ORIGINAL ARTICLE

VITAMIN E SHIELDS AGAINST ALCOHOLIC TOXICITY BY SAFEGUARDING HEPATIC PARENCHYMAL MORPHOLOGY AND LOWERING BLOOD ALT LEVELS

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Background: Alcohol consumption can have detrimental effects on the liver, as it plays a crucial role in processing and detoxifying substances in the body, including alcohol. Alcohol has the potential to hinder the liver's capacity, which results in a variety of metabolic imbalances and deficiencies. This research aimed to investigate alterations in the liver tissue due to alcohol administered orally, along with exploring the potential protective effects of vitamin E against these alterations. **Methods:** An assortment of male pet rabbits (totaling 18) was part of the study. The categorized groups included: Control group A, which received normal saline as a placebo treatment. Experimental group B, administered with an oral dose of a 30% ethanol solution mixed with normal saline. Experimental group C, given an oral dose of a combination containing a 30% ethanol solution, vitamin E, and normal saline. At the end of experiment, blood samples were obtained to assess ALT levels, and liver tissue sections were stained with Hematoxylin and Eosin for microscopic analysis of hepatocyte structure. **Results**: Highly significant differences between the blood ALT levels, hepatocyte count/size/nuclear count/size, sinusoids size in control and experimental groups were observed. Vitamin treated rabbits showed preserved morphology of hepatocytes as compared to non-vitamin treated rabbits during alcohol consumption by showing less ballooning of hepatocytes and shrinkage of nuclei which are the main initial signs of hepatocytes damage. **Conclusion**: Frequent alcohol intake leads to swift alterations in liver tissue and blood ALT levels over brief spans, yet these effects may be reduced through the antioxidative properties of vitamin E.

Keywords: Alcohol toxicity; Hepatocytes; ALT; Vitamin E

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INTRODUCTION

Alcohol consumption can have detrimental effects on the liver, as it plays a crucial role in processing and detoxifying substances in the body, including alcohol.[1] Alcohol has the potential to hinder the liver's capacity to efficiently detoxify substances and process nutrients, which may result in a variety of metabolic imbalances and deficiencies.[2] When consumed, alcohol is metabolized by the liver through a series of chemical reactions that convert it into acetaldehyde, a toxic substance that damages liver cells.[3] Continuous excessive alcohol consumption can trigger liver inflammation, termed alcoholic hepatitis, manifesting symptoms like jaundice, abdominal discomfort, fever, queasiness, and vomiting.[4] The severity of alcoholic hepatitis can vary from mild to severe, posing a potential threat to life. Chronic alcohol consumption can lead to inflammation and damage to liver tissue over time, potentially progressing to more severe conditions like hepatitis and cirrhosis.[5]

Hepatocytes, the primary operational units of the liver, play a crucial role in several metabolic functions such as detoxification, synthesizing proteins, and producing biochemicals essential for digestion.[6] Alcohol consumption can profoundly impact hepatocytes, largely because of the harmful substances generated during alcohol metabolism. The breakdown of alcohol produces reactive oxygen species (ROS) and other detrimental compounds, initiating oxidative stress within hepatocytes.[7] This oxidative stress can compromise cellular functionality, leading to liver damage and inflammation. Long-term alcohol consumption can cause damage to these cells, affecting their ability to detoxify substances and produce essential proteins.[5]

Prolonged alcohol intake may also result in fatty changes in hepatocytes, leading to fat buildup within the liver cells. This condition, known as

alcoholic fatty liver disease, can further impede liver function and increase the risk of severe complications.[8] Excessive alcohol consumption can also impact hepatic sinusoids, specialized blood vessels within the liver. Chronic alcohol use can cause inflammation and damage to these sinusoids, disrupting effective blood and nutrient transport which contributes to conditions such as portal hypertension - increased pressure in certain blood vessels within the liver.[9] ALT, or alanine transaminase, alternatively referred to as SGPT or serum glutamate-pyruvate transaminase, is an enzyme predominantly present in the liver and holds a critical function in amino acid metabolism. Elevated levels of ALT in the bloodstream may signal liver damage or illness. Prolonged and excessive alcohol intake can contribute to liver damage and inflammation, leading to a persistent increase in ALT levels.[10]

Vitamin E is recognized for its antioxidative properties, which can be advantageous for liver health.[11] It helps shield liver cells from oxidative harm due to alcohol metabolism and decreases inflammation in the liver.[12] Moreover, Vitamin E has been found to contribute to diminishing fibrosis (formation of scar tissue) in the liver, a consequence of chronic alcohol use.[13] Understanding the complete impact of alcohol on the structure and function of the liver is crucial for healthcare providers and individuals alike, highlighting responsible alcohol consumption as well as early detection of signs indicating potential liver damage.

