International Journal of Forensic Medicine

E-ISSN: 2707-4455
P-ISSN: 2707-4447
IJFM 2024; 6(1): 37-40
www.forensicpaper.com
Received: 16-12-2023
Accepted: 30-01-2024

Mohammed Ageeli Sheikh
Department of Forensic Medicine, Northern Border University, Rafha, Saudi Arabia

S Zeb
Department of Forensic Medicine, Northern Border University, Rafha, Saudi Arabia

Abdullah Hakami
Department of Forensic Medicine, Northern Border University, Rafha, Saudi Arabia

Corresponding Author:
Mohammed Ageeli Sheikh
Department of Forensic Medicine, Northern Border University, Rafha, Saudi Arabia

Ethanol production analysis in postmortem liver and muscle tissues of rats

Mohammed Ageeli Sheikh, S Zeb and Abdullah Hakami

DOI: https://doi.org/10.33545/27074447.2024.v6.i1a.78

Abstract
This study investigated ethanol production in postmortem liver and muscle tissues of rats. The analysis aimed to assess the presence and quantity of ethanol in these tissues after death. A total of 100 rats were included in the study, and their liver and muscle tissues were subjected to ethanol analysis using gas chromatography-mass spectrometry (GC-MS). The results revealed varying levels of ethanol production in both liver and muscle tissues, with ethanol detected in 65% of liver tissue samples and 45% of muscle tissue samples. The concentration of ethanol ranged from 0.05 mg/g to 0.2 mg/g in liver tissue and from 0.03 mg/g to 0.15 mg/g in muscle tissue. Additionally, the time course of ethanol production postmortem showed a gradual increase in ethanol levels up to 24 hours postmortem. These findings contribute to our understanding of ethanol metabolism in postmortem tissues and have implications for forensic investigations involving alcohol-related deaths.

Keywords: Ethanol production, postmortem, liver tissue

Introduction
Ethanol, widely recognized as alcohol, is extensively studied within the contexts of both living and deceased subjects because of its significant implications in forensic science, toxicology, and medical research. Typically metabolized in the liver through enzymes such as alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1), ethanol's behavior after death varies significantly as physiological processes halt and microbial activity potentially contributes to either the production or modification of ethanol levels. This distinction is crucial in forensic toxicology where determining if ethanol was consumed before death or produced postmortem affects the outcomes in legal interpretations and insurance claims, especially in cases involving accidental deaths and suicides.

One of the primary challenges in postmortem ethanol analysis is microbial fermentation, which can occur in various tissues and lead to endogenous ethanol production. This introduces difficulties in accurately establishing ethanol levels at the time of death. Factors like environmental conditions, including temperature and humidity, and the time elapsed between death and tissue analysis further complicate the quantification of ethanol.

Previous studies have underscored these challenges. For instance, research by Zilg *et al*. revealed that elevated temperatures could accelerate microbial fermentation, resulting in higher ethanol levels, while Hartmann and Aradottir investigated ethanol synthesis rates in rat liver and muscle tissues postmortem, finding significant differences that highlight the impact of tissue-specific enzymatic activities and microbial actions.

Despite advances in analytical techniques and a deeper understanding of postmortem changes, there are still significant gaps, particularly in the standardization of protocols for sample collection, preservation, and analysis. Future research needs to focus on developing more sensitive and specific analytical methods, such as advanced versions of gas chromatography-mass spectrometry (GC-MS). There's also a need for establishing universal protocols for handling and analyzing postmortem tissues to reduce variability caused by external factors and conducting more detailed studies on the role of microbial flora in ethanol production under various postmortem conditions.

Furthermore, enhancing the ethical framework around postmortem studies to ensure they respect donor wishes and comply with legal requirements is essential. Ethanol production analysis in postmortem tissues remains a complex field that straddles biochemistry, forensic science, and ethical considerations.

As technologies and understanding of biochemical processes postmortem continue to evolve, the accuracy of forensic interpretations related to ethanol will improve, better serving the fields of law and medicine. Future research aimed at overcoming the current challenges is crucial for advancing the field and providing more reliable data for forensic and toxicological analyses.

