

# ARCHITECTURAL CHANGES OF LIVER IN RESPONSE TO ALCOHOL

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## ABSTRACT

**Background:** Alcohol is a known hepatotoxic agent and cause different varieties of liver damages. The objective of this study is to determine the effects of alcohol on liver histology in albino rats.

**Material & Methods:** This study was an experimental Randomized Control Trial conducted at the experimental research laboratory of University of Health Sciences Lahore, from January to December 2010. Sixteen male albino rats of 6-8 weeks old, weighing 130-230 gm each were divided into two groups of eight rats each. Group A served as control and was given normal rat diet; Group B was given ethanol at a dose of 0.8ml/100gm/day for 10 weeks. At the end of the experiment, blood was drawn from each animal by cardiac puncture for liver function tests. Each animal was then sacrificed under chloroform anaesthesia and its liver was removed.

**Results:** Mean values of ALT and GGT in gp A were  $26.06 \pm 7.13$  and  $23.33 \pm 3.05$  respectively while in gp B these values were  $82.83 \pm 10.89$  and  $76.33 \pm 4.37$  respectively. The mean size of hepatocytes in gp A & B was  $19.03 \pm 0.38$  and  $26.23 \pm 0.54$  respectively and the size of central vein in gp A & B was  $78.5 \pm 0.99$  and  $79.16 \pm 1.35$  respectively. Students "t" test showed statistically significant increase in the mean values of ALT and GGT in group B as compared with those in group A,  $p < 0.05$ . Liver was normal in appearance in all animals. Statistically significant difference ( $p < 0.05$ ) for weight and volume of the liver of group B when compared with those in group A was observed. Histological study of hepatocytes showed large number of cytoplasmic vacuoles, pyknotic nuclei and lymphocytes infiltration of portal areas. Students "t" test showed statistically significant difference in the mean value of the size of hepatocytes; Fischer exact test also showed statistically significant difference in the percentage of hepatocytes containing cytoplasmic vacuoles, pyknotic nuclei and percentage of portal areas showing lymphocytic infiltration, in the of the liver of the animals in group B, when compared with those in group A ( $p < 0.05$ ).

**Conclusion:** Ethanol is hepatotoxic in albino rats as evident from the functional derangement and structural changes in the liver.

**KEY WORDS:** Ethanol, Hepatotoxicity, Hepatocytes.

## INTRODUCTION

Alcohol overconsumption causes fatty liver, alcoholic hepatitis, and chronic hepatitis with hepatic fibrosis or cirrhosis.<sup>1</sup> At least 80% of heavy drinkers have been reported to develop steatosis, 10%-35% alcoholic hepatitis, and approximately 10% liver cirrhosis.<sup>1,2</sup>

The first stage of alcoholic liver damage is fatty liver, which is reversible with abstinence from alcohol (ethanol) but may progress to cirrhosis if excessive use of alcohol continues. Fatty acids can be seen as fatty globules under the microscope. Accumulation of large fatty globules occurs throughout the liver and can begin to occur after a few days of heavy drinking. On gross examination, the fatty liver is a large, soft organ, which is yellow and greasy. Fatty liver is prone to the development of inflammation and alcoholic hepa-

titis.<sup>3</sup> In Alcoholic Hepatitis there is widespread inflammation and destruction of liver tissue. Patients may develop fibrosis, where scar tissue begins to replace healthy liver tissue. Lymphocytes and macrophages enter portal tracts and spill into the hepatic parenchyma. Alcoholic hepatitis is usually associated with proliferation of sinusoidal stellate cells and portal tract fibroblast, giving rise to sinusoidal and perivenular fibrosis.<sup>4,5</sup> The advanced and irreversible form of alcoholic liver damage is alcoholic cirrhosis, in which collagen deposition occurs in the space of Disse, and around central veins, which prevents blood from traveling freely through liver, leading to portal hypertension and its complication.<sup>6,7</sup>

Liver injury in chronic alcoholics is produced on account of oxidative stress.<sup>8</sup> Progress of disease involves repeated injury to hepatocytes, fol-

lowed by impaired liver functions and fibrosis.<sup>9,10</sup> It had been reported earlier that ethanol increased weight and volume of the liver,<sup>11</sup> produced fat vacuoles in hepatocytes.<sup>12</sup> Apoptosis had been observed in the liver after treatment with ethanol.<sup>11</sup> The present study was designed to make comprehensive investigations on hepatotoxic effects of ethanol, which included functional derangement of the liver and changes in its structure, it is hoped that it might serve as warning to those, who have the tendency to indulge in drinking.