MATERIAL AND METHODS

After approval from Institutional Review Board, the research was conducted at the Anatomy department of Peshawar Medical College in Peshawar, Pakistan from February 2022 to June 2023. The study involved eighteen healthy male rabbits of the domestic breed, approximately one year old and weighing between 1 and 1.5 kg. Female Rabbits were excluded because of breeding issue as they are induced ovulators. Rabbits were housed in meticulously crafted iron enclosures featuring a natural ground base, and maintained within consistent environmental settings throughout the research period. All rabbits had access to a specialized laboratory diet and drinking water. To ensure systematic approach, they were divided into three main groups, each further subdivided into two sub-groups.

The rabbits were divided into three different categories: A, B, and C. Group A was the control group and received a normal saline placebo. Groups B and C were given 30% ethanol at a dose of 30ml per kg/day through a pediatric Nasogastric tube.[14] In addition to this, group C underwent an extra treatment where they were given vitamin E in distilled water at a dosage of 50mg/kg/day via nasogastric tube.[15] Each category was further divided into two subcategories - I and II based on the duration of the experiment: one for an 8-week period labeled as "category E8," while another for a 4-week period labeled as "category E4." This classification allowed for more detailed analysis over varying time frames within each category. At the conclusion of the experiment, anesthesia was administered to the animals through isoflurane inhalation. Subsequently, samples of blood were obtained from the external jugular vein to quantitatively analyze the levels of Alanine aminotransferase (ALT) in the blood. Normal saline and 4% paraformaldehyde were used for cardiac perfusion. Following this, the complete liver was meticulously dissected, extracted for subsequent processing, and readied for microscopic examination.

The liver specimens were sliced and then placed in 10% neutral buffered formalin for a day for fixation. Following this, they were moved to freshly made 10% neutral buffered formalin. A detailed paraffin processing and embedding procedure was carried out, ensuring that each liver sample resulted in blocks suitable for further slicing. Tissue sections measuring 5 µm in thickness were carefully prepared using a microtome. Subsequently, these sections underwent Masson Trichrome staining for future analysis. To perform light microscopic examination, three slides were randomly chosen from each specimen and observed at 4x, 10x, and 40x magnifications. The hepatocytes count and size along with hepatocyte nuclei size, were measured at 40X using ImageJ Fiji software in 10 random fields on each slide. Statistical comparisons among the groups were carried out using the One-way ANOVA test, while analyses within each group utilized independent sample t-tests. All statistical analyses were conducted with SPSS-22 software, using a significance level of $p<0.05$ to determine statistical significance.

RESULTS

The mean Blood ALT levels of subgroups categorized as E4 and E8 are depicted in Figure 1-A. In E4 animals' category, there was a high statistically significant difference between the blood ALT levels of control and experimental groups as the *p*-value was 0.000. Additionally, there was a statistically significant difference in ALT levels between experimental groups B-II and C-II presented by *p*-value of 0.013. In the E8 animals'

category, a statistically high significant difference presented by *p*-value of 0.000 was observed in ALT levels between experimental and control groups. Furthermore, *p*-value of 0.015 was noted between subgroups B-I and C-I which shows significant difference in ALT levels between these groups. similarly, significant difference was evident when comparing experimental groups B-I and B-II as *p*-value was 0.005, and likewise, *p*-value of 0.05 between groups C-I and C-II in terms of ALT levels. The mean count of hepatocytes across all groups is depicted in Figure 1-B. Within the E4 animal category, a remarkably high and statistically significant difference in hepatocyte count was observed between the experimental and control groups, as indicated by a *p*-value of 0.000. Moreover, a similarly high and statistically significant difference in hepatocyte count was found between subgroups B-II and C-II, with a *p*-value of 0.000. In the E8 animal category, a statistically significant difference in hepatocyte count between experimental and control groups was noted with a *p*-value of 0.000. Additionally, a significant difference in hepatocyte count was observed between subgroups B-I and C-I, with a *p*-value of 0.000, indicating high statistical significance. Similarly, a significant difference was detected when comparing experimental groups B-I and B-II, with a *p*-value of 0.05. Likewise, a *p*-value of 0.05 was found between groups C-I and C-II concerning hepatocyte count, signifying a significant difference.

