Available online on 15.05.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Investigation of the Efficacy of Zonisamide in Alcohol Induced Hepatotoxicity

Nilüfer Dönmezdil *, Selçuk Tunik, Fırat Aşır, Senem Çetin Duran, Dilara Akinci, Enis Duran, Eda Yıldızhan, Nurullah Peker, Mehmet Obut

Department of Histology and Embryology, Medical Faculty, Dicle University, Diyarbakır, Turkey

Article Info:

Article History:

Received 23 Feb 2024
Reviewed 06 April 2024
Accepted 26 April 2024
Published 15 May 2024

Cite this article as:

Dönmezdil N, Tunik S, Aşır F, Duran SC, Akinci D, Duran E, Yıldızhan E, Peker N, Obut M, Investigation of the Efficacy of Zonisamide in Alcohol Induced Hepatotoxicity, Journal of Drug Delivery and Therapeutics. 2024; 14(5):64-67

DOI: http://dx.doi.org/10.22270/jddt.v14i5.6594

*Address for Correspondence:

Nilüfer Dönmezdil, Department of Histology and Embryology, Medical Faculty, Dicle University, Diyarbakır, Turkey

Abstract

Subject: This research aims to evaluate the histological changes in liver tissue induced by alcohol consumption and to assess the effectiveness of zonisamide in treating alcohol-related hepatotoxicity.

Materials and Methods: The study utilized 40 adult Wistar albino rats, divided into four groups: a control (sham), an ethanol group, a zonisamide group, and a combination of ethanol and zonisamide (ethanol+zonisamide). Each group underwent a four-day binge drinking protocol to mimic excessive alcohol intake observed in humans. Zonisamide was administered at a dose of 100 mg/kg daily. Post-treatment, the liver tissues were collected, stained with hematoxylin and eosin, and examined for histopathological changes.

Results: The control and zonisamide-only groups showed no abnormal liver or vascular alterations, indicating zonisamide's safety. In contrast, the ethanol group displayed significant liver damage, including vascular dilatation, congestion, and extensive cellular degeneration. Conversely, the ethanol+zonisamide group exhibited substantial histological improvements with noticeable reductions in vascular impairments and signs of hepatocyte regeneration, suggesting that zonisamide mitigates the detrimental effects of alcohol on the liver.

Conclusion: The study concludes that zonisamide has a protective effect against alcohol-induced liver damage. It appears to preserve the structural integrity of hepatocytes and supports cellular survival, potentially through its anti-inflammatory and antioxidant properties. These promising results advocate for further exploration of zonisamide as a therapeutic option for managing liver injuries associated with alcohol abuse. This study contributes significant insights into the therapeutic potential of zonisamide, encouraging more comprehensive investigations into its clinical applications in hepatology.

Keywords: Alcohol, Zonisamide, Hepatotoxicity

INTRODUCTION

Chronic alcohol use is globally recognized as a significant public health issue and is cited as a major contributor to numerous diseases and deaths [1]. According to the World Health Organization, alcohol consumption is responsible for millions of deaths and the emergence of hundreds of diseases annually [2]. Alcohol, similar to other addictive substances, has the potential to cause dependency across various age groups [3].

The impact of alcohol on the liver is particularly severe, with alcohol use disorder being a primary cause of diverse hepatic diseases [4]. The prevalence of alcohol-related problems is closely linked to socio-cultural factors and must be addressed as a worldwide health concern [5].

The detrimental effects of chronic alcohol consumption on the liver are multifaceted. Alcohol directly acts as a hepatotoxin, damaging liver cells. Prolonged and excessive intake can lead to serious conditions such as fatty liver disease, alcoholic hepatitis, and cirrhosis, a terminal stage liver disease [5]. Acetaldehyde, produced during alcohol metabolism, initiates cellular damage and subsequent inflammation, further exacerbating damage through increased oxidative stress and the suppression of antioxidant defenses in the liver [6].

