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ORIGINAL ARTICLE

Exploring protective potential of Vitamin E in mitigating liver steatosis in alcoholic liver injury.

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ABSTRACT… Objective: To investigate and assess the efficacy of Vitamin E in preventing or reducing fatty liver changes associated with alcoholic liver injury. **Study Design:** Experimental study. **Setting:** Department of Anatomy and Animal Facility of Peshawar Medical College, Peshawar. **Period:** February 2018 to April 2020. **Methods:** The study involved eighteen male domestic rabbits (Oryctolagus cuniculus), organizing them into categories based on the time frames specified for the research. Animals in "Category E8" were subjected to an 8-week time period, while those in "Category E4" underwent a 4-week experimental duration. Each category was further divided into three groups: "Control Group A" received standard laboratory food and daily access to normal saline as drinking water, "Experimental Group B" received standard nutrition, a 30% ethanol solution in distal water (30ml per kg/day) and normal saline for drinking, and "Experimental Group C" was treated with the necessary standard diet, a 30% ethanol solution in distal water (30ml per kg/day), and "Vitamin E" (50mg dissolved in 2ml distal water per kg/day) via nasogastric tube. Liver tissue specimens from all animals were stained with H&E and Masson's trichrome stain for quantification of fatty change. **Results:** A significant difference in steatosis development was observed among the E4 groups and among the E8 groups having a respective p-values of 0.001 and 0.003. This underscored the impact of alcohol within the context of alcohol-induced liver injury. However, no appreciable differences were noted between BI & CI and BII & CII (p-values > 0.05) indicating no significant distinction in liver steatosis between subjects treated with vitamin E and those not receiving vitamin E. **Conclusion:** In the context of alcohol-induced liver injury, the study failed to deliver anticipated protective benefits of vitamin E. There is a possibility of adverse effects, potentially rendering its use counterproductive.

Key words: Alcohol, Liver Steatosis, Vitamin E.

INTRODUCTION

Alcoholic liver injury refers to the damage and inflammation that can occur in the liver due to excessive and prolonged alcohol consumption.[1] The liver plays a crucial role in processing and metabolizing alcohol, but chronic alcohol abuse can overwhelm the liver's ability to function properly, leading to various forms of liver damage.[2] Fatty Liver Disease (Alcoholic Steatosis) is the early stage of alcoholic liver disease and is characterized by the accumulation of fat in liver cells. It often has no symptoms, but it can progress to more severe conditions if alcohol consumption continues.[3] Chronic alcohol consumption can disrupt the balance of fat metabolism in the liver. The breakdown of fatty acids is impaired, leading to an increased accumulation of fat within liver cells.[4] If alcohol consumption continues, alcoholic steatosis may progress to more severe forms of liver disease, such as alcoholic hepatitis and cirrhosis. Therefore, early intervention and lifestyle changes are crucial to prevent further damage.[5]

As the detrimental consequences of daily alcohol consumption become increasingly apparent specially in west, understanding how vitamin E antioxidant properties may mitigate the development of fat accumulation in liver cells presents a crucial avenue for potential intervention and improved liver health in the context of alcohol-related liver damage.[6] This exploration aims

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to shed light on the intricate interplay between vitamin E and alcoholic liver steatosis, paving the way for informed strategies to counteract this prevalent health concern.[7] This study delves into the investigation of the protective capabilities of vitamin E in alleviating liver steatosis amidst alcoholic liver injury.

METHODS

The study was conducted in the anatomy department of Peshawar Medical College, Pakistan after ethical approval vide letter no: Prime/IRB/2017-547. A selection was made of exclusively male, healthy adult rabbits, totaling 18 individuals, 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 meticulously crafted iron cages featuring a natural soil base and a standardized controlled environment. Each rabbit within every group had access to specially formulated laboratory feed and drinking water without any restrictions.