Objective

The main objective of analyzing ethanol production in postmortem liver and muscle tissues of rats is to investigate the presence and levels of ethanol in these tissues after death.

Previous Studies

Takayasu *et al*. (1995) [1] - This study employed deuterium-labeled ethanol to investigate postmortem changes in ethanol concentrations, finding that ethanol levels peak shortly after death and decrease over time, influenced by microbial activity as tissues putrefy.

Petković *et al*. (2005) [2] - Research showed that postmortem ethanol production varies with temperature, with significant increases at higher temperatures, providing important implications for forensic investigations (Petković *et al*., 2005) [14].

Nanikawa *et al*. (1982) [3] - This study highlighted that ethanol concentrations can be detected in skeletal muscle postmortem and that these concentrations increase with the storage temperature of the carcasses.

French *et al*. (2005) [4] - Investigated gene expression changes in the liver of rats fed with ethanol, showing how ethanol impacts liver function at the molecular level.

Raskin & Sokoloff (1972) [5] - Examined the activity of enzymes involved in ethanol metabolism across various tissues, providing insights into how different body tissues process ethanol.

Williamson *et al*. (1969) [6] - This research explored the metabolic effects of ethanol in perfused rat liver, particularly how ethanol influences glucose, fatty acid oxidation, and the citric acid cycle.

Salem *et al*. (2001) [7] - Studied the efficacy of using fatty acid ethyl esters as postmortem markers for ethanol intake, which persist in liver and adipose tissues after death .

Schaffert *et al*. (2010) [8] - Analyzed how ethanol exposure affects fibrogenic responses in precision-cut liver slices, noting significant increases in oxidative stress and cytokine production.

Channareddy *et al*. (1996) [9] - Investigated the saturable binding of ethanol in rat liver microsomes, demonstrating that ethanol has a specific and significant binding capacity in liver tissues.

Reilly *et al*. (2000) [10] - This study examined the comparative effects of acute ethanol dosage on liver and muscle protein metabolism, showing different impacts on these tissues.

Ward *et al*. (1985) [11] - Focused on the impact of ethanol on leucine oxidation in rat tissues, indicating that ethanol alters enzyme activities and metabolic ratios affecting protein synthesis.

Cook *et al*. (1995) [12] - Explored muscle protease activities in cirrhotic rats and how they respond to ethanol and its metabolites, highlighting how ethanol influences muscle degradation processes.

Materials and Methods

Study Design and Sample Collection: A total of 100 rats were used to investigate ethanol production and concentration in postmortem tissues. All rats were humanely euthanized following approved ethical guidelines. Subsequent to euthanasia, liver and muscle tissues were meticulously harvested from each rat. To ensure the preservation of biochemical integrity, the collected tissues were immediately stored under controlled conditions optimal for postmortem analysis.

Ethanol Extraction and Analysis: The analysis of ethanol concentrations in the collected tissues was carried out using gas chromatography-mass spectrometry (GC-MS). This method involves several key steps:

1. **Extraction of Ethanol:** Ethanol was extracted from the liver and muscle tissues using a standardized solvent extraction technique. This method ensures the efficient isolation of ethanol from the tissue matrices, minimizing contamination and loss of ethanol.
2. **GC-MS Analysis:** Following extraction, the isolated ethanol was analyzed using GC-MS. This technique separates ethanol from other co-extracted substances and quantifies it based on its unique mass spectral signature. GC-MS is renowned for its precision and sensitivity, making it ideal for detecting and quantifying ethanol even at low concentrations.

Data Collection and Statistical Analysis: The concentrations of ethanol were quantified in each tissue sample at various postmortem time points. This approach allowed for a comparative analysis across different time intervals, providing insights into the dynamics of ethanol production and degradation after death. The data were statistically analyzed to determine significant differences in ethanol concentrations between the liver and muscle tissues and among various postmortem intervals. Statistical significance was assessed using appropriate statistical tests, with a p-value of less than 0.05 considered indicative of significant differences.

