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ORIGINAL ARTICLE

Collagen Deposition Reduction by Vitamin E in Alcoholic Liver Injury

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ABSTRACT

Background: Alcohol encourages fibrotic changes in liver by initiating collagen deposition in liver parenchyma. Vitamin E can slow down progress of fibrosis in alcoholic liver injury and play a defensive part against these changes.
Aim To observe difference in fibrotic changes in livers of vitamin E treated and non-treated rabbits exposed to intra-gastric administration of alcohol.
Study Design: Analytical experimental study
Place and duration of study: Department of Anatomy, Peshawar Medical College, Peshawar Pakistan from 1st May 2019 to 30th October 2019.
Methodology: Eighteen domestic type male rabbits (Oryctolaguscuniculus) were included. All animals were divided in two categories on the basis of experiment time frame. “Category E8” animals having 8 weeks’ time period and “Category E4” animals having 4 weeks experimental duration. Each category was then clustered in “Control Group A” (appropriate food as per standard laboratory requirements, and normal saline as drinking water was provided daily), “Experimental Group B” (proper standard nutrition, 30% solution of ethanol made in distal water in the dosage of 30ml per kg/day through nasogastric tube and normal saline for drinking purpose were provided daily) and “Experimental Group C” (treated with suitable standard required diet, 30% of ethanol solution made in distal water in the dosage of 30ml per kg/day via NG tube and “Vitamin E” 50mg dissolved in 2ml distal water per kg/day with same nasogastric tube daily and normal saline for drinking purpose were given).
Results: Collagen fibers deposition (fibrosis) in liver stroma was detected and recorded according to Knodel histological activity index. Quantitative difference in the collagen deposition among all groups from both “Category E4” and “Category E8” presented vastly significant “P” values.
Conclusion: Alcohol can cause major fibrotic changes in “liver” even if it is consumed for the minor duration of 4 to 8 weeks, and these alterations can be minimised by the proper use of vitamin e through its “antioxidant effect”.
Keywords: Alcohol, Liver, Fibrosis, Vitamin E, Hepatocyte, Collagen

INTRODUCTION

Worldwide Alcohol consumption has increased drastically in the recent years, predominantly in emerging countries and it is currently the fifth primary cause of deaths all around the globe[1].

Among all other reasons, alcohol numbers the 5th top most danger to the global disease load and incapacities. Internationally, 12.1% of the total injuries are alcohol-related disease problems and a current review of “International emergency room studies” revealed that up to “59%” of injuries accessed to the emergency section were alcohol associated[2].

Alcohol causes toxicity in different body tissues either by the action of toxic derivatives that are produced from alcohol metabolism or by the direct action of alcohol on cells and cellular components. Both of these actions provide base for upsurge in the assembly of free radicals or “Reactive Oxygen Species” (ROS), decrease antioxidant activities and enhance oxidative stress in many tissues[3]. Free radicals or ROS are major metabolites that are responsible for oxidative injury in different body tissues. Important free radicals causing the oxidative injury in body are superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2)[3].

A common clinical problem of longstanding alcohol consumption is “Alcoholic Liver Disease” (ALD). Consequences of ALD may arise in the form of “Alcoholic Fatty Liver” (steatosis) along with “Alcoholic Hepatitis” which can lead to end Alcoholic Cirrhosis[4]. In liver, one of primary target of alcohol is hepatic sinusoidal and perisinusoidal cells causing activation of fibroblasts and Ito cells in space of disse which leads to development of fat accumulation and collagen deposition after damaging hepatic stroma and parenchyma[5].

In all natural body defense mechanisms, vitamins like A, B, C and E are utmost noticeable “Antioxidant” defense systems working as free radical hunters[6]. These vitamins help many other enzymes by operating as cofactor to protect normal structure and function of body cells and tissues against “Free Radical” facilitated damage[6].