The figure depicted in Figure 1-C illustrates the mean size of hepatocytes across all groups. In E4 animals, there was a highly significant disparity in hepatocyte size between the experimental groups and control group in E4 category as the *p* value was 0.000. Likewise, there existed a notable contrast in the size of hepatocytes between experimental groups B-II and C-II, given the statistical significance with a *p*-value of 0.027. Within category E8, there was also a highly significant distinction in hepatocyte size between the control and experimental groups (p=0.000), and there was also a notable difference in size between experimental groups B-I and C-I, indicated by a *p*-value of 0.005. Moreover, notable variances in hepatocyte size were observed between the B-I and B-II subgroups, with a *p*-value of 0.001 indicating significance, as well as between the C-I and C-II groups, with a *p*-value of 0.017 demonstrating statistical significance.

The mean size of hepatocyte nuclei for all groups is depicted in Figure 1-D. There was no noteworthy difference detected in the size of hepatocyte nuclei between the experimental groups and the control group in E4 animals, given the *p*-value of 0.512. Similarly, there was no significant difference noted in between subgroups B-II and C-II as the *p*-value between them was 0.238. In the E8 category of animals, there was a notable difference in the size of hepatocyte nuclei between the experimental and control groups, as indicated by a *p*-value of 0.001. Additionally, subgroups B-I and C-I also exhibited significance, with *p*-values of 0.030. Additionally, there was a significant difference noted in hepatocyte nuclear size between sub groups B-I and B-II indicated by *p*-value of 0.001, as well as between sub groups C-I and C-II signified by *p*-value of 0.004.

The mentioned values signifies that there were noticeable differences in the count and size of hepatocytes, as well as the size of hepatocyte nuclei, between the control (A) and alcohol (B) groups, as shown in Figure 2, 3 & 4. Additionally, noticeable disparities are also observed between the alcohol (B) and vitamin E treated (C) groups. The alcohol-treated group exhibited a lower count of hepatocytes compared to the group treated with both alcohol and vitamin E. This difference can be attributed to increased ballooning and swelling in the alcohol-treated group, which resulted in greater space occupied by hepatocytes and fewer appearing in specific fields, as illustrated in figures 3 and 4. Likewise, there was more pronounced shrinkage observed in the nuclei of hepatocytes in group B, treated solely with alcohol, in comparison to group C, which received both alcohol and vitamin E treatment.

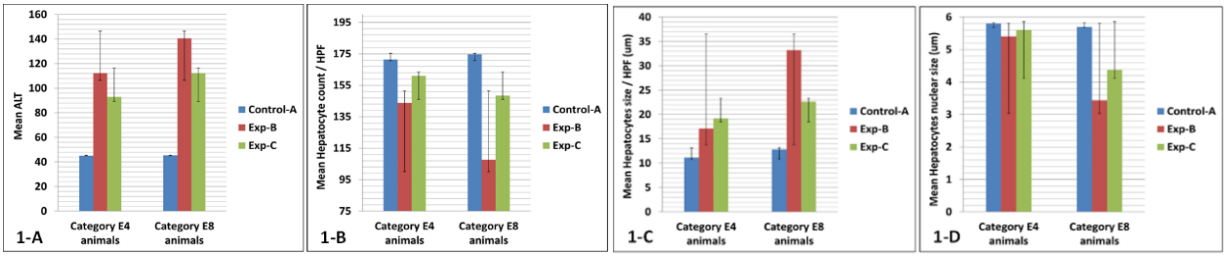

Figure-1: Mean and standard deviations of (1-A) blood ALT levels, (1-B) hepatocytes count per high power field/40X, (1-C) hepatocytes size per high power field/40X, (1-D) hepatocytes nuclei size per high power field/40X.

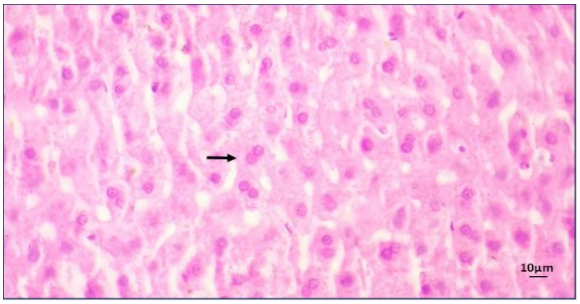

Figure-2: Photomicrographs of 5µm thick H&E-stained section of liver from control group showing normal hepatocytes morphology having arranged in hepatic cords at 400x magnification (scale bar 10µm). Arrow points at bi-nucleated hepatocyte with normal appearance of cell and nuclei.

