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Research Article

Chronic alcohol consumption: Increases the liver marker enzymes and hepatic damage on the albino Wister rat liver

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ABSTRACT

To investigate the effect of ethanol induced liver toxicity in male Wister albino rats. The liver toxicity was induced by the chronic administration of ethanol to the animals at the 20%v/v, 10%v/v respectively for 28 days at daily basis. The liver toxicity was assessed by the estimation of liver marker enzymes and liver histopathological studies. The chronic induction of ethanol in rats, liver marker enzymes like *aspartate* aminotransferase (AST), alanine aminotransferase (ALT), alkaline *phosphatase* (ALP) , lactate dehydrogenase (LDH), total bilirubin (TB), direct bilirubin (DB) levels were significantly elevated (P<0.0001) when, compared to the normal animals. Ethanol is one of the most widely used and abused drugs and increasing lipid levels in humans and experimental animals. Liver damage seen in chronic ethanol consumption appears to be modulated by kupffer cell activation. Chronic ethanol treatment has been shown to enhance oxidative stress in liver tissues. Ethanol induced oxidative stress is a major role in the mechanism of hepatotoxicity. In the present study was revealed that the chronic consumption of ethanol treated rats shows significantly increased liver marker enzymes and severe damage to liver tissues.

Keywords: Ethanol; Serum Enzymes; Hepatotoxicity; Histopathology.

INTRODUCTION

Ethanol induced hepatotoxicity is one of the major problems in worldwide and especially developing countries. The spectrum of alcoholic liver disease ranges from fatty liver to alcoholic hepatitis, ultimately fibrosis and cirrhosis (Tuma DJ and Sorrell M, 2004). Previous studies showed 80% of heavy drinkers had been reported to develop steatosis, 10-35% alcoholic hepatitis and approximately 10% liver cirrhosis (Ruth A. Roberts et al., 2007). The animal models suggest that liver injury in chronic alcoholics is due to oxidative stress that leads to fibrosis, impaired liver functions and increased apoptosis condition (Juliane I. Beier and Craig J. McClain, 2010). Ethanol induced apoptosis sensitizes rat hepatocytes to lipopolysaccharide-mediated cytotoxicity (Sanjoy Roychowdhury et al., 2013). Chronic alcohol consumption leads in the progressive of alcoholic liver disease and characterized by the development of hepatic steatosis and an increase in the number of inflammatory mediators, including cytokines, reactive oxygen species, and nitrogen species (Pablo Muriel, 2009). Chronic ethanol increases macrophage infiltration and inflammatory cytokine expression (Resstel LB et al., 2006). Ethanol induced tissue injury in both adipose and liver tissue (Yuanyuan Qin et al., 2013).

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Long-term use of alcohol leads to the development of steatosis, alcoholic hepatitis and cirrhosis resulting in weight and volume changes (Radan Bruha et al., 2012). Activation of kupffer cells directly or indirectly by toxic agents results in the release of an array of inflammatory mediators, growth factors, and reactive oxygen species (Debra L. Laskin et al., 2011). Chronic ethanol consumption is associated with cardiovascular dysfunctions independent of other known risk factors (Katia Colombo Marchi et al., 2014). Regular use of ethanol is associated with inadequate control of blood pressure in treating hypertensive patients (Gulliver SB et al., 2006; De Biasi M and Salas R, 2008).

Epidemiological and clinical studies have established a positive relationship between long-term ingestion of ethanol leads to development of hypertension (Malhi H et al., 2010), brain ischemia, and stroke (Nagata K et al., 2007; Deaciuc IV et al., 2001; Dey A, Cederbaum AI, 2006). Liver damage seen in chronic ethanol consumption appears to be modulated by kupffer cell activation. More recent evidence has noted a contributory role of kupffer cell activation in the process of hepatic carcinogenesis. Ethanol dependence is characterized by an abstinence syndrome in which withdrawal symptoms resulting from