EFFECT OF SILYMARIN ON SERUM LEVELS OF ALT AND GGT IN ETHANOL INDUCED HEPATOTOXICITY IN ALBINO RATS

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Background: Alcoholic liver disease is a worldwide health problem. At least 80% of heavy drinkers have been reported to develop steatosis, 10–35% alcoholic hepatitis, and approximately 10% liver cirrhosis. The objective of this study was to determine the effect of silymarin on the levels of serum ALT and GGT in ethanol induced hepatotoxicity in albino rats. This study was an experimental Randomised Control Trial (RCT), and was conducted at the experimental research laboratory of University of Health Sciences, Lahore, from January 2007 to December 2007. **Methods:** Eighteen male albino rats of 6–8 weeks age, weighing 150–200 gm each were divided into 3 groups of 6 rats each. Group A served as control, Group B was given ethanol at a dose of 0.6 ml (0.5 gm)/100 gm/day and group C was given ethanol and silymarin at a dose of 0.5 gm/100 gm/day, and 20 mg/100 gm/day respectively for 8 weeks. At the end of the experiment, each animal was euthenised with chloroform. Blood was drawn from each animal by cardiac puncture for liver function tests (ALT and GGT). After taking blood sample, each euthenised animal was sacrificed and then its liver was removed for gross and histological examination. **Results:** The mean values of serum alanine-aminotransferase (ALT) in groups A, B and C were 28.16±7.13, 82.33±10.89 and 49.66±6.12 U/L respectively, whereas, the mean values of gamma-glutamyl transferase (GGT) in groups A, B and C were 27.33±3.05, 79.33±4.37 and 45.66±1.85 U/L respectively. ANOVA showed significant (p<0.05) difference in mean value of these serum enzymes among groups. Post Hoc test, using the Tukey honestly significant difference (HSD) showed that there was significant (p<0.05) increase in mean value of ALT and GGT in group B as compared to group A and C. This test also showed that there was significant (p<0.05) decrease in mean value of these enzymes in-group C as compared to group B. **Conclusion:** Silymarin tends to normalise liver function test in alcoholic liver disease.

Keywords: Ethanol, Silymarin, Alanine-aminotransferase (ALT), Gamma-glutamyl transferase (GGT)

INTRODUCTION

Alcoholic liver disease is a worldwide health problem.[1–3] The three most widely recognized forms of alcoholic liver diseases are fatty liver/steatosis, alcoholic hepatitis and liver cirrhosis. At least 80% of heavy drinkers have been reported to develop steatosis, 10–35% alcoholic hepatitis, and approximately 10% liver cirrhosis.[4–6]

Various experimental studies described that ethanol caused accumulation of reactive oxygen species like super oxide, hydroxyl radical,[7–9] and hydrogen peroxide in hepatocytes that oxidized the reduced glutathione, which in turn lead to lipid per oxidation of cellular membranes, and oxidation of protein and DNA resulting in hepatocytes injury.[10,11]

Numbers of works have been published their data showing that certain fruits, vegetables, herbs, and plants exhibited hepatoprotective effects. One of these is silymarin, which is isolated from the fruits and seeds of the milk thistle. Silymarin acts as an antioxidant, cell membrane stabiliser, and promoter of ribosomal RNA synthesis and hence, stimulate liver regeneration in various liver diseases. [12–16]

Ethanol-induced hepatotoxicity has been reported to cause significant increase in the values of serum alanin- aminotransferase and gamma-glutamyl transferase. The current study is, therefore, designed to determine the effect of silymarin on the serum levels of ALT and GGT in ethanol induced hepatotoxicity in albino rats.