Grouping of Experimental Animals

To ensure a systematic approach, the rabbits were categorized into three groups:

"CONTROL GROUP A" consisting of six animals, received a consistently standardized laboratory diet, and normal saline was supplied for drinking. Additionally, this group was further subdivided into two subgroups based on the experimental period. Subgroup A-I comprised three rabbits with an experimental duration of 8 weeks (category E8), while Subgroup A-II consisted of three rabbits with a 4-week (category E4) experimental duration.

"EXPERIMENTAL GROUP B" in the experimental set comprised six rabbits. These rabbits were administered a standardized laboratory diet, received a daily oral dose of 30% ethanol solution (30ml/kg/day)[8] through a pediatric Nasogastric tube, and were provided with normal saline as their drinking water. Additionally, this group was further divided into two subgroups based on the experimental period. Subgroup B-I included three rabbits with an 8-week (category E8) experimental duration, while Subgroup B-II consisted of three rabbits with a 4-week (category E4) experimental duration.

"EXPERIMENTAL GROUP C", designated as the experimental group, consisted of six rabbits provided with unrestricted access to a standard laboratory diet. Additionally, they were administered 30% ethanol at a dosage of 30ml per kg/day[8], along with 50mg per kg/day vitamin E[9] dissolved in 2ml of distilled water through a nasogastric tube on a daily basis. Normal saline served as their drinking water. Within this group, there were two subgroups based on the experimental period. Subgroup C-I included three rabbits with an 8-week (category E8) experimental duration, while Subgroup C-II comprised three rabbits with a 4-week (category E4) experimental duration."

Tissue Processing

An official chemical supplier provided ethanol, manufactured with a purity of 99.9% by BDH Laboratories, England. Subsequently, a 30% ethanol solution was created by diluting it in distilled water. Vitamin E in powder form was procured from Abbott Pharmaceuticals, Pakistan. To prepare the Vitamin E solution, 50mg of the powder was mixed with 2ml of distilled water. Animals in Category E4 were anesthetized after 4 weeks, while those in Category E8 were anesthetized after 8 weeks. Cardiac perfusion with normal saline and 4% paraformaldehyde was performed. The livers were dissected into segments and immersed in 10% neutral buffered formalin for 24 hours to undergo fixation. Subsequently, they were transferred to freshly prepared 10% neutral buffered formalin. A portion of each subject's liver was meticulously processed and embedded in paraffin to create blocks for subsequent sectioning.

Microscopy

Thin sections of tissues, 5μm in thickness, were prepared using a microtome. Masson's Trichrome and H&E staining were conducted to quantify fatty changes. For microscopic examination, three slides were randomly chosen from each specimen and observed under 4x, 10x, and 40x

magnifications. Fatty change in hepatocytes was graded as no change = 0, mild change = 1, moderate change =2 and severe change = 3.[10] Scoring numbers were given to each grade for calculating mean and standard deviation. Statistical analysis for comparison between the groups employing the One-way ANOVA test and within the groups, independent sample T test, was performed using SPSS-22, with a P-value of <0.05 considered statistically significant.

RESULTS

Figure-1 displays the means and standard deviations for all study groups. The application of the One-way ANOVA test revealed a statistically significant p-value of 0.001 between groups AII, BII, and CII. Likewise, a highly significant p-value of 0.003 was observed between groups AI, BI, and CI. This outcome underscored the impact of alcohol within the context of alcohol-induced liver injury, revealing its capacity to initiate fatty changes (steatosis) in the liver. Notably, these alterations can be manifested even in a relatively brief timeframe, spanning 4 to 8 weeks. The findings emphasize the swift onset of alcohol-induced steatosis, shedding light on the rapidity with which alcohol can contribute to liver fat accumulation in the context of liver injury.

The Independent T-test was not performed between group AI and AII as both of these groups served as control groups, exhibiting normal liver architecture without any occurrence of fatty changes. There was no statistically significant difference between groups BI and CI, as well as groups BII and CII, with p-values of 1.00 for both comparisons. These findings indicate that there was no significant distinction in liver steatosis (fatty change) between subjects treated with vitamin E and those not receiving vitamin E. This implies that vitamin E does not play a preventive role in the early development of liver steatosis resulting from alcohol consumption as shown in Figure-2, 3 and 4.

