ORIGINAL ARTICLE

INFLUENCE OF VITAMIN E ON PANCREATIC ACINAR CELL MORPHOLOGY AND SERUM AMYLASE CONCENTRATIONS IN ALCOHOL-INDUCED PANCREATIC TOXICITY

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Background: Misusing alcohol can cause damage to different tissues in the body, resulting in conditions like alcoholic liver disease, pancreatitis, cardiomyopathy, neurotoxicity, muscle wasting, weakened immune system, hormonal disruptions, birth defects, and bone loss. The objective of this research was to evaluate how alcohol affects the exocrine pancreas histology and the levels of amylase in the blood serum. Additionally, it aimed to explore whether vitamin E provides a safeguard against alcohol-induced harm to the pancreas in rabbits. **Methods:** A laboratory-based experimental investigation was carried out at Peshawar Medical College involving eighteen healthy adult male domestic rabbits weighing between one to one and a half kilograms each. The rabbits were divided into three groups. Group A, serving as the control, received normal saline as a placebo. Group B was administered a daily dose of 30 percent ethanol solution (30 ml/kg/day) in normal saline. Group C received a daily oral dose of 30% ethanol solution (30 ml/kg/day) in normal saline along with vitamin E (50 mg/kg/day). Blood samples were collected for serum amylase analysis, while morphological assessment of acinar cells involved evaluating cell count, acinar size, acinar cell size, and acinar nucleus size. **Results:** Serum amylase levels did not exhibit a statistically significant variance between the control and experimental groups as *p*-value was >0.05. Furthermore, no notable distinctions were noted in the size and number of pancreas acini, cells of pancreatic acini, and pancreatic acinar cells nuclei between the control and experimental groups in both category E4 and Category E8, as $p > 0.05$. **Conclusion:** There were no significant variations noted in the size and number of acini in pancreas, cells in pancreatic acini, and nuclei of cells in pancreatic acini. Consequently, the protective role of vitamin E against alcohol-induced pancreatic damage was not conclusively identified.

Keywords: Serum Amylase; Alcohol; Pancreatic acini; Vitamin E

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INTRODUCTION

Misusing alcohol can cause damage to different tissues in the body, resulting in conditions like alcoholic liver disease, pancreatitis, cardiomyopathy, neurotoxicity, muscle wasting, weakened immune system, hormonal disruptions, birth defects, and bone loss.[1] The pancreas is particularly susceptible to alcohol's effects, with prolonged consumption causing irreversible damage, primarily through recurrent acute pancreatitis episodes. Alcohol metabolism yields harmful metabolites, inducing oxidative stress and tissue damage. Vitamin E, functioning as an antioxidant, scavenges peroxyl radicals, thwarting free radical-induced tissue harm.[2] The breakdown of alcohol produces diverse chemicals across bodily tissues, generating reactive oxygen species, commonly known as free radicals, exacerbating tissue damage.[3] Moreover, alcohol heightens the activity of cytochrome P450s, notably ethanol-stimulated cytochrome P4502E1 (CYP2E1), a pivotal contributor to alcohol-induced tissue damage via reactive oxygen species, thereby inducing oxidative stress.[4] Additionally, ethanol increases gut permeability, facilitating the entry of endotoxins generated by gut bacteria.[5]

Although alcohol is known to induce severe pancreatic damage, the precise pathways through which it initiates this damage remain incompletely understood.[6] A key byproduct formed when the body metabolizes alcohol, acetaldehyde, causes harm to pancreatic acinar cells, affecting the generation and function of oxygen-based free radicals.[7] Free radicals generated during alcohol metabolism disturb the structure of the cell membrane's lipid bilayer by triggering peroxidation, which weakens the membrane's integrity. Continuous alcohol intake decreases bicarbonate levels and the volume of fluid in pancreatic secretions, causing the formation of protein and calcium crystals in pancreatic ducts, leading to blockage of the ducts over time.[8] This obstruction contributes to the destruction of pancreatic acini, leading to pancreatitis primarily associated with acinar cell damage.[9]