MATERIAL AND METHODS

This study was an experimental Randomized Control Trial conducted at the Experimental Research Laboratory of University of Health Sciences Lahore. Eighteen male albino rats of 6–8 weeks old, weighing 150–200 gm each were procured from National Institute of Health, Islamabad. They were kept under controlled temperature (23–25 ºC), humidity (60%) and light and dark cycles of 12 hours each and allowed to acclimatize for one week. The animals were fed on standard rat diet and water ad libitum, and weighed both at the start and end of the experiment. The animals were randomly divided into 3 groups, having 6 rats in each group. Group A served as control whereas group B and C as experimental. Group B was given orally 2 ml/100 gm body weight per day of 30% v/v of aqueous solution of

ethanol containing 0.6 ml (0.5 gm) of ethanol, whereas group C was given orally 2 ml/100 gm/day of 30% v/v of aqueous solution of ethanol containing 0.6 ml (0.5) gm ethanol along with 20 mg/100 gm per day of silymarin for 8 week. At the end of experiment, each animal was taken out of cage and was euthanized under chloroform. Six ml of blood was taken in 10 ml disposable syringe by cardiac puncture. The blood sample was allowed to stand for one hour and centrifuged at a speed of 3,000 rpm for 10 minutes. The clear serum was collected with the help of a clean dropper in sterilised disposable plastic tubes. These plastic tubes were properly labelled and stored at -20 ºC in freezer for testing on a later date. Serum alanine-aminotransferase and gamma-glutamyl-transferase levels were measured by using commercially available kits of Human Company.

The liver of each animal was also removed from the body and examined for gross changes and histological study.

The data was analysed using SPSS version 15. Statistical analysis was performed using ANOVA followed by Post Hoc Tukeys and Fischer exact tests. Differences between groups were considered to be statistically significant at $p<0.05$.

RESULTS

All animals of group A were healthy and active, whereas, the animals of group B and C suffered slight degree of drowsiness. Ethanol induced hepatotoxicity and its prevention by silymarin was confirmed by gross and microscopic examination of the liver of the 3 groups. ANOVA and Fischer exact test showed significant ($p<0.05$), increase in weight and volume of the liver, size of hepatocytes and number of cytoplasmic vacuoles in group B when compared to group A. (Figures 1, 2 and 3). However in group C, these changes were statistically insignificant, ($p>0.05$).

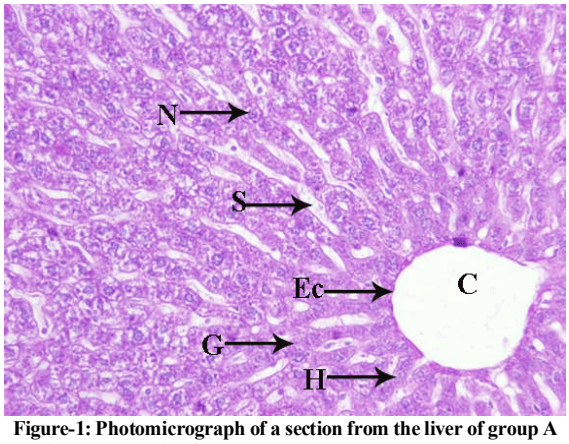

Figure-1: Photomicrograph of a section from the liver of group A showing central vein (C) lined by endothelial cells (Ec), surrounded by cords of hepatocytes (H), enclosing hepatic sinusoids (S); hepatocytes contain glycogen granules (G) and central nuclei (N). PAS stain. ×200.

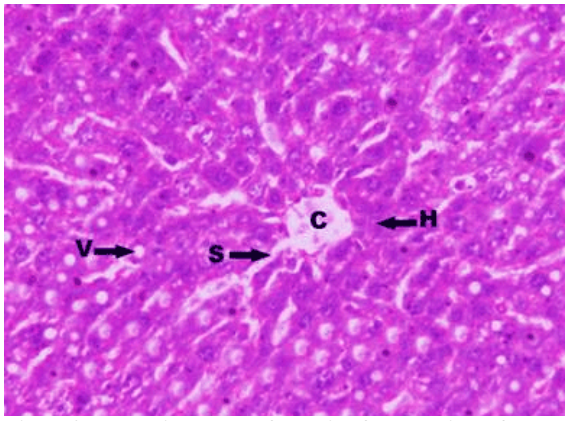

Figure-2: Photomicrograph of a section from the liver of group B, showing central vein (C), continuing with hepatic sinusoids (S); surrounded by cords of hepatocytes (H) that contain cytoplasmic vacuoles (V). H and E stain. ×200