Results and analysis

Table 1: Ethanol Detection and Concentration in Postmortem Liver and Muscle Tissues of Rats

Tissue Type	Ethanol Detected (%)	Ethanol Concentration (mg/g) Range
Liver	65	0.05 - 0.2
Muscle	45	0.03 - 0.15

The analysis revealed varying levels of ethanol production in postmortem liver and muscle tissues of rats. Ethanol was detected in 65% of liver tissue samples and 45% of muscle tissue samples. The concentration of ethanol ranged from 0.05 mg/g to 0.2 mg/g in liver tissue and from 0.03 mg/g to 0.15 mg/g in muscle tissue. Additionally, the time course of ethanol production postmortem showed a gradual increase in ethanol levels up to 24 hours postmortem.

Discussion

The findings of this study suggest that ethanol production may occur in postmortem liver and muscle tissues of rats. The presence of ethanol in postmortem tissues has important

implications for forensic investigations, as it can affect the interpretation of toxicological results and determination of cause of death. The mechanisms underlying postmortem ethanol production and metabolism warrant further investigation to improve our understanding of this phenomenon. The results presented in Table 1 provide valuable insights into the ethanol production in postmortem liver and muscle tissues of rats.

The detection of ethanol in postmortem tissues is noteworthy, as it suggests the possibility of endogenous ethanol production following death. In this study, ethanol was detected in 65% of liver tissue samples and 45% of muscle tissue samples. This indicates that ethanol production may occur in both liver and muscle tissues postmortem, albeit to varying extents.

The concentration of ethanol in postmortem tissues ranged from 0.05 mg/g to 0.2 mg/g in liver tissue and from 0.03 mg/g to 0.15 mg/g in muscle tissue. These findings demonstrate the variability in ethanol levels among different tissue types and individual rats. The observed concentrations are within the range typically encountered in postmortem toxicology analyses and are consistent with previous studies investigating postmortem ethanol production.

The detection of ethanol in postmortem tissues has significant implications for forensic investigations, particularly in cases involving alcohol-related deaths. The presence of ethanol in tissues can influence the interpretation of toxicological findings and the determination of the cause of death. Moreover, the variability in ethanol levels among different tissues and individuals underscores the importance of considering multiple factors when interpreting postmortem toxicology results.

The gradual increase in ethanol levels observed up to 24 hours postmortem suggests ongoing ethanol production or redistribution within the body following death. This temporal pattern of ethanol production postmortem warrants further investigation to elucidate the underlying mechanisms and factors influencing postmortem ethanol metabolism. It is important to acknowledge the limitations of this study, including the small sample size and the use of rats as animal models. Further research with larger sample sizes and different animal models is needed to validate these findings and explore the mechanisms underlying postmortem ethanol production. Additionally, future studies could investigate the impact of environmental factors, such as temperature and humidity, on postmortem ethanol metabolism to enhance our understanding of this phenomenon.

In conclusion, the results of this study provide valuable insights into ethanol production in postmortem liver and muscle tissues of rats. These findings contribute to our understanding of postmortem ethanol metabolism and have implications for forensic investigations involving alcohol-related deaths. Further research is warranted to elucidate the mechanisms and significance of postmortem ethanol production in various tissue types and under different conditions.

Conclusion

The analysis of ethanol detection and concentration in postmortem liver and muscle tissues of rats illuminates the complex interplay of biochemical processes occurring after death, presenting both challenges and opportunities for forensic toxicology. One significant challenge is distinguishing between antemortem ethanol consumption and postmortem ethanol production, as variations in ethanol concentrations across different tissues can lead to misinterpretations about the circumstances of death. These issues are further complicated by the enzymatic activities that continue after death and the environmental factors that can influence microbial fermentation.

Moreover, there is a pressing need for the standardization of protocols for handling, storing, and analyzing postmortem tissues to ensure that changes in ethanol levels due to external factors are minimized. This standardization is essential for providing consistent and reliable results across forensic investigations.

Looking ahead, future research should focus on developing more precise analytical techniques and exploring the biochemical mechanisms behind ethanol synthesis and degradation in postmortem conditions. Such advancements could significantly improve the accuracy of forensic analyses, helping to clarify the timing and consumption patterns of ethanol prior to death and better inform legal and medical conclusions. This growing understanding will ultimately enhance the interpretive power of forensic toxicology, providing clearer insights into postmortem physiological processes.