Zonisamide, a sulfonamide-structured anticonvulsant used primarily for treating various neurological conditions, including epilepsy, modulates seizures by regulating abnormal brain electrical activity and reducing neuronal hyperexcitability [7]. It is also noted for its anti-inflammatory and antioxidant properties, which may theoretically protect against alcohol-induced liver damage [8]. This makes zonisamide a potential candidate for the prevention and treatment of liver injuries associated with alcohol use [9].

In recent years, there has been a surge in research into novel therapeutic agents aimed at treating chronic alcohol abuse and liver diseases [10]. The potential hepatoprotective effects of zonisamide are drawing significant interest from the scientific and medical communities [11]. A comprehensive approach, encompassing everything from lifestyle modifications to pharmacological interventions, is required to prevent and treat alcohol-related liver injuries [12,13]. Understanding the role of zonisamide in this context could significantly aid in developing effective treatment strategies and provide new insights into managing alcohol-induced liver conditions [14].

MATERIAL AND METHOD

In this study, 40 male Wistar albino rats were used. The experimental animals were divided into 4 groups (n:10). The groups were formed as follows;

Group1: Sham group: The rats in this group were administered with an amount of saline equivalent to ethanol given orally 3 times a day for 4 days and zonisamide given orally 1 time a day.

Group 2: Ethanol group: Rats in this group were given ethanol orally 3 times a day for 4 days; instead of zonisamide, saline equivalent to the dose of zonisamide was given once a day.

Group3: Zonisamide group: Rats in this group were given zonisamide (100 mg/kg) orally once a day for 4 days; saline equivalent to the dose of ethanol was given 3 times a day.

Group4: Ethanol+Zonisamide group: Rats in this group were given ethanol orally 3 times a day for 4 days. At noon, 1 hour before the ethanol dose, 100 mg/kg zonisamide was administered once a day.

The experimental animals had unlimited access to food and water and were kept under control in 12 hours of daylight/12 hours of darkness, 8:00 am-8:00 pm, 23±2 oC. Zonisamide (Zalipin 100 mg) was obtained from Generica. Ethanol administration in the groups was based on Majchowicz's excessive alcohol protocol [15]. Three hours after the last alcohol administration, the animals were sacrificed by intracardiac blood sampling under general anesthesia.

Tissue Tracking

The liver tissues were first placed in 10% neutral formalin solution and routine paraffin tissue follow-up was started. After fixation (24 hours), the tissues were kept under running tap water for 6 hours. The tissues were passed through 50%, 70%, 80%, 90%, 96% and absolute ethyl alcohol series and kept in xylene 3 times for 45 minutes for transparency. Finally, the tissues were placed in paraffin oil at 58°C for infiltration. The tissues were then embedded in paraffin blocks and sections of 4-6 µm thickness were taken for Hematoxylin-Eosin staining using a microtome (catalog number: Leica RM2265, Wetzlar, Germany).

Hematoxylin-Eosin Staining

Liver tissue sections from paraffin blocks were placed in a bain-marie set at 37°C. The sections were kept in an oven at 58-62°C for 6 hours to melt the excess paraffin on the slide. The sections were deparaffinized in xylene for 3x15 minutes. The sections were passed through decreasing alcohol series (100%, 96%, 90%, 70%, 50% ethyl alcohol) for 10 minutes each and then brought to distilled water and kept for 5 minutes. Harris Hematoxylin stain was applied for 8 minutes and then the sections were washed under running water for 5 minutes. The sections were rinsed and kept in alcoholic eosin stain for 6 minutes. The sections were rapidly immersed in increasing alcohol series (80%, 90%, 96% ethyl alcohol series) and kept in absolute alcohol for 2 minutes [16]. Finally, the sections were soaked in xylene for 3x15 minutes and Entellan was dripped onto the tissue and covered with a coverslip.

