ORIGINAL ARTICLE

ANNALS OF PUNJAB MEDICAL COLLEGE

Preserving Hepatic Glycogen Stores: Investigating the Therapeutic Influence of Vitamin E in Alcoholic Liver Injury-Induced Intracellular Carbohydrate Depletion

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Submitted for Publication: 06-04-2024
Accepted for Publication 23-05-2024

How to Cite: Wazir N, Khawaja TM, Khaliq H, Hayat W, Shehzad K, Faisal MS. Preserving Hepatic Glycogen Stores: Investigating the Therapeutic Influence of Vitamin E in Alcoholic Liver Injury-Induced Intracellular Carbohydrate Depletion. APMC 2024;18(2):85-88. **DOI: 10.29054/APMC/2024.1594**

ABSTRACT

Objective: The study aimed to explore and evaluate the effectiveness of Vitamin E in preventing or minimizing glycogen depletion in hepatocytes associated with alcoholic liver damage. **Study Design:** Experimental study. **Settings:** The study was conducted in the department of anatomy and animal facility of Peshawar Medical College, Peshawar Pakistan. **Duration:** February 2022 to April 2023. **Methods:** The study included eighteen male domestic rabbits (Oryctolagus cuniculus), classified according to the duration of the experiment. Rabbits in "Category E8" were exposed to an 8-week time frame, while those in "Category E4" experienced a 4-week experimental duration. Each classification was further segmented into three sections: "Control Group A" which was administered normal saline as a placebo; "Experimental Group B" received a 30% ethanol solution in distal water (30ml per kg/day) through a nasogastric tube; and "Experimental Group C" treated with a 30% ethanol solution in distal water (30ml per kg/day) administered along with "Vitamin E" (50mg dissolved in 2ml distal water per kg/day). Liver tissue specimens from all subjects underwent staining with PAS stain to quantify glycogen (carbohydrates) in hepatocytes. **Results:** A statistically significant variance in glycogen depletion within hepatocytes was noted between animals treated with vitamin E and those not treated with vitamin E in both E4 and E8 categories. Nonetheless, vitamin E treated rabbits exhibited higher glycogen levels compared to their non-vitamin E treated counterparts. **Conclusion:** In the realm of alcohol-induced liver damage, vitamin E provides the expected protective benefits by safeguarding glycogen (carbohydrate) stores within hepatocytes.

Keywords: *Alcoholic liver injury, Glycogen, PAS stain, Vitamin E.*

INTRODUCTION

Glycogen, a polysaccharide acts as a mechanism for energy storage in animals, including humans.[1] It is a storage form of glucose, stored in the liver and muscles for use when the body needs energy.[2] Glycogen within hepatocytes functions as a flexible storehouse of glucose, enabling the liver to adapt to fluctuations in energy requirements and uphold glucose balance in the body.[3]

The liver, facilitated by hepatocytes, assumes a vital role in maintaining glucose homeostasis.[4] Serving as a glucose buffer, it releases glucose as required and uptakes excess amounts, contributing to the regulation of blood glucose levels and ensuring a stable energy supply for the body. Following a rise in blood glucose levels, such as post-meal, insulin prompts hepatocytes to absorb glucose from the bloodstream. Subsequently, these hepatocytes convert the surplus glucose into glycogen through a process known as glycogenesis.[5] Glycogen essentially serves as a storage form of glucose, enabling the liver to reserve energy for later utilization.[6] In instances of decreased blood glucose levels, like during intervals

between meals or physical activity, the liver expels glucose into the bloodstream by initiating the breakdown of glycogen, a process termed glycogenolysis.[6] This action ensures a consistent provision of glucose for diverse tissues and organs, including the brain,[7]

The intake of alcohol can influence glycogen levels in the body, especially within the liver.[8] The presence of alcohol hinders the process of glycogen synthesis by liver.[9] Typically, after a meal, the body transforms surplus glucose into glycogen to store for later use.[10] Nonetheless, alcohol disrupts this normal course, resulting in diminished glycogen synthesis.[11] This may result in glycogen depletion, leading to diverse health consequences such as disturbances in energy metabolism, heightened susceptibility to hypoglycemia (low blood sugar), and potential long-term impacts on liver function.[12]

Research has explored the potential advantages of Vitamin E in addressing specific liver conditions, such as alcoholic liver disease (ALD).[13] Renowned for its antioxidant properties, Vitamin E can potentially counteract detrimental free radicals within the body.[14] This research examines how vitamin E may protect against glycogen depletion in the liver during episodes of alcoholic liver injury.

METHODS

The study was conducted in the Anatomy department of Peshawar Medical College, Peshawar. A selection was made of exclusively male, healthy adult rabbits belonging to the domestic breed. These rabbits were approximately 1-year-old, with a weight ranging from 1 to 1.5 kg. The chosen rabbits were accommodated in a controlled environment with free access to specially formulated laboratory feed and drinking water.

