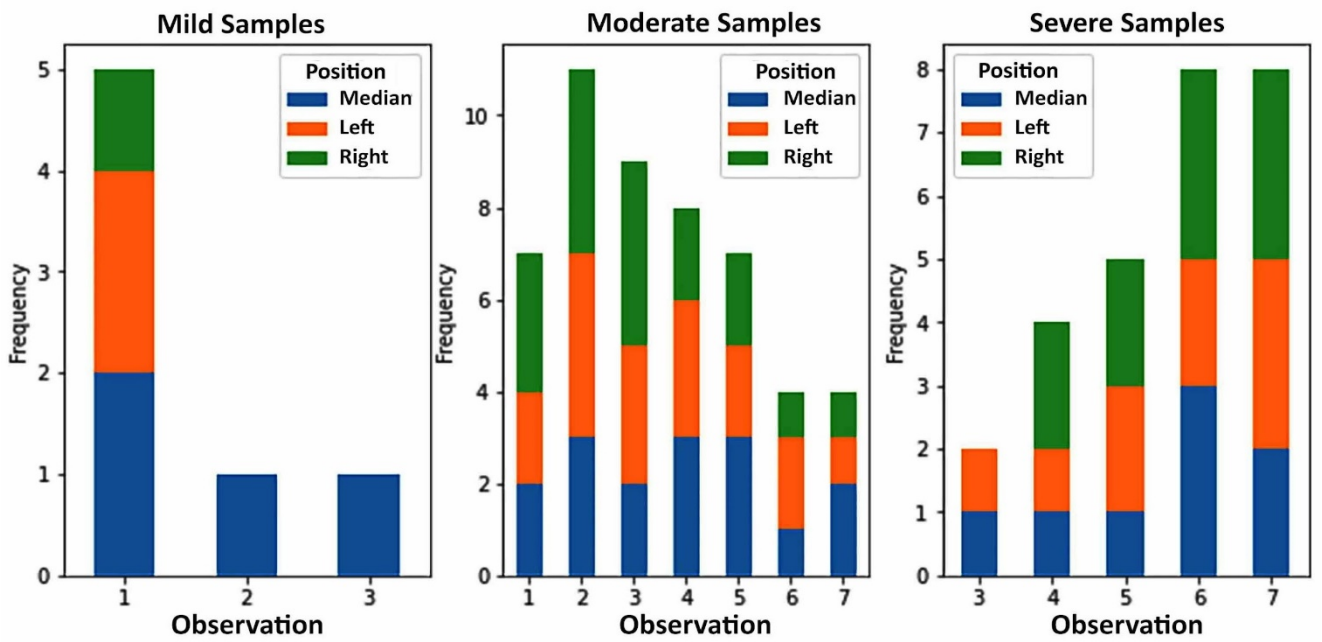

Fig 5. Damage Severity in Liver Lobes per Observation Period

However, as observed and presented in a heat map in Figure 6, specific indicators of liver damage were used. The figure separately shows the effect of liquid and solid diets with ethanol. The first damaged lobes were the right and median lobes under ethanol solid diet compared to those treated with ethanol liquid diet. This is characterized by marked microvesicular steatosis, inflammation, and hyperthrophy. In addition, the areas covered with black colors reflect the severity of damage in each lobe. Microvesicular damage is apparent in both right and median lobes, with a score ranging from 2.5-4.0 in the right lobe and a higher frequency of 3.5-4.0 score in the median lobe in ethanol-fed animals with a solid diet. While hypertrophy scored 3.5-4.0 with high frequency in both right and left lobes, solid diet.

3.4 Liver Damage between Male and Female Samples

The experimental study was composed of a male and female rat population treated with alcohol. Liver damage was assessed in every observation period. The result of the study (Figure 7) indicates that the liver of male rats appears to be damaged earlier than that of females. The male liver showed apparent severe liver damage on the third observation of alcohol exposure, and this observation was consistent until the 7th observation period. Milder samples were recorded at the early stages for the females ranging from 50% frequency on the first observation period with an increase of moderate damage frequency to 75% to 80% in the 3rd to 4th observation period, while moderate to severe samples were recorded for the males with 75% to 100% of moderate damage frequency in the first and second observation period and an increase in severity in the succeeding observation periods (3-7) giving a final severity index of 50% frequency at 6th and 7th observation. However, it should also be noted that more female samples were recorded to have severe levels at the later part of the experiment with 80%-90% damage frequency (6-7), indicating that on the earlier exposure to alcohol, females may have slow progress of damage in the liver but seem to cause severe damage in the long-term exposure. Meanwhile, male alcohol intake has an apparent effect on the liver during the earlier exposure. Still, it shows more resistance to further damage at the later stages, as shown by the low severe level at the later stages of observation.