Findings

Hematoxylin Eosin staining of the liver sections of the groups is shown in Figure 1. In the sections of the control group, the vena centralis was centrally located in the middle of the liver lobule, and the sinuses were normal and not dilated. Hepatocytes were generally binucleated and hexagonal in appearance (Figure 1a). In the zonisamide group, findings similar to the control group were observed. In this group, liver histology and vascular structures were normal. Hepatocytes were arranged radially (Figure 1b). In the ethanol group, vascular dilatation and congestion and impaired vascular integrity in the vena centralis, dilatation and congestion in the hepatic sinuses, leukocyte infiltration, pyknosis and degeneration in hepatocytes were observed due to alcohol toxicity. Disorders in the radial arrangement organization of hepatocytes and dense vacuolar structures in the cytoplasm were noted (Figure 1c). In the group treated with zonisamide after ethanol toxicity, the pathologies healed and restoration of liver tissue was observed. Reduction in vascular dilatation and congestion, regeneration in hepatocytes and normal appearance of the vena centralis were observed (Figure 1d).

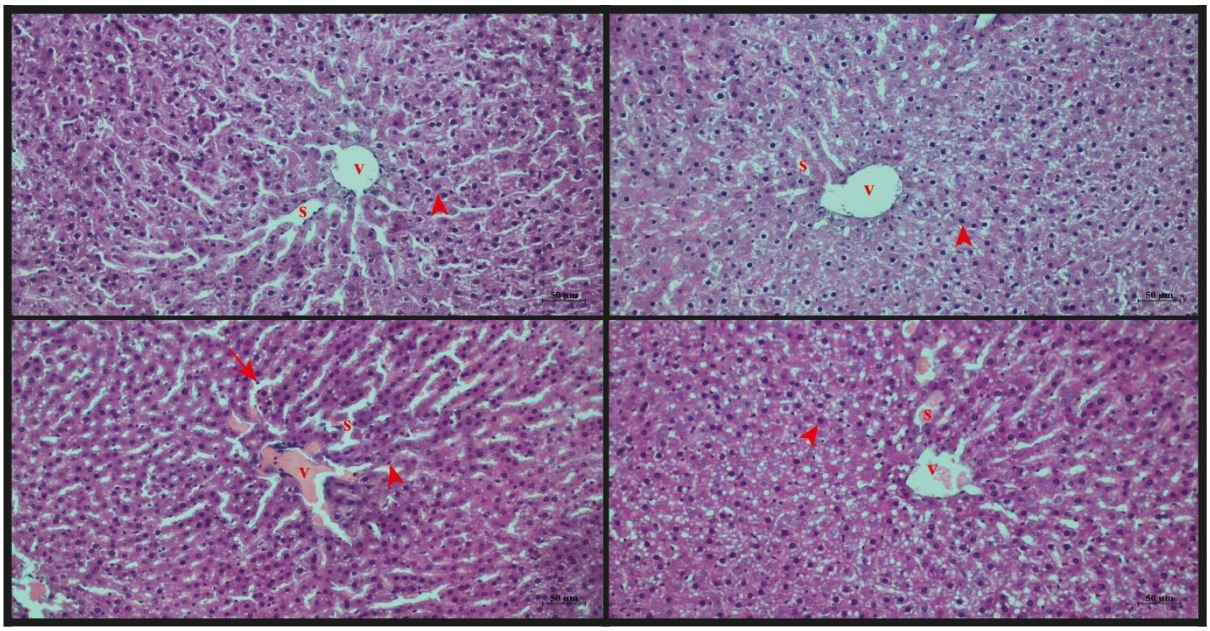

Figure 1. Sections of liver tissue. A) Control, B) Zonisamide, C) Ethanol, D) Ethanol+Zonisamide group. Zonisamide decreased alcohol toxicity. s: sinusoids, v: vena centralis, arrow: leukocyte infiltration, arrowhead: hepatocytes

DISCUSSION

In the findings of this study, normal liver tissue and vascular structures were observed in the control and zonisamide groups, while histopathologic findings in the liver tissue due to alcohol use were recorded in the ethanol group. It was observed that zonisamide treatment histologically improved the pathologies caused by alcohol toxicity and provided tissue regeneration.