Vitamin E, as an antioxidant has an action of scavenging peroxyl radical. It avoids the proliferation of “Free Radicals” in tissues by countering and converting them to tocopheryl radical. These tocopherol radicals, by the action of hydrogen molecule donors like vitamin E and C, are reduced[7]. “Vitamin E” is fat-soluble vitamin and it incorporates into cell membrane of cells and play key role in protecting cells from oxidative destruction[8].

MATERIALS AND METHODS

Only male healthy adult rabbits (n=18) of domestic type, round about 1-year-old and weighing 1-1.5 kg were carefully chosen. Precisely designed iron cages having natural soil base and standard controlled setting was provided to each rabbit in all groups. Each animal was given free access to specially prepared laboratory feedstuff and drinking water. Animals from both categories were divided into three groups and each group then into two sub-groups.

Control Group A which included six animals. These animals were regularly fed on a proper standardised laboratory diet and normal saline was provided for drinking purpose. Further division of this group into 2 subgroups centred on experimental period was done.

Subgroup AI contained three rabbits, having 8 weeks experimental duration.

Subgroup AII contained three rabbits, having 4 weeks experimental duration.

Experimental Group B included six rabbits which were provided with standardised laboratory diet, 30% ethanol solution (30ml/kg/day) orally daily[9] given through a paediatric Nasogastric tube and normal saline as drinking water. Further division of this group into 2 subgroups centred on experimental period was done.

Subgroup BI contained three rabbits, having 8 weeks experimental duration.

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Subgroup BII contained three rabbits, having 4 weeks experimental duration.

Experimental Group C: This group also included six rabbits which were given free access to standard laboratory diet, 30% ethanol 30ml per kg/day along with 50mg per kg/day dissolved in 2ml distal water (through nasogastric tube) daily[9-10] and normal saline as drinking water. This group was further divided into 2 subgroups based on experimental period.

Subgroup CI contained thee rabbits, having 8 weeks experimental duration.

Subgroup CII comprised of 3 rabbits, having 4 weeks experimental duration.

According to experimental duration, the subgroups were categorised into:

Category E4 animals having 4 weeks experimental duration which included subgroups A-II, B-II, and C-II.

Category E8 animals having 8 weeks experimental duration which included subgroups A-I, B-I, and C-I.

From an official chemical dealer Pure (99.9% factory-made by BDH laboratories England) ethanol was bought and then 30% solution was made in distilled water. Vitamin E was purchased in powder form from Abbott Pharmaceuticals Karachi, Pakistan.

Solution from Powder vitamin E was prepared by mixing 50mg vitamin E with 2ml distilled water. Each rabbit in the experimental group "**C**" was given this dose of 50mg vitamin E through the similar gastric tube.

End of experiment: Category E4 animals after 4 weeks and Category E8 animals after 8 weeks were anesthetised and cardiac perfusion with normal saline and 4% paraformaldehyde for fixation was done. Whole liver was dissected and taken out for processing and microscopy.

Tissue processing: livers were cut into pieces and were kept in 10% neutral buffered formalin for 24 hours for fixation and then shifted to newly prepared 10% neutral buffered formalin. A piece from each subject liver was processed and then carefully embedded in paraffin for blocks formation to sectioning it.

Tissues were sliced in 5 µm thin section using microtome. These sections were then picked carefully on clean "glass slides". Six slides from each specimen were prepared for staining. Slides were kept in hot air oven at 100^0 C for 15 minutes to clear-out unnecessary paraffin wax and were then shifted to "slide boxes" for storage till staining.

Masson's Trichrome stain was applied for identification of collagen fibres. After staining, sections were mounted with DPX solution and carefully covered with glass coverslips for better handling, storage, and protection of the sections.

For microscopy, 3 slides from each specimen were examined under 4x, 10x and 40x powers in microscopes. Statistical analysis was performed using the SPSS-25. Chi-square test was applied. <0.05 "P-value" was considered as "Statistically Significant".