In the current study, the effect of ethanol-binge feeding on liver injury was focused on the pattern of cellular morphology damage in the lobes of liver tissue using Wistar rats affected by alcohol intake, time of exposure, gender, and diet. Histological changes in terms of severity of damage in the features of the liver lobes (right, middle, and left) in Wistar rats under prolonged chronic binge ethanol feeding in different observation periods show that microvesicular, macrovesicular steatosis, and hypertrophy changes appear to be the first stage of hepatic damage. It becomes severe when all the other parameters, such as inflammation, increase. Steatosis is the earliest response to heavy drinking and is characterized by the deposition of fat in hepatocytes[10]. This result is corroborated by Patel and Mueller 2023[7] that alcoholic liver disease covers a spectrum

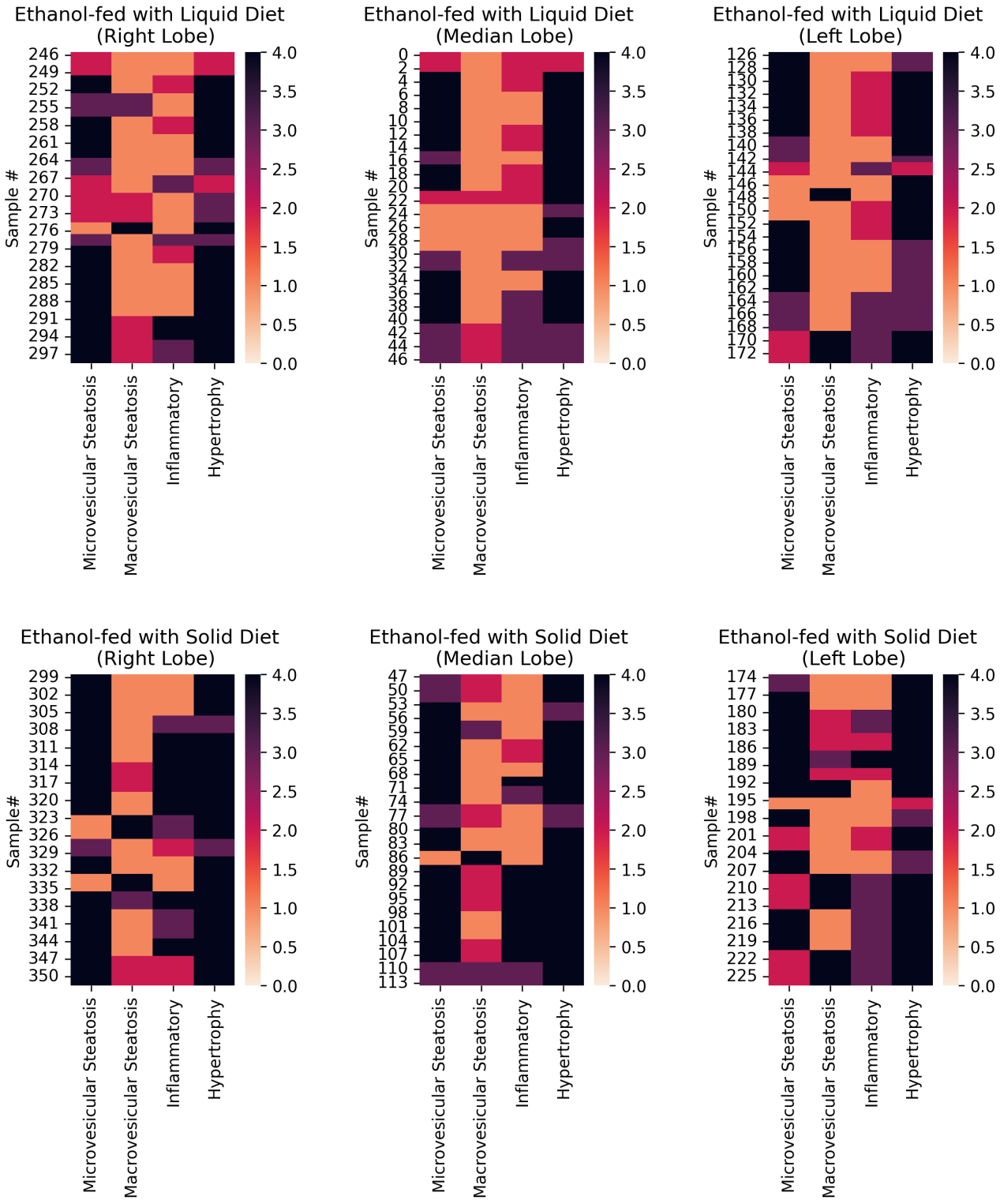

Fig 6. Specific histological damage in the liver's right, median, and left lobes

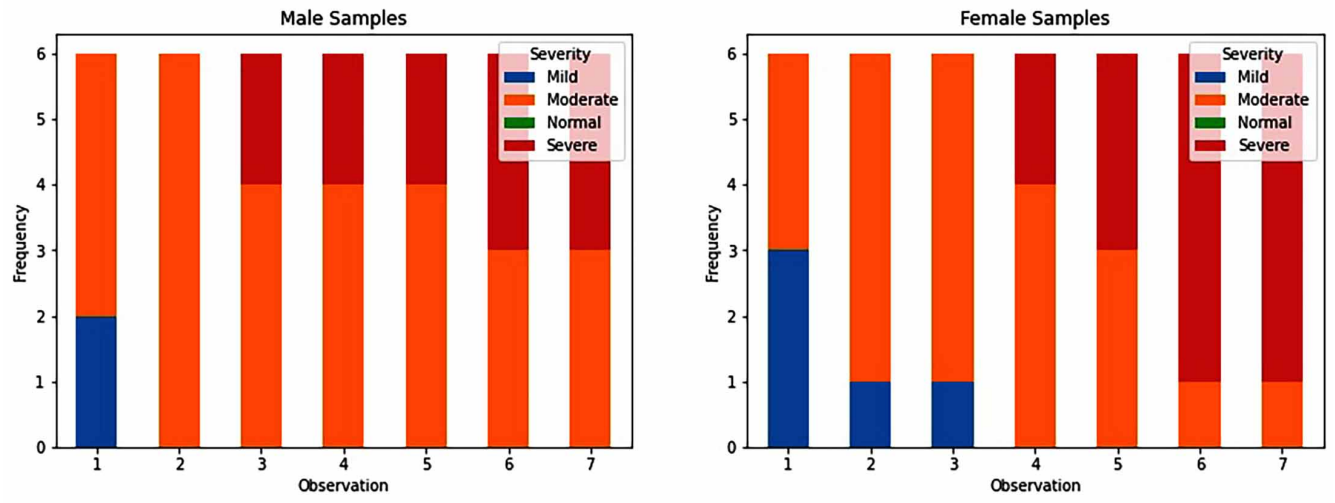

Fig 7. Liver damage between male and female samples

of disorders that begins with fatty liver, progressing at times to alcoholic hepatitis, and culminating in alcoholic cirrhosis. Further progression of liver damage is marked by steatosis and acute inflammation. It is shown by various experimental research that the metabolism of alcohol through the enzyme alcohol dehydrogenase (ADH) is a very important