Alcohol is known to cause a range of harmful effects on acinar cells. These cells have been seen to metabolize alcohol using both oxidative and nonoxidative pathways.[10] Even after thorough research on whether differences in ethanol-metabolizing enzymes play a role in chronic pancreatitis, a conclusive connection hasn't been confirmed.[11] The metabolites and byproducts produced have a damaging effect on the pancreas, causing both short-term and long-term changes.[12] However, the specific susceptibility factor responsible for triggering overt illness remains unidentified. In addressing recurring pancreatitis and pancreatic discomfort not associated with gallstones, micronutrient antioxidant therapy emerges as a promising therapeutic approach.[13]

MATERIAL AND METHODS

Following approval from the Institutional Review Board (Prime/IRB/2017-547), analytical experimental research was conducted at the Pathology, Anatomy, and Pharmacology Departments of Peshawar Medical College in Peshawar, Pakistan, from January 1, 2018, to June 30, 2021. For this study, we selected eighteen male adult domestic rabbits weighing between one and one and a half kilograms. Female rabbits were omitted from the study due to concerns related to induced ovulation associated with breeding. Moreover, rabbits younger than 6 months or older than 2 years were not included. The rabbits were accommodated in custom-designed iron cages with natural earth flooring and supplied with a standardized laboratory diet and water. Subsequently, they were segregated into three primary groups for subsequent examination.

Group A (control), consisting of six rabbits, was fed a standard diet and administered saline. Later, this group was split into two subgroups based on the trial's duration. Subgroup A-I comprised three rabbits undergoing an 8-week trial, while Subgroup A-II consisted of three rabbits undergoing a 4-week trial. Group B (experimental) in the experiment included rabbits given a regular diet along with daily oral doses of a 30% ethanol solution (30 ml/kg/day) and normal saline.[14] Like Group A, this group was divided into two subgroups based on the length of the trial. Subgroup B-I consisted of three rabbits tested for 8 weeks, while Subgroup B-II comprised three rabbits tested for 4 weeks. Group C (experimental), consisting of six rabbits, received a standard diet supplemented with a daily oral dose of a 30% ethanol solution (30 ml/kg/day), along with vitamin E (50 mg/kg/day), and normal saline.[14,15] Similar to the other groups, Group C was split into two subgroups based on the length of the trial. Subgroup C-I comprised three rabbits for an 8-week experimental period, while Subgroup C-II included three rabbits for a 4-week experimental period.

The previously mentioned subgroups were categorized into two main groups according to the length of the experimental period. Category E4 comprises animals from subgroups A-II, B-II, and C-II, all of which underwent a 4-week experimental period. On the other hand, Category E8 consists of animals from subgroups A-I, B-I, and C-I, all of which were subjected to an 8-week experimental period. On the final day of the experiment, each rabbit was administered anesthesia using ketamine HCL via intramuscular injection at a dosage of 1 ml/kg of body weight. Blood samples were then collected from the external jugular vein for quantitative analysis of serum amylase levels. Subsequently, a meticulous dissection of abdomen was carried out. The viscera in abdomen were recognized, and special care was taken to carefully remove the left lobe of the pancreas. The left lobe was specifically chosen for study due to its significance, as the majority of the rabbit's pancreatic tissue is located within it, while the right lobe is more dispersed and therefore unsuitable for detailed examination. After processing and sectioning, tissues staining was performed by H&E stain. Microscopic analysis of tissues was performed and microscopic pictures were taken on 10X, 40X and 100X magnifications. Two slides from each subject were made in each group and in each slide, measurements and calculations were made in five random fields by ImageJ Fiji software. Cells count in ten acini/field at 40X, acini size (10 acini/field) at 40X, size of acinar cells (in 10 acini/field) at 40X, and size of acinar cells nuclei (in 10 acini/field) at 40X. The histological assessment data mentioned earlier was inputted into SPSS version 22 for analysis. Descriptive statistics, including means and standard deviations, were computed. As the data satisfied parametric assumptions, a One-way ANOVA test was employed to compare data across groups AI, BI, CI, and AII, BII, CII. Additionally, an Independent Samples T-test was utilized to compare data within each group A, B, and C. A significance level of less than 0.05 was deemed significant.