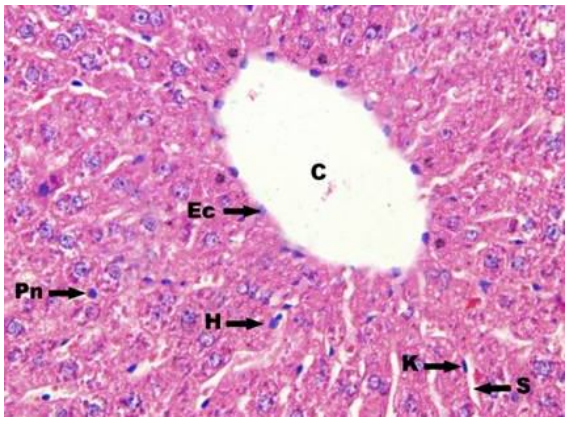

Figure-3: Photomicrograph of a section from the liver of group C, showing central vein (C) lined by flattened endothelial cells (Ec) surrounded by radiating cords of hepatocytes (H) enclosing sinusoids (S) that contain Kupffer cells (K); pyknotic nuclei (Pn) are also observed. H and E stain. ×200.

The mean value of serum ALT in groups A, B and C were 28.16±7.13, 82.33±10.89 and 49.66±6.12 U/L respectively, whereas, the mean value of GGT in groups A, B and C were 27.33±3.05, 79.33±4.37 and 45.66±1.85 U/L respectively. ANOVA showed statistically significant ($p<0.05$) difference in the mean value of serum ALT serum GGT among groups B and C (Table-1) when compared with control group A.

Post Hoc test, using the Tukey honestly significant difference (HSD) also showed that there was significant increase in the mean value of ALT and GGT in group B as compared to group A and C ($p<0.05$). This test also showed that there was significant ($p<0.05$) increase in the mean value of these enzymes in group C as compared to group A. However the levels of ALT & GGT was significantly ($p<0.05$) decreased in group C as compared to group B.

Table-1: Values of serum alanine-aminotransferase (ALT) and serum Gamma-glutamyl transferase (GGT) in U/L (Mean±SE)

Serum Enzymes	Group A	Group B	Group C
ALT (U/L)	28.16±7.13	82.33±10.89*	49.66± 6.12*
GGT (U/L)	27.33±3.05	79.33±4.37*	45.66±1.85*

*$p<0.05$

DISCUSSION

The serum level of ALT and GGT in group B was significantly increased ($p<0.05$) as compared to those animals in the control group A. This was presumably due to production of reactive oxygen species, inducing protein oxidation and lipid per oxidation which resulted in hepatocytes injury. These observations were comparable to those reported by Enomoto[17] in 2003. On the other hand, in group C, there was significant increase ($p<0.05$) in the value of serum ALT and GGT as compared to control group A, however, as compared to group B, the value of these enzymes were significantly decreased ($p<0.05$) in group C, showing that silymarin decreased the leakage of enzymes

In 2006, Song[18] *et al*, studied the protective role of silymarin in ethanol-induced hepatotoxicity in mice and observed that acute ethanol administration caused prominent hepatic micro vesicular steatosis with mild necrosis and an elevation of serum enzymes, so they reported the same that, silymarin protects ethanol induced hepatotoxicity. Our results were comparable to these findings and confirmed hepatoprotective effects of silymarin.

CONCLUSION

Silymarin decreases the level of raised serum enzymes in alcoholic liver disease.

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