It can be concluded that zonisamide reduces alcohol-induced cellular damage by protecting the structural integrity of hepatocytes and by supporting cytoprotective effects and cell survival.

The findings of this study provide important evidence for the protective effects of zonisamide against alcohol-induced liver injury. These results show several similarities and differences when compared with previous studies in the literature. For example, a study by He et al. focused on the antioxidant properties of zonisamide and showed that this drug protects nerve cells against oxidative stress [17]. This finding suggests that the antioxidant properties of zonisamide are not limited to the nervous system and may also protect against oxidative damage in other organs such as the liver. This is consistent with the findings obtained in our study, where zonisamide was observed to reduce alcohol-induced oxidative stress and protect the structural integrity of liver tissue.

Research on alcohol-induced liver disease requires ongoing evaluation and comparison of treatment approaches. For example, a study by Pandey et al. examined the potential benefits of antioxidant-based therapies such as silymarin, vitamin E and polyphenols in alleviating alcohol-induced liver injury [18]. This study suggests that antioxidants may play important roles in the prevention and treatment of liver injury by neutralizing free radicals and reducing inflammation. However, another study by Huang et al. emphasized that zonisamide may have protective effects on alcohol-induced liver injury [10]. The study by Huang et al. investigates whether zonisamide (ZNS) therapy reduces metabolic outcomes and nonalcoholic fatty liver disease in epilepsy patients. ZNS is a chemical with antiepileptic and antioxidant activities. This study evaluates the efficacy of ZNS therapy in reducing obesity and lowering vascular disease risks. The results of the study are characterized by ≥5% body weight reduction observed in patients after 12 and 24 weeks of ZNS therapy. ZNS treatment showed a significant reduction in serum levels of body weight, body mass index (BMI), HbA1c, triglycerides, hs-CRP and hepatic steatosis index (HSI) when adjusted for age, gender, time and baseline values. These results suggest that ZNS may be beneficial in patients with obesity and metabolic syndrome with high vascular risk. The results of the study support that agents that specifically modulate alcohol metabolism and oxidative stress may exert positive effects on the liver. However, a direct comparison of the results of these studies emphasizes the complexity of therapeutic strategies used in the management of alcohol-induced liver disease and that the effectiveness of these approaches may vary according to individual disease states. The findings of Huang and colleagues are a sign that the different findings in the existing literature indicate that more research is needed to support the protective effects of specific agents such as ZNS against alcohol-induced liver injury.

Our findings demonstrate a potential hepatoprotective effect of zonisamide against alcohol-induced liver injury and contrast with previous concerns regarding its hepatotoxicity. Although zonisamide, a sulfonamide anticonvulsant, is generally recognized for its neuroprotective roles, concerns regarding its hepatotoxic risks have persisted due to limited and inconsistent findings in the literature. For example, a review study by Kamitaki et al. highlights this uncertainty by noting that although zonisamide has been associated with potential liver injury, conclusive evidence is sparse and hepatotoxic risk has not been adequately characterized [19]. Our data contribute to this dialog by suggesting that, contrary to these hesitations, zonisamide may in fact play a protective role in the context of alcohol-induced liver injury. This protective effect may be attributed to its anti-inflammatory and antioxidant properties, which may reduce oxidative stress and cellular damage typically exacerbated by alcohol consumption. These observations warrant a reassessment of the hepatotoxic risk profile of zonisamide and emphasize the need for further studies to clarify its safety and efficacy in liver protection.

In the literature, there are many studies on the efficacy of various ingredients with antioxidant properties for the management of alcohol-induced liver injury. For example, a study by Granado et al. demonstrated the potential benefits of certain antioxidants in reducing alcohol-induced oxidative stress and improving liver function [20]. This study is consistent with our findings supporting that the protective effects of zonisamide against alcohol-induced liver injury may be mediated through antioxidant mechanisms.