Conflict of Interest
Not available

Financial Support
Not available

References

1. Takayasu T, Ohshima T, Tanaka N, Maeda H, Kondo T, Nishigami J, et al. Postmortem degradation of administered ethanol-d6 and production of endogenous ethanol: experimental studies using rats and rabbits. Forensic Science International. 1995;76(2):129-140. https://doi.org/10.1016/0379-0738(95)01806-4
2. Petković S, Simić M, Vujić D. Postmortem production of ethanol in different tissues under controlled experimental conditions. Journal of Forensic Sciences. 2005;50(1):204-208. https://doi.org/10.1520/JFS2004109
3. Nanikawa R, Ameno K, Hashimoto Y, Hamada K. Medicolegal studies on alcohol detected in dead bodies--alcohol levels in skeletal muscle. Forensic Science International. 1982;20(2):133-140. https://doi.org/10.1016/0379-0738(82)90054-5
4. French B, Dedes J, Bardag-Gorce F, Li J, Wilson L, Fu P, et al. Microarray analysis of gene expression in the liver during the urinary ethanol cycle in rats fed ethanol intragastrically at a constant rate. Experimental and Molecular Pathology. 2005;79(2):87-94. https://doi.org/10.1016/j.yexmp.2005.07.001
5. Raskin N, Sokoloff L. Enzymes catalysing ethanol metabolism in neural and somatic tissues of the rat. Journal of Neurochemistry; c1972. p. 19.
6. Williamson J, Scholz R, Browning E, Thurman R, Fukami M. Metabolic effects of ethanol in perfused rat liver. Journal of Biological Chemistry. 1969;244(18):5044-5054.
7. Salem R, Refaai M, Cluette-Brown J, Russo JW, Laposata M. Fatty acid ethyl esters in liver and adipose

tissues as postmortem markers for ethanol intake. Clinical Chemistry. 2001;47(4):722-725. https://doi.org/10.1093/clinchem/47.4.722
8. Schaffert C, Duryee M, Bennett R, DeVeney AL, Tuma D, Olinga P, et al. Exposure of precision-cut rat liver slices to ethanol accelerates fibrogenesis. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2010;299(3):G661-8. https://doi.org/10.1152/ajpgi.00324.2009
9. Channareddy S, Jose SS, Eryomin V, Rubin E, Taraschi T, Janes N. Saturable ethanol binding in rat liver microsomes. Journal of Biological Chemistry. 1996;271:17625-17628. https://doi.org/10.1074/jbc.271.29.17625
10. Reilly M, Mantle D, Salisbury J, Peters T, Preedy V. Comparative effects of acute ethanol dosage on liver and muscle protein metabolism. Biochemical Pharmacology. 2000;60(12):1773-1785. https://doi.org/10.1016/S0006-2952(00)00463-0
11. Ward L, Ramm G, Mason S, Daly R. Ethanol and leucine oxidation--II. Leucine oxidation by rat tissue *in vitro*. International Journal of Biochemistry. 1985;17(2):195-201.
12. Cook E, Gove C, Panos M, Williams R, Preedy V. Skeletal muscle protease activities are unaltered in cirrhotic rats but altered in response to ethanol and acetaldehyde *in vitro*. Alcohol and Alcoholism. 1995;30(2):203-209. https://doi.org/10.1093/oxfordjournals.alcalc.a045673
13. Gao Q, He F, Wang H, Huang W, Dong H. A primary study of ethanol production in postmortem liver and muscle tissue of rats. Journal of Forensic and Legal Medicine. 2024 Feb 15:102653. https://doi.org/10.1016/j.jflm.2024.102653
14. Petković SM, Simić MA, Vujić DN. Postmortem production of ethanol in different tissues under controlled experimental conditions. Journal of Forensic Sciences. 2005 Jan 1;50(1):JFS2004109-5. https://doi.org/10.1520/JFS2004109

How to Cite This Article
Sheikh MA, Zeb S, Hakami A. Ethanol production analysis in postmortem liver and muscle tissues of rats. International Journal of Forensic Medicine. 2024;6(1):37-40.