**MATERIAL AND METHODS**

This study was an experimental Randomized Control Trial (RCT) conducted at the Experimental Research Laboratory of University of Health Sciences Lahore. Sixteen male albino rats 6-8 week old, weighing 130-230 gm, each was procured from National Institute of Health, Islamabad and was randomly divided into two groups, having eight rats each. Group A served as control and were given 2ml/100gm/day distilled water by mouth, in addition to water ad libitum. Group B served as experimental and was given 2ml/100gm body weight per day of 30% v/v of aqueous solution of ethanol containing 0.8ml (0.5gm) of ethanol by mouth for 10 week. The body weight of each animal was recorded twice weekly and at the end of the experimental period.

Blood samples from each group were collected by cardiac puncture in vacuum tubes and allowed to stand for one hour to separate the serum, using test tube stand. The test tubes were centrifuged at the speed of 3000 revolutions per minute, the clear serum was collected with the help of clear dropper in plastic tubes and stored in freezer at -20°C for testing on a later date. Serum alanin- aminotransferase (ALT) and gamma glutamyl transferase (GGT) levels were measured by using commercially available kits of "Human Company.

The histological preparations of liver were stained using Eosin and Haematoxylin and Periodic Acid Schiff.<sup>13</sup> The data was analyzed using SPSS version 15.0. Mean±SE was given for quantitative variables. Frequencies and percentages were given for qualitative variables. Two independent sample "t" tests were applied to observe group mean differences between two groups. Fischer exact test was applied to observe association between qualitative variables.

**RESULTS**

In group A and B, the mean body weight of the animals at the start was 138.67± 0.52 and 139.28± 0.656 respectively; whereas at the end of experiment it was 145.05±1.07and 150.87± 1.89 respectively. Students't' test did not show statistically significant difference among the groups at the start of experiment (p>0.05) however it became statistically significant at the end of experimental period, p<0.05. (Table1)

In-group A, the mean values of serum Alanin Amino Transferase (ALT) and serum Gamma Glutamyl Transferase (GGT) were 26.06±7.13 and 23.33±3.05U/L respectively; where as in-group B, the mean values of these enzymes were 82.83 ±10.89 and 76.33 ± 4.37 U/L respectively. Students't' test showed that there was statistically significant increase in the enzyme levels in group B, when compared to those in group A, p <0.05 (Table 2).

**Histological examination of liver:** The liver of group A, showed normal hepatolobular architecture. The mean size of hepatocytes in groups A and B was 19.03 ± 0.38 and 26.23 ±0.54 respectively, the difference between the groups was statistically significant p<0.05. The mean diameter of central vein in group A and B was 78.50±0.99µm and 79.16 ±1.35µm respectively which was not statistically significant p>0.05. Cytoplasm of the

**Table 1: Mean body weight of animals in gm at the start and at the end of experiment.**

Body weight of animals	Group A (n=8) Mean ±S.E	Group B (n=8) Mean ±S.E	p. value
At the start	138.67 ± 0.52	139.28 ± 0.656	0.8311
At the end	145.05 ± 1.07	150.87 ± 1.89	<0.041

**Table 2: Mean value of serum Alanin Amino Transferase (ALT) and serum Gamma Glutamyl Amino Transferase (GGT) in U/L.**

Serum Enzymes	Group A (n=8) Mean ±S.E	Group B (n=8) Mean ±S.E	p. value
ALT	26.06 ± 7.13	82.83 ± 10.89	<0.001
G GT	23.33 ± 3.05	76.33 ± 4.37	<0.001

**Table 3: Mean size of Hepatocytes, and central veins in microns ( $\mu\text{m}$ ).**

Components of lobules	Group A (n=8) Mean $\pm$ S.E	Group B (n=8) Mean $\pm$ S.E	p.value
Hepatocytes	19.03 $\pm$ 0.38	26.23 $\pm$ 0. 54	<0.001
Central vein	78.5 $\pm$ 0.99	79.16 $\pm$ 1.35	>0.035