RESULTS

Figure 1-A illustrates the mean serum amylase levels of subgroups labeled E4 and E8. When comparing the serum amylase levels between the control and experimental groups within the E4 and E8 categories, no significant differences were found, with *p*-values of 0.980 and 0.901, correspondingly. Mean counts of pancreatic acinar cells in both categories are illustrated in Figure 1-B. The analysis showed statistically insignificant result in pancreatic acinar cell counts among the experimental and control groups in category E8 and E4, with *p-values* of 0.051 and 0.318, respectively. Similarly, the mean size of pancreatic acini in both categories is displayed in Figure 1-C. The comparison indicated non-significant differences between the control and experimental groups in category E4 and E8, with *p*-values of 0.053 and 0.348, respectively. The mean size of

pancreatic acinar cells in both categories is presented in Figure 1-D, showing statistically insignificant differences between the control and experimental groups in category E4 and E8, with *p*-values of 0.733 and 0.057, respectively. Lastly, the mean size of pancreatic acinar cell nuclei in both categories is depicted in Figure 1-E, with insignificant differences observed between the control and experimental groups in category E4 and E8, having *p*-values of 0.440 and 0.160, respectively. The values mentioned above indicate that there is insignificant alteration in the morphology and function of pancreatic acini, the exocrine part of the pancreas, between rabbits treated with vitamin E and those not treated, during alcohol consumption, as illustrated in Figure 2.

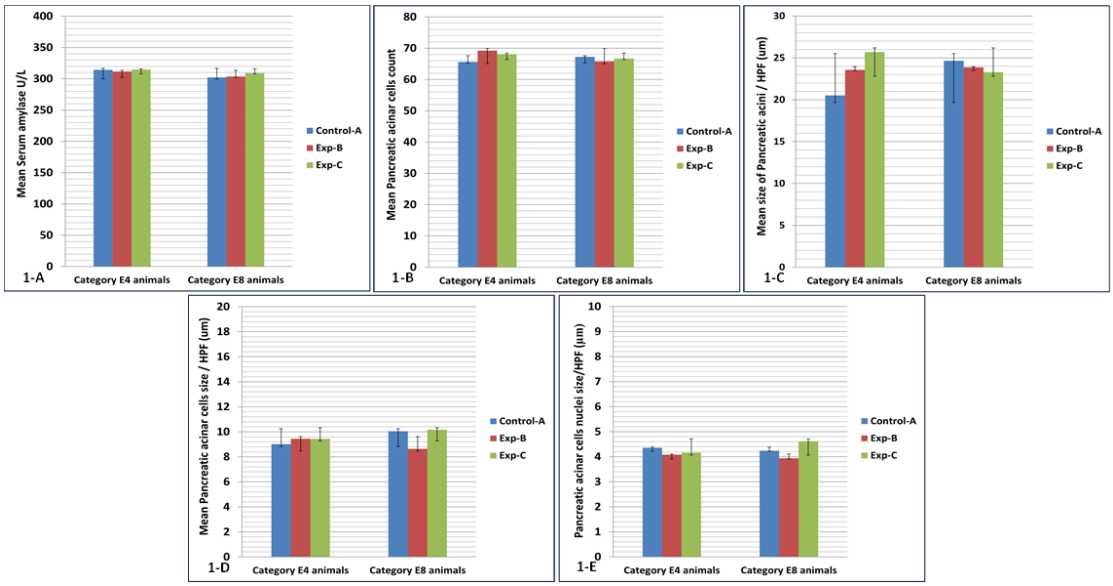

Figure-1: Mean and standard deviations of (1-A) serum amylase, (1-B) cells count in pancreatic acini, (1-C) pancreatic acini size, (1-D) cells size in pancreatic acini and (1-E) cells nuclei size in pancreatic acini in both category E4 & E8.