Grouping of Experimental Animals: To ensure a systematic approach, the rabbits were categorized into three main groups (each comprised of six rabbits).

"CONTROL GROUP A" received normal saline as a placebo through a pediatric NG tube. *"EXPERIMENTAL GROUP B"* received a daily oral dose of 30% ethanol solution (30ml/kg/day).[15] *"EXPERIMENTAL GROUP C"* received a daily oral dose of 30% ethanol solution (30ml/kg/day), along with 50mg/kg/day of vitamin E dissolved in 2ml of distilled water.[15-16] Each group was further divided into two sub-groups I and II, based on the duration of experiment. Sub-group AI, BI, and CI each comprised three rabbits had an experimental duration of 8-weeks (Category E8), while Sub-group AII, BII and CII each comprised three rabbits had an experimental duration of 4-weeks (category E4).

At the end of experiment, animals were anesthetized with isoflurane inhalation and cardiac perfusion maintained with normal saline and 4% paraformaldehyde. Following this, the entire liver was dissected and extracted for further processing.

Tissue Processing: The liver specimens were divided into segments and placed in 10% neutral buffered formalin for 24 hours for fixation. Following this, they were moved to newly prepared 10% neutral buffered formalin. A portion of each liver was meticulously processed and embedded in paraffin to create blocks for subsequent sectioning. Tissue sections, measuring 5µm in thickness, were created using a microtome. Subsequently, these sections underwent staining with Periodic acid-Schiff (PAS).

Microscopy: To conduct microscopic examination, three slides were randomly selected from each specimen and observed under 4x, 10x, and 40x magnifications. The quantification of glycogen in hepatocytes was performed at 10x, represented as a percentage of the total area covered by glycogen in the section, using ImageJ (Fiji) software.

Statistical comparisons between the groups were conducted utilizing the One-way ANOVA test, and within-group analyses were performed using independent sample t-tests. The statistical analysis was executed using SPSS-22, and a significance level of $P < 0.05$ was adopted.

RESULTS

Figure 1 displays the means and standard deviations for all study groups. In category E4, statistically highly significant differences in glycogen content percentages were observed among AII, BII, and CII groups, as well as between groups BII and CII, with P values for both comparisons being 0.000. Similarly, in category E8, statistically highly significant differences in glycogen content percentages were observed among AI, BI, and CI groups, as well as between groups BI and CI, with P values for both comparisons being 0.000. A noteworthy distinction in glycogen content within hepatocytes was observed between experimental groups BI and BII, with a significant P value of 0.013. Likewise, a significant difference in hepatocyte glycogen content was evident in the comparison between experimental groups CI and CII, with a P value of 0.001. This shows the time dependent increase in glycogen depletion in alcoholic livery injury. The outcomes of our study demonstrated notable therapeutic benefits of vitamin E in restricting glycogen depletion within hepatocytes during alcoholic liver injury, as illustrated in Figure 2.

Figure 1: Means and standard deviations of percentage of glycogen in hepatocytes of all groups in both category E4 and E8 animals

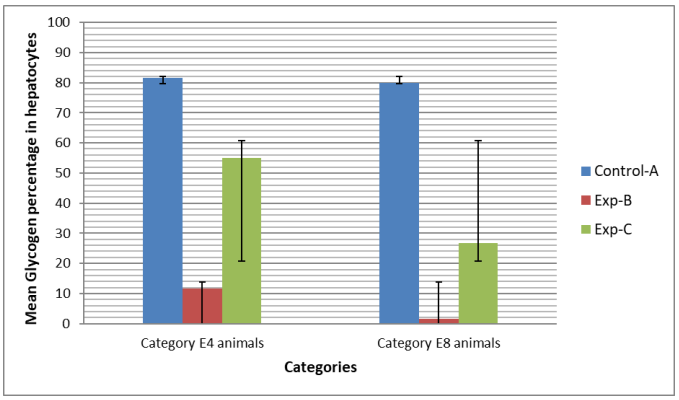
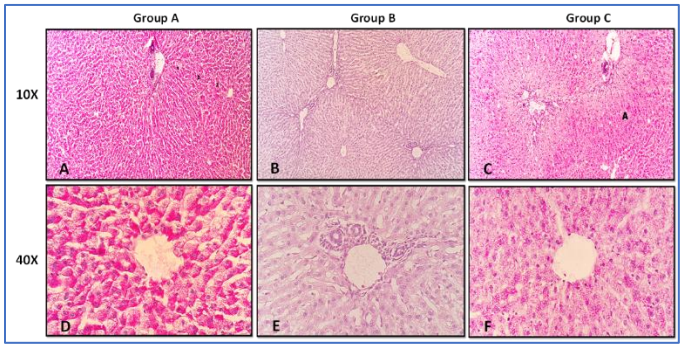