CONCLUSION

This study, which evaluated the effects of zonisamide on alcohol-induced liver injury, presents results that are both consistent with the existing literature and different in several aspects. These differences call for a more in-depth investigation of the effects of zonisamide on the liver and the discovery of new approaches for the treatment of alcohol-induced liver injury. In this context, further research is needed to obtain more comprehensive information on the potential therapeutic use of zonisamide.

Acknowledgement

This study was part of doctoral thesis of Ph.D. candidate Nilüfer Dönmezdil. The ethical approval was taken from Local Ethics Committee of Animal experimantation (DUSAM), Dicle University (approval number: 75, date: 28/02/2023).

Conflıcts of Interest:

The authors do not have any conflicts of interests.

REFERENCES

1. Rossow I, Mäkelä P. Public health thinking around alcohol-related harm: Why does per capita consumption matter? J Stud Alcohol Drugs. 2021;82(1):9-17.https://doi.org/10.15288/jsad.2021.82.9 PMid:33573718

2. Shmulewitz D, Aharonovich E, Witkiewitz K, Anton RF, Kranzler HR, Scodes J, et al. The World Health Organization Risk Drinking Levels Measure of Alcohol Consumption: Prevalence and Health Correlates in Nationally Representative Surveys of U.S. Adults, 2001-2002 and 2012-2013. Am J Psychiatry [Internet]. 2021 Jun 1 [cited 2024 Apr 28];178(6):548-59. https://doi.org/10.1176/appi.ajp.2020.20050610 PMid:33472388 PMCid:PMC8222066

3. Şimşek Şeref, Dönmezdil Süleyman, Kakdaş Erkan, Özen Şakir. Diyarbakır Çocuk ve Ergen Madde Bağ. Araştırma ve Tedavi... Bağımlılık Dergisi. 2014;15(1):23-7.

4. Crabb DW, Im GY, Szabo G, Mellinger JL, Lucey MR. Diagnosis and Treatment of Alcohol-Associated Liver Diseases: 2019 Practice Guidance From the American Association for the Study of Liver Diseases. Hepatology [Internet]. 2020 Jan 1 [cited 2024 Apr 28];71(1):306-33. https://doi.org/10.1002/hep.30866 PMid:31314133

5. Stokłosa I, Więckiewicz G, Stokłosa M, Piegza M, Pudlo R, Gorczyca P. Medications for the Treatment of Alcohol Dependence-Current State of Knowledge and Future Perspectives from a Public Health Perspective. Int J Environ Res Public Health [Internet]. 2023 Feb 1 [cited 2024 Apr 28];20(3).

https://doi.org/10.3390/ijerph20031870 PMid:36767234 PMCid:PMC9915396

6. Pohl K, Moodley P, Dhanda AD. Alcohol's impact on the gut and liver. Nutrients. 2021 Sep 1;13(9). https://doi.org/10.3390/nu13093170 PMid:34579046 PMCid:PMC8472839

7. Dries SS, Seibert BS, Bastiani MF, Linden R, Perassolo MS. Evaluation of oxidative stress biomarkers and liver and renal functional parameters in patients during treatment a mental health unit to treat alcohol dependence. Drug Chem Toxicol. 2022;45(2):861-7. https://doi.org/10.1080/01480545.2020.1780251 PMid:32567384

8. Drugs and Lactation Database (LactMed®) - NCBI Bookshelf [Internet]. [cited 2024 Apr 28]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK501922/

9. Hossain MM, Weig B, Reuhl K, Gearing M, Wu LJ, Richardson JR. The anti-parkinsonian drug zonisamide reduces neuroinflammation: Role of microglial Na v 1.6. Exp Neurol. 2018 Oct 1;308:111-9. https://doi.org/10.1016/j.expneurol.2018.07.005 PMid:30017881 PMCid:PMC7404626