factor in liver damage. ADH metabolizes ethanol to acetaldehyde, a toxic substance that can be oxidized to acetate through acetaldehyde dehydrogenase (ALDH). Acetaldehyde is one of the ethanol metabolites that leads to liver damage[(11)]. This claim is shown in the research findings of Balkrishna 2021[(12)], in which exposure of HepG2 cells to acetaldehyde caused increased intracellular lipid and triglyceride accumulation, leading to steatosis. Biochemically, this lipid and triglyceride accumulation observed in the hepatocytes caused by alcohol is produced during the metabolism of alcohol to acetaldehyde; a co-enzyme nicotinamide adenine dinucleotide (NAD+) is needed to metabolize acetaldehyde to acetate. This causes the change in the NAD+/NADH+ ratio, causing hepatic triglyceride accumulation and fatty acid synthesis in the liver[(11)]. Furthermore, acetaldehyde induces liver damage by promoting glutathione depletion and ROS generation, which are evident in alcohol-induced liver damage. This increase of ROS in the hepatocytes leads to the oxidation of DNA, protein, and cellular membrane, which consequently marks an increase in liver damage and a decrease in glutathione[(11)]. This alcohol metabolism in the liver has a direct molecular and biochemical change in the liver cells, triggering pathological responses such as steatosis, inflammation, various types of cell deaths, fibrogenesis, and even liver regeneration[(13)].