RESULTS

Category E4 animals: Mean fibrosis score in liver according to Knodel histological activity index in "Control group A-II" was 0.00±0.00, "Experimental Group B-II" was 3.00±0.00 and "Experimental Group C-II" was 1.00±0.00. Difference in fibrosis in "Experimental Group B-II" and "Experimental Group C-II" showed significant value (P=0.050) [Tables 1-2].

Category E8 animals: Mean fibrosis score in liver according to Knodel histological activity index in control group A-I was 0.00±0.00, experimental group B-I was 4.00±.00 and experimental group C-I was 3.00±0.00. Difference in fibrosis in experimental group B-II and C-II showed significant value (P=0.050).

Table 1: Score of fibrosis according to knodel histological activity index

Category	Group	Mean	N	S.D
E4 Animals	Control Group A-II	.00	3	.000
	Experimental Group B-II	3.00	3	.000
	Experimental Group C-II	1.00	3	.000
	Total	1.33	9	1.323
E8 Animals	Control Group A-I	.00	3	.000
	Experimental Group B-I	4.00	3	.000
	Experimental Group C-I	3.00	3	.000
	Total	2.33	9	1.803

Table 2: Score of fibrosis according to knodel histological activity index in experimental group B vs C (Chi-square Test)

		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (1-sided)
Category E4	Pearson Chi-Square	6.000	1	.014	.050
	Continuity Correction	2.667	1	.102	
	Likelihood Ratio	8.318	1	.004	
	Fisher's Exact Test				
	Linear-by-Linear Association	5.000	1	.025	
	N of Valid Cases	6			
Category E8	Pearson Chi-Square	6.000	1	.014	.050
	Continuity Correction	2.667	1	.102	
	Likelihood Ratio	8.318	1	.004	
	Fisher's Exact Test				
	Linear-by-Linear Association	5.000	1	.025	
	N of Valid Cases	6			

Fig. 1: Score of fibrosis according to Knodel histological activity index

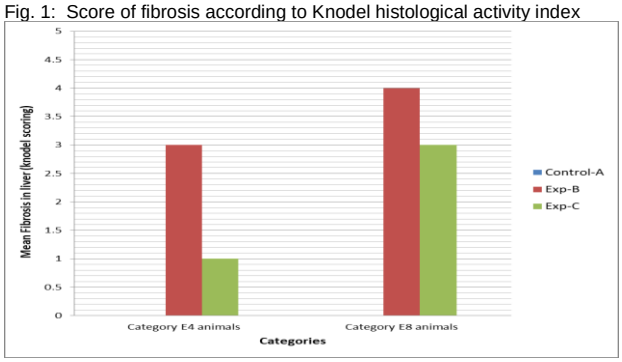
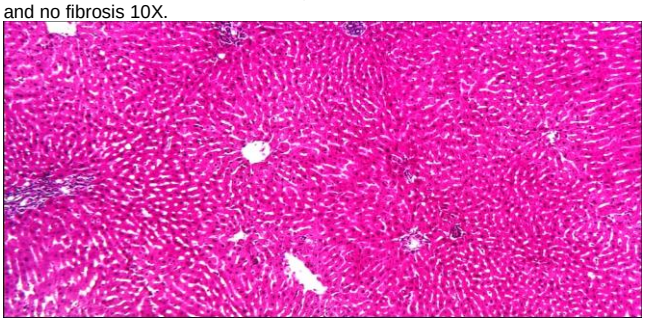


Fig. 2: 5µm thick section of control group A-II rabbit liver, stained with "MASSON'S TRICHROME" showing NORMAL hepatic lobules architecture and no fibrosis 10X.