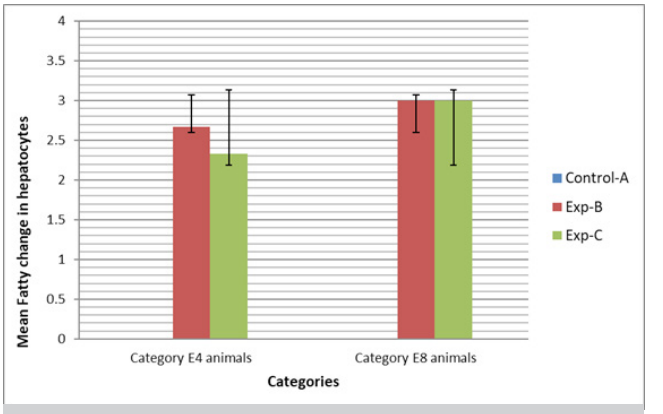

Figure-1. Means and standard deviations of fatty change in hepatocytes of all groups in both category E4 and E8 animals.

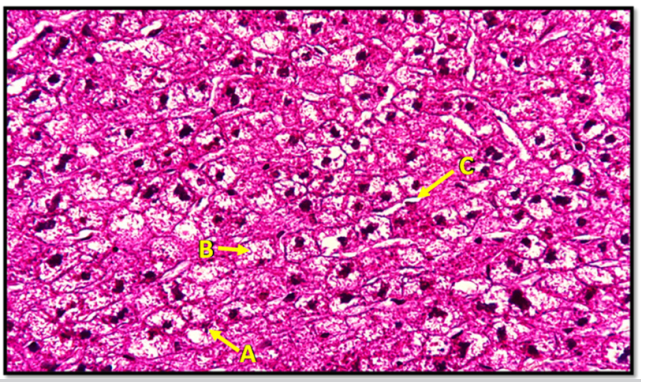

Figure-2. Photomicrograph of 5μm thick Masson's Trichrome stained section from the rabbit liver of group B-II showing A: Enlarged hepatocyte with shrunken small nucleus. B: Micro-vesicular fatty change in hepatocyte. C: Decreased size of sinusoids 450X.

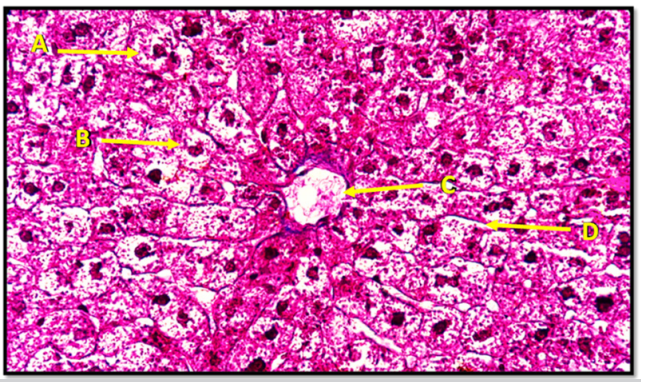

Figure-3. Photomicrograph illustrating a 5μm thick section of a Masson's Trichrome stained sample from the rabbit liver of group C-II. The image reveals: A. An enlarged hepatocyte with a shrunken picnotic nucleus, B. Micro vesicular fatty change observed in hepatocytes, C. Reduction in the size of the central vein accompanied by the presence of fibrous tissue around it & D. Decrease in the size of hepatic sinusoids, observed at 40X magnification.