On the other hand, in the current study, alcohol-related liver damage was assessed alongside dietary intake. The result confirms that a solid diet combined with alcohol binge feeding leads to a faster progression of alcoholic liver disease compared to the formulated liquid Lieber-DeCarli diet. It was earlier discussed that the enzyme ADH plays a significant role in the process of alcohol metabolism, which leads to various biochemical pathways that affect the liver. ADH is found to be highly expressed in the liver, where alcohol is mainly metabolized, though alcohol digestion and absorption happen first in the digestive tract. ADH is found in a minimal amount in the stomach and other organs in the body, such as the kidney[(14)], [(15)], [(16)]. However, ADH levels are higher in the fed nutritional state than in the fasted state, and when ADH levels are higher, the ability of the substrate shuttle mechanism to transport reducing equivalents into the mitochondria is elevated[(16)]. Hence, the presence of a solid diet while drinking alcohol increases liver blood flow and slows the time of emptying the stomach. This digestion process of alcohol with solid diets allows for more extended periods of alcohol in the stomach, which causes slower alcohol absorption, sustaining a longer alcohol concentration in the bloodstream. This increase in the ADH during food uptake while drinking will favor the catabolism of ethanol to acetaldehyde and increases the by-product, such as the concentration of NAD/NADH+ ration that was noted to affect the production of detrimental oxidants may have been a significant factor in the severity index result in rat treated with solid diet and alcohol drinking throughout the study.

Furthermore, the Lieber-DeCarli Formula – Liquid diet, according to Lieber 1989[(17)], the liquid diet technique is one of the most efficient techniques for most experimental studies of chronic alcohol consumption. This type of diet provides a controlled nutritional condition that allows alcohol consumption of clinical relevance. The technique also facilitates the comparison with controls by simplifying the pair feeding. It is the best procedure available for the study of the toxic effects of alcohol and their interactions with deficiency or excess of various nutrients. The quality and composition of diet are known to have a significant

influence on the histology of the ethanol-treated mice[16]. There is no sufficient amount of related data that will thoroughly explain the specific component of food intake and alcohol metabolism in detail; however, several studies support that food components may affect the absorption of alcohol as well as the production of ADH in the stomach, which consequently hasten the process of metabolism and production of metabolites of ethanol that will be available in the blood and been noted to be the causative agent in the apparent changes in the liver damage. Thus, this may lead to a further investigation into the possible effects of various food intake while drinking alcohol, which can be a preventive mechanism for further damage to the liver for drinkers by choosing the right kind of food component to take while drinking.

The liver is the most affected organ because it is the primary site of ethanol metabolism, and most alcohol-metabolizing enzymes are the highest[13],[16]. Several studies have been done to establish how the liver is affected medically during alcohol intake; however, in this study, the lobe of the liver was assessed to establish which part of the liver is initially and adversely affected, which is not yet well recognized in any related studies on alcohol liver damage. The current study reveals that the median lobe of the liver is initially and adversely affected by alcohol damage. Rats are the animal model utilized in this study.

Anatomically, the liver lobe of rats is almost the same as that of humans and is similar to the portal branches that supply them. Blood is provided adequately by the hepatic artery. Physiologically, alcohol is a small water-soluble molecule slowly absorbed from the stomach and rapidly absorbed by the small intestine[18]. Thus, chronic alcohol consumption is mainly processed by the medial lobe since it is the segment of the liver responsible for receiving blood from the entire gastrointestinal tract, being the recipient of blood alcohol to be detoxified[19]. This result establishes a better understanding of the specific areas of the liver primarily affected by alcohol intake. This may lead to further research on the metabolic processes in this particular liver area for possible intervention methods and therapies.