10. Huang CR, Chuang HY, Chen NC, Chen SF, Hsu CY, Chuang YC. Zonisamide Therapy Reduces Metabolic Consequences and Diminishes Nonalcoholic Fatty Liver Disease in Patients with Epilepsy. J Clin Med [Internet]. 2021 Jul 30 [cited 2024 Apr 28];10(15). https://doi.org/10.3390/jcm10153380 PMid:34362163 PMCid:PMC8347271

11. Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, et al. Hepatic steatosis index: A simple screening tool reflecting nonalcoholic fatty liver disease. Digestive and Liver Disease. 2010 Jul;42(7):503-8. https://doi.org/10.1016/j.dld.2009.08.002 PMid:19766548

12. Hamada K, Lasek AW. Receptor Tyrosine Kinases as Therapeutic Targets for Alcohol Use Disorder. Neurotherapeutics. 2020 Jan 1;17(1):4-16. https://doi.org/10.1007/s13311-019-00795-4 PMid:31617071 PMCid:PMC7007457

13. Anekwe C, Chandrasekaran P, Stanford FC. Ketogenic Diet-induced Elevated Cholesterol, Elevated Liver Enzymes and Potential Non-alcoholic Fatty Liver Disease. Cureus [Internet]. 2020 Jan 9 [cited 2024 Apr 28];12(1). https://doi.org/10.7759/cureus.6605 PMid:32064187 PMCid:PMC7008768

14. Cannella N, Ubaldi M, Masi A, Bramucci M, Roberto M, Bifone A, et al. Building better strategies to develop new medications in Alcohol Use Disorder: Learning from past success and failure to shape a brighter future. Neurosci Biobehav Rev. 2019 Aug 1;103:384-98. https://doi.org/10.1016/j.neubiorev.2019.05.014 PMid:31112713 PMCid:PMC6692218

15. Faingold CL. The majchrowicz binge alcohol protocol: An intubation technique to study alcohol dependence in rats. Curr Protoc Neurosci. 2008;(SUPPL. 44). https://doi.org/10.1002/0471142301.ns0928s44 PMid:18634000

16. Doğru C, Aşir F. Effect of ellagic acid on damage caused by hepatic ischemia reperfusion in rats. Eur Rev Med Pharmacol Sci [Internet]. 2022 [cited 2024 Apr 28];26(22):8209-15. Available from: https://pubmed.ncbi.nlm.nih.gov/36459004/

17. He J, Zhang X, He W, Xie Y, Chen Y, Yang Y, et al. Neuroprotective effects of zonisamide on cerebral ischemia injury via inhibition of neuronal apoptosis. Braz J Med Biol Res [Internet]. 2021 [cited 2024 Apr 28];54(4):1-8. https://doi.org/10.1590/1414-431x202010498 PMid:33656055 PMCid:PMC7917778

18. Liver Journal E, Pandey B, Baral R, Kaundinnyayana A, Panta S. Promising hepatoprotective agents from the natural sources: a study of scientific evidence. SpringerB Pandey, R Baral, A Kaundinnyayana, S PantaEgyptian Liver Journal, 2023•Springer [Internet]. 2023 Dec 1 [cited 2024 Apr 28];13(1). https://doi.org/10.1186/s43066-023-00248-w

19. Kamitaki BK, Minacapelli CD, Zhang P, Wachuku C, Gupta K, Catalano C, et al. Drug-induced liver injury associated with antiseizure medications from the FDA Adverse Event Reporting System (FAERS). Epilepsy and Behavior. 2021 Apr 1;117. https://doi.org/10.1016/j.yebeh.2021.107832 PMid:33626490

20. D Salete-Granado, C Carbonell, D Puertas-Miranda, VJ Vega-Rodríguez, M García-Macia, Oxidative Stress, and Alcoholic Liver Disease: A Systematic Review and Potential Clinical Applications. Antioxidants https://doi.org/10.3390/antiox12071425 PMid:37507963 PMCid:PMC10376811