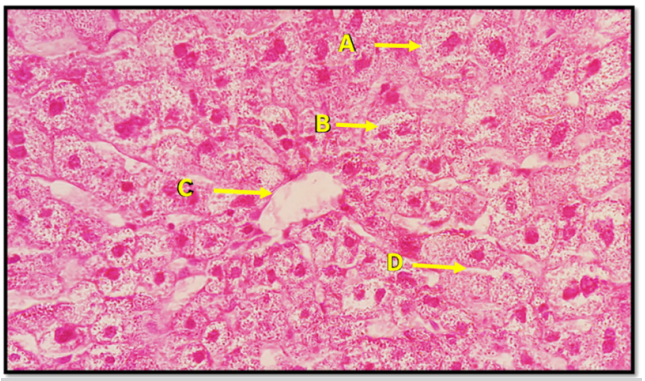

Figure-4. A photomicrograph depicts a 5μm thick H&E-stained section from the rabbit liver of group B-I. The image reveals: A) Enlarged hepatocyte with a condensed nucleus, B) Hepatocyte exhibiting micro-vesicular fatty change, C) Reduction in the size of the central vein, and D) Reduction in the size of hepatic sinusoids at 40X magnification.

DISCUSSION

Liver steatosis refers to the accumulation of fat in liver cells, and it is a common feature of alcoholic liver disease. In this study, the degree of fatty alteration was consistent across all experimental groups for animals in categories E4 and E8. This finding leads to the conclusion that vitamin E does not have a mitigating effect on alcohol-induced fatty changes in hepatocytes over a short duration. We observed a micro-vesicular fatty change, whereas Rabinowich L and Shibolet O reported macro-vesicular changes in hepatocytes induced by alcohol consumption.[11] Duly A and Alani B conducted a study on mice, which also noted the presence of macro-vesicular fatty changes in the liver as a result of alcohol consumption.[12] Both these studies therefore do not corroborate our study as far as the type of fatty change is concerned and the reason might be the difference in the animals used in their experiment. Upon reviewing the existing literature, we could not identify studies that specifically assessed the protective effects of vitamin E against hepatic steatosis in the context of alcoholic liver injury. Consequently, we compared our study findings with research that has explored the protective role of vitamin E in non-alcoholic hepatic steatosis.

The outcomes of our study diverge from the research findings that indicated the effective prevention of the transition from simple steatosis to steatohepatitis in mice lacking Phosphatidylethanolamine N -methyltransferase (PEMT) through vitamin E treatment.[13] The current findings exhibit parallels with a previous study, revealing that the supplementation of vitamin E did not successfully mitigate the histological characteristics of non-alcoholic steatohepatitis (NASH) and did not curtail the progression of NASH pathogenesis in genetically obese mice. Contrarily, it led to an elevation in certain markers associated with metabolic dysfunction.[14] Our study outcomes diverge from the research findings that illustrate the significant reduction in various parameters, including AST, ALT, ALP, GGT, TC, TG, LDL, VLDL, plasma glucose, hepatic lipid peroxidation (MDA), hepatic mRNA expression of inflammatory cytokines (IL-1β and TNFα), and mRNA expression of hepatic SREBP-1c in rats treated with varying doses of choline and vitamin E, as compared to the non-alcoholic fatty liver disease (NAFLD) group. Additionally, the treated groups exhibited a noteworthy increase in total protein and albumin levels, HDL, insulin, antioxidant enzymes, mRNA expression of fatty acid oxidation genes, and mRNA expression of hepatic PEMT compared to the non-alcoholic fatty liver disease group.[15]

Our findings may indicate that the use of vitamin E, in this specific scenario of alcohol-induced liver injury, does not yield the expected protective benefits. It is crucial to analyze these outcomes within the framework of the study design, methodology, and the particular species under investigation. Further research and clinical trials may be needed to validate these findings and to explore alternative approaches for managing liver steatosis in the context of alcohol-related liver injury. Additionally, healthcare professionals should consider these results when making recommendations for patients with alcoholic liver disease and liver steatosis.

CONCLUSION

Our study's observations suggest that vitamin E, often acknowledged for its antioxidant properties and presumed protective benefits, might not prove effective and could potentially worsen liver

steatosis when dealing with alcoholic liver injury.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Muhammad Saleh Faisal	Study design, Data analysis, Manuscript write-up.	
3	Mohammad Tamhid	Statistical analysis, Bibliography.	
4	Hafsa Khaliq	Data analysis and curation.	
5	Zainab Irshad	Critical review, Editing final draft.