Sociological research claims that alcoholism is commonly associated with men since men display a higher prevalence of alcohol liver disease. Although a low population of women is inclined to drinking habits, women have been noted to suffer a greater risk of organ damage brought by the adverse metabolic effects of alcohol[20].

This notion of the effect of alcohol damage in male and female Wistar rats treated with alcohol is congruent with the study results. However, in this study, the liver pattern of cellular morphology and length of exposure were observed and analyzed to understand the damage pattern concerning exposure. Thus, this will provide insight into the tolerance of males and females in alcohol consumption and liver damage that may lead to further evaluation that can be utilized in the proper dispensing of alcohol among genders.

Males and females are different in their ability to metabolize alcohol. Alcohol dehydrogenase (ADH) and Mitochondrial Aldehyde Dehydrogenase (ALDH2) are enzymes present in the liver at higher levels and lower amounts in many other tissues in the body, including the gut. These enzymes metabolize the bulk of ethanol in the body[13]. ADH is a major oxidizing enzyme located in the cytosol of the parenchymal cells of the liver.

Penaloza 2020[21] supports this study's current result; gender-related differences in total liver ADH and ALDH activity among different animal species have been observed. The group compared the male and female ADH, ALDH, and cytochrome isoenzyme (Cyp2e2) mRNA expression. Cyp2e2 is highly expressed in females, with females producing 15% more ROS than males. However, males have double the GSH and a ROS scavenger. This result supports the fact that there is a difference in the enzymatic activity between male and female patients. A similar analysis was done by Vatsalya et al.2022[22] where the authors concluded that women are more impaired than men after alcohol administration in both males and females. This finding is partly attributed to the differences in total body water content between genders. Furthermore, Llamosa-Falcon et al. 2022[23] cited that the tolerance of males is higher than that of females; however, with the same amount of alcohol exposure, females have a higher risk of developing liver cirrhosis than males. One of the critical factors in the process of alcohol metabolism is genetics, and there are some genes related to CYP2E1. Penaloza 2022[21] described CYP2E1 as a member of the P450 family with higher catalytic activity with ethanol. CYP2E1 is a minor pathway of ethanol oxidation as it catalyzes the two-electron oxidation of ethanol to acetaldehyde. CYP2E1 metabolizes and activates many toxicological substrates, including ethanol, to more reactive toxic products. This is one of the major factors that make women more sensitive to alcohol, according to Penaloza 2022[21]. Research findings show that females express approximately 8X more CYP2E1. Furthermore, Penaloza 2022[21] mentioned that female cells produce approximately 15% more ROS (reactive oxygen species) than male cells. ROS metabolism has a direct biochemical change in the liver cells, triggering pathological responses such as steatosis, inflammation, cell death, fibrogenesis, and even liver regeneration[24].

4 Conclusion

This study adapted the NIAAA model to extend the protocol to 90-day chronic binge ethanol exposure to demonstrate the progressive, lobe-specific, and sex-dependent liver damage in Wistar rats using liquid and solid diets. Histopathological changes, including microvesicular and macrovesicular steatosis, inflammation, and hepatocellular hypertrophy, were observed as early as

Day 11, and the damage progressed over time. Notably, histological scores for hypertrophy and steatosis reached severity levels of 3.5–4.0 by Day 90, particularly in the median and right lobes. Under a solid diet, the median lobe consistently exhibited the highest severity scores, suggesting increased vulnerability under specific nutritional conditions.

Quantitative analysis revealed that 75% to 80% of female rats at observation 4 exhibited severe pathological damage earlier than males, with consistently recorded 80%-90% damage frequency in females on the 6-7 observation, highlighting sex as a significant factor in alcohol-induced liver injury. This sex difference, alongside lobe-specific responses, emphasizes the complexity of alcohol-related hepatic pathology.

These findings underscore the potential of this refined model for studying the progression of alcohol-induced liver injury. As a recommendation, future work should incorporate molecular markers and automated image analysis to develop predictive models for early diagnosis and progression monitoring of alcohol-related liver disease. Furthermore, dietary modulation and sex-specific interventions should be explored as targeted strategies for prevention and therapy.

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