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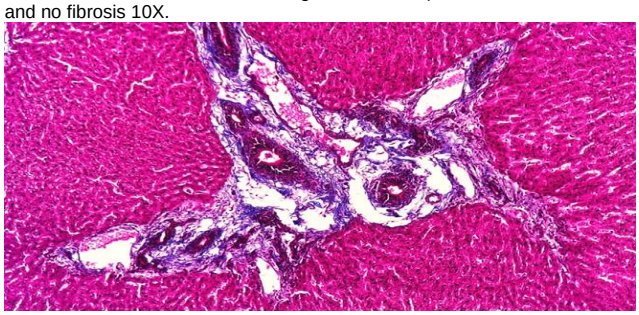


Fig. 3: 5µm thick section of Group B-I, Category E8 rabbit liver stained with "MASSON'S TRICHROME" showing nodule formation. A: Dense irregular collagen fibers, completely surrounding the nodule. B: Degenerating hepatocytes inside the nodule 10X.

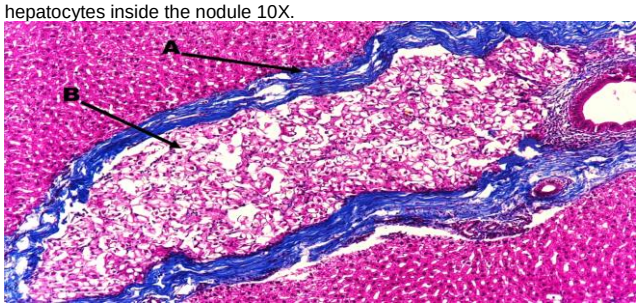
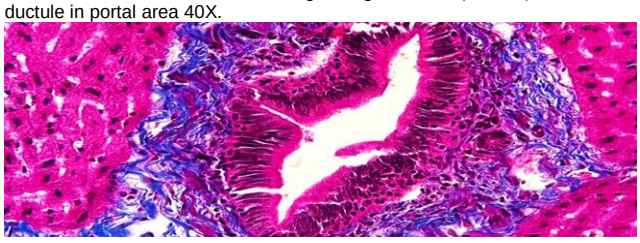


Fig. 4: 5µm thick section of Category E8, Group C-I rabbit liver, stained with "MASSON'S TRICHROME" showing collagen fibers (fibrosis) around bile ductule in portal area 40X.



DISCUSSION

The extent of fibrosis and score of portal inflammation in liver was calculated according to knodel histological activity index. There was marked fibrosis in "Experimental Group B" (treated only with alcohol) and "Experimental Group C" (treated with alcohol along with vitamin E) but the degree of fibrosis was high in experimental group B as compared to experimental subgroups C in both categories.

In the present study in category E8 animals, experimental subgroup B-I shows nodule formation in liver which denotes cirrhotic change while in experimental group C there was no nodule formation. This observation reveals a therapeutic benefit of "Vitamin E" in diminishing alcohol toxicity induced collagen fibers deposition in liver.

A study carried out by Lu and Ward[11] support our study as they reported collagen deposition in mice liver after 3 weeks of alcohol administration but they haven't observed any nodule formation. Explanation for this variation might be the difference in experimental duration and alcohol dose as well as experimental animal. Ashwani et al[12] have stated that in chronic hepatitis C patients, "Vitamin E" was given for compete 8 weeks which prevented the progress of fibrogenesis cascade as mirrored by decrease in malonaldehyde levels and decrease in activity of hepatic stellate cells.

CONCLUSION

The alcohol, if taken in high doses regularly has abundant influence on liver and can cause abnormal changes in the normal architecture and morphology of both liver parenchyma and stroma by its toxic metabolites like Reactive Oxygen Species (ROS). These alterations in liver morphology due to "Alcohol Toxicity" ranges from less harmful and treatable steatosis (fatty change) to irreversible non treatable fibrosis of liver parenchyma which can lead to cirrhosis and ultimately liver failure even in a short duration of 8 weeks. Liver failure is a life threatening condition because liver is one of the vital organs of our body.

The degree of damaging alterations and level of injury in liver by alcohol intake in the form of collagen deposition in response to different toxic metabolites generation can be reduced by regular intake of suggested dose of "Vitamin E" which acts as an antioxidant agent for deoxidization of free radicals and thus reducing reactive oxygen species load, in return decreasing the severity of injury and destruction in liver caused by liver toxicity.

Conflict of interest: Nil

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