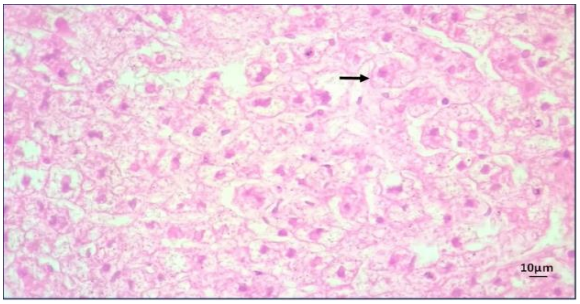

Figure-3: Photomicrographs of 5µm thick H&E-stained section of liver from experimental group B category E8 rabbit, showing hepatocytes ballooning with shrunken nuclei at 400x magnification (scale bar 10µm). Micro vesicular fatty steatosis can also be seen in hepatocytes. Arrow points at a swollen hepatocyte with shrunken pyknotic nucleus.

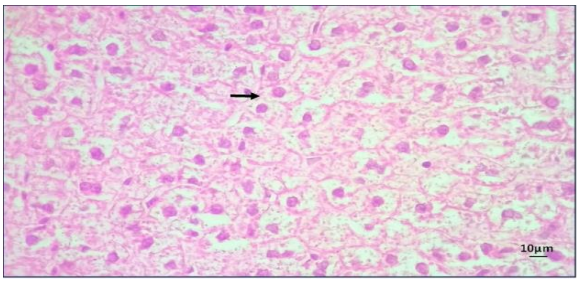

Figure-4: Photomicrographs of 5µm thick H&E-stained section of liver from experimental group C category E8 rabbit, showing hepatocytes ballooning with almost normal nuclei at 400x magnification (scale bar 10µm). Micro vesicular fatty steatosis can also be seen in hepatocytes. Arrow points at a swollen hepatocyte with normal nucleus.

DISCUSSION

This study provides strong evidence of the beneficial effects of vitamin E in preventing alcohol-related liver damage. The research methodology and results highlight the potential role of vitamin E in maintaining liver function and reducing the negative impact of alcohol on this organ. By focusing on hepatic parenchymal morphology and decreasing blood ALT levels, the study offers important insights into how vitamin E works to protect the liver. Understanding these mechanisms could be crucial for developing interventions and therapies for alcohol-induced liver injury. Our research findings supported previous studies indicating an elevation in ALT levels among alcoholic individuals[16]. Nevertheless, our findings opposed those of another research which discovered that vitamin E did not have a notable effect on decreasing ALT levels in cases of alcoholic liver disease, attributing it to vitamin E's limited antioxidant effectiveness against toxic alcohol metabolites.[17] The provision of vitamin E led to enhancements in serum aminotransferase levels, and the restoration of ALT levels typically correlates with better histological outcomes in disease activity, as per our findings.[18] Another study investigated the effect of vitamin E on liver damage following renal ischemia-reperfusion. It was observed that the increase in plasma AST and ALT induced by ischemia-reperfusion was mitigated in mice consuming vitamin E, indicating a reduction in liver damage to some extent due to vitamin E supplementation.[19] In our current study, the mean size of hepatocytes was highest in experimental group B (exposed only to alcohol), indicating hepatocyte ballooning, a characteristic feature of alcoholic hepatocyte injury. On the contrary, group B exhibited the lowest count of hepatocytes compared to group C in both categories. This highlights the promising role of vitamin E in averting or reducing hepatocyte death or damage induced by alcohol. Similar findings were reported in a human study conducted by researchers, demonstrating hepatocyte ballooning and necrotic nuclei in hepatocytes due to alcohol abuse.[20]

The results of this study add to the increasing research on the possible impact of vitamin E in supporting liver wellness. This connection may lead to new treatment methods for safeguarding the liver from alcohol-related harm. With growing interest in liver health, this study emphasizes the need for more exploration into how vitamin E could potentially protect the liver from alcohol-induced damage.

CONCLUSION

In conclusion, the study's findings shed light on the promising role of vitamin E in protecting the hepatic parenchymal morphology and reducing blood ALT levels, which are crucial factors in preventing alcohol-induced liver damage. This reinforces the potential for vitamin E to be incorporated into interventions and therapies aimed at preserving liver function in individuals at risk of alcohol-related liver injury. As research in this area continues to evolve, further exploration of the mechanisms by which vitamin E protects the liver is warranted. Ultimately, these insights may pave the way for novel strategies to promote

liver wellness and mitigate the deleterious effects of alcohol consumption on hepatic health.

AUTHORS' CONTRIBUTION

NUW: Conception of study or design of study. SH: Interpretation of the study. FS: Acquisition of study and data analysis. MH: Drafting the work. MK: Critical Review. SW: Final review and approval.

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