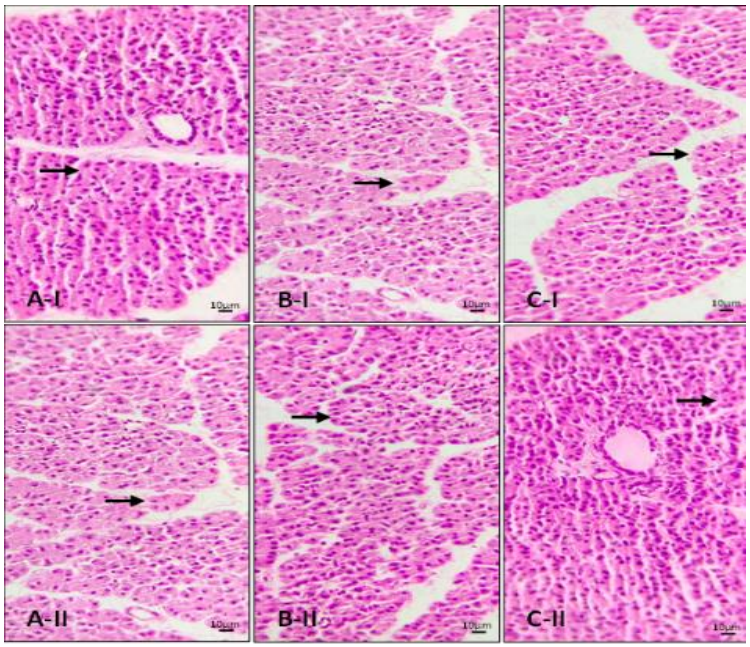

Figure-1: Photomicrographs of 5µm thick H & E stained sections of pancreas from all groups showing pancreatic acini with almost equal size and number of pancreatic acini, cells and nuclei count and size in pancreatic acini at 40x magnification (scale bar 10µm). Arrows points at pancreatic acini having acinar cells with nuclei.

DISCUSSION

Vitamin E is a well-known antioxidant that has been studied for its potential benefits in various health conditions[16]. It has been shown to protect cells from damage caused by oxidative stress and free radicals.[16] In the context of alcohol-induced toxicity, the role of vitamin E in mitigating damage to the pancreas is of particular interest.[13] The present study investigated the impact of Vitamin E on serum amylase levels and pancreatic acini morphology in alcohol-induced toxicity. In this study, the researchers found that there was insignificant change in these parameters with the administration of Vitamin E. These results suggest that Vitamin E may not have a direct protective effect on pancreatic function in alcohol-induced toxicity. Same kind of results are demonstrated in a recent study where researchers have found no benefits of antioxidants like vitamin E in overcoming the chronic pancreatitis.[17] This finding contradicts previous studies that have shown the potential of Vitamin E as an antioxidant and its ability to protect against oxidative stress.[18] There is existing research that demonstrates the antioxidant properties of Vitamin E and its ability to mitigate oxidative stress in various organs and tissues. Some studies have also shown an association between Vitamin E supplementation and improved pancreatic health in non-alcohol-induced models of pancreatic damage.[19]

This study adds to the existing literature on the effects of Vitamin E in alcohol-induced toxicity. Furthermore, the study highlights the importance of considering other factors such as alcohol metabolism and glucose metabolism in understanding the overall impact on pancreatic function. Further understanding the impact of non-enzyme-based antioxidants in alcohol-induced pancreatic toxicity could provide valuable insights into the potential therapeutic interventions for managing pancreatic function. It's important to recognize that the effects of Vitamin E on pancreatic function can be influenced by various factors such as dosage, duration of supplementation, and the specific conditions being studied. Additionally, the interplay between different antioxidants and their combined effects on pancreatic health needs further exploration. There is evidence to suggest that a combination of antioxidants, including Vitamin E, may have a more pronounced impact on mitigating oxidative damage in the pancreas.

Further research is necessary to delve deeper into the potential benefits of Vitamin E in different contexts and to understand its role in pancreatic health beyond alcohol-induced toxicity. It's also important to consider the broader implications of antioxidant therapy in managing pancreatic function and exploring potential synergies between different antioxidants. This will provide a more comprehensive understanding of the complex mechanisms involved in maintaining pancreatic health and in developing targeted therapeutic strategies for pancreatic diseases.

CONCLUSION

In conclusion, the findings of this study suggest that Vitamin E has no significant impact on serum amylase levels and pancreatic acini morphology in alcohol-induced toxicity. This indicates that Vitamin E may not be an effective intervention in mitigating the harmful effects of alcohol on the pancreas. Further research is needed to explore alternative potential treatments for alcohol-induced pancreatic damage. Understanding the mechanisms underlying the impact of alcohol on the pancreas is crucial for developing effective therapeutic strategies to mitigate its detrimental effects.

AUTHOR CONTRIBUTION

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

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