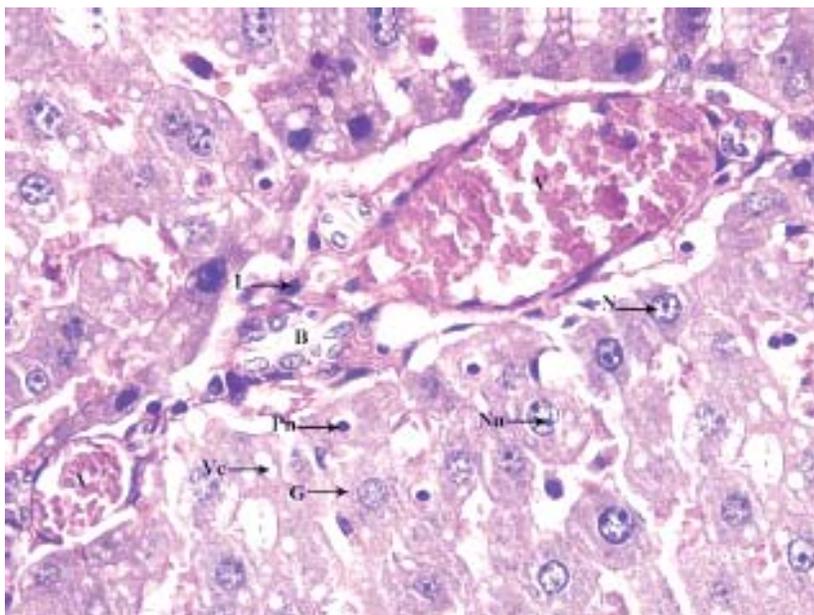


Fig.1: Photomicrograph of a section from the liver of group B, showing portal area comprising portal vein (V), hepatic artery (A) and bile duct (B); the surrounding cells contain glycogen granules (G) and cytoplasmic vacuoles (Vc), nuclei (N) contain one or two nucleoli (Nu); there is lymphocyte infiltration (L) in portal area, pyknotic nuclei (Pn) are also observed. PAS stain. X400.

cells of group B, however, contained large number of micro and macro vacuoles involving whole of the hepatic lobule, Pyknotic nuclei were also observed (Fig.1). The portal area showed lymphocyte infiltration; the portal vein and hepatic artery contained erythrocytes and bile duct was lined with cuboidal cells and appeared normal (Fig.1).

## DISCUSSION

In the current investigations we observed that ethanol produced hepatotoxic effects in rats, as manifested by significant increase in the serum levels of Alanine Amino Transferase (ALT) and Gamma Glutamyl Transaminase (GGT) in group B, when compared to those in group A ( $p < 0.05$ ); this may be because of oxidative stress. Accumulation of reactive oxygen species, cause lipid peroxidation of cellular membranes which resulted in hepatocyte injury. These observations were comparable to those reported by Enomoto<sup>12</sup>, who observed the effect of Pioglitazone in prevention of ethanol induced liver injury in rats.

There was significant increase in weight and volume of the liver of group B, when compared with those of group A, this may be due to accumulation of fats, water and increased size of hepatocytes. Our observations showed statistically significant increase in size of hepatocytes, this was presumably due to accumulation of fats, protein and water; our results corroborate those reported earlier by Thurman<sup>6</sup> who reported that ingestion of ethanol along with low carbohydrate diet in rats produced vacuoles and inflammatory changes in the liver. There was no statistically significant change in the diameter of central vein in group B as compared to group A ( $p > 0.05$ ). pyknotic nuclei were observed in group B; the difference in number of pyknotic nuclei of the group B was statistically significant, when compared with those in group A ( $p < 0.05$ ).

These observations were in accord with those of Stewart<sup>4</sup> who observed the effects of ethanol on hepatocyte cultures and reported ethanol induced apoptosis characterized by pyknotic nuclei which appeared as dark and irreversible con-

densation of chromatin. Liver preparation from rats in group B showed lymphocytes infiltration around the bile duct called periportal inflammation; this finding was also reported by Yin<sup>11</sup> who observed periportal lymphocyte infiltration in ethanol induced hepatotoxicity in rats. Evidence of liver cirrhosis was not observed in animal of our experimental group B which possibly would have developed eventually, if the animals were given ethanol for a longer period.

## CONCLUSION

Ethanol treated albino rats showed a fair degree of derangement of liver functions, associated with concomitant changes in the histological structure of the organ.

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