Figure 2: A photomicrograph depicting a 5µm thick PAS-stained sections from category E8 groups is presented at both 10X and 40X magnifications. In control group labeled as "Group A," images A and D reveal normal hepatocytes brimming with glycogen. In contrast, images B and E highlight a significant depletion of glycogen in "Group B", while vitamin E treated "Group C" exhibit a contrasting demonstration of glycogen abundance in images C and E



DISCUSSION

While concrete evidence directly supporting the positive therapeutic impact of vitamin E on alcoholic liver glycogen depletion is limited, vitamin E is recognized for its antioxidant properties. Several studies propose that it may play a protective role in liver health, particularly in conditions associated with alcohol consumption. Depletion of liver glycogen is frequently observed in alcoholic liver disease (ALD), playing a role in the disease's advancement. Vitamin E, a fat-soluble antioxidant, has been investigated for its potential therapeutic benefits in different liver conditions, including ALD. Nevertheless, the precise impact of vitamin E on liver glycogen depletion in ALD lacks well-established evidence. Liver glycogen plays a crucial role in regulating blood glucose levels to maintain homeostasis. Inadequate storage of liver glycogen can impact the body's energy regulation and overall well-being. Insufficient liver glycogen translates to a diminished supply of glucose for energy synthesis, potentially causing fatigue, weakness, and a noticeable decline in endurance. The role of liver glycogen in blood glucose level regulation becomes crucial, and poor storage may lead to erratic blood sugar levels, increasing the risk of hypoglycemia (low blood sugar) during fasting intervals or between meals.

The present study successfully confirms the initial hypothesis that vitamin E supplementation has positive effects on preventing liver glycogen depletion in individuals with alcohol use disorder. This is supported by the observed increase in liver glycogen levels in the group receiving vitamin E compared to the non-vitamin E treated group. Our study findings show resemblance with study in which researchers report the therapeutic effects of vitamin E and C in inhibiting liver amino acids reduced by fatigue-induced glycogen depletion[17]. The findings of current study are consistent with another investigation that demonstrated a noteworthy reduction in MDA levels and PKm2 expression, along with a substantial elevation in insulin levels, antioxidant markers (SOD and GPx), and GSk3 beta gene expression, when vitamin E, zinc, and selenium were administered concurrently. When compared to the diabetic group, these results underscore the potential hypoglycemic effects of the combined supplementation of vitamin E, zinc, and selenium. Furthermore, they emphasize the role of these supplements in the regulation of carbohydrate metabolism through the control of Pkm2 and GSk3 beta gene expression, contributing to the improvement of the antioxidant status in liver tissues[18].

By bolstering antioxidant defenses and reducing oxidative stress, vitamin E contributes to the preservation of hepatic glycogen levels, thereby potentially averting complications associated with glycogen depletion. The antioxidant properties of vitamin E may not only protect against glycogen depletion but also contribute to overall liver function and resilience in the face of alcohol-induced stress. However, further research is warranted to explore optimal dosage, long-term efficacy, and potential interactions with other treatments or interventions. Additionally, considering the complex nature of alcohol use disorder, a holistic approach that includes lifestyle modifications and other medical interventions should be considered for comprehensive management.

CONCLUSION

The study displayed an important role of vitamin E in mitigating adverse impact of alcohol on liver glycogen levels. It suggests that vitamin E supplementation could potentially prevent liver glycogen depletion, which is a common issue in alcohol-induced liver injury. This highlights the therapeutic potential of vitamin E as an adjunctive treatment for alcoholics, providing a non-

invasive and accessible intervention to improve liver health.

LIMITATIONS

The study's scope may have been constrained by a relatively small sample size, potentially impacting the applicability of its findings. Additionally, the duration of the intervention involving vitamin E treatment might not have been sufficiently prolonged to thoroughly evaluate its efficacy in safeguarding hepatic glycogen reserves. Furthermore, differences in compliance with the treatment regimen, including adherence to vitamin E supplementation, could vary among both human subjects and animal models, potentially influencing the outcomes of the study.

SUGGESTIONS / RECOMMENDATIONS

This study can be further strengthened by analyzing various biomarkers of liver injury. Key biomarkers include liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which can indicate liver cell damage. Additionally, assessing inflammatory markers like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) can provide insights into the inflammatory response associated with alcoholic liver injury. Measuring oxidative stress markers, such as malondialdehyde (MDA) and glutathione (GSH), will help evaluate the oxidative damage and antioxidant capacity within the liver. These comprehensive biomarker analyses will provide a detailed understanding of the therapeutic effects of Vitamin E in preserving hepatic glycogen stores and mitigating liver injury.

CONFLICT OF INTEREST / DISCLOSURE

There is no conflict of interest to declare in this research.

ACKNOWLEDGEMENTS

We express our heartfelt appreciation to everyone who contributed to the fulfillment of this research. We would like to offer particular recognition to Dr. Jahanzeb Khan, our supervisor, for his invaluable support, guidance, and assistance during the course of this study. Without his steadfast dedication and expertise, this research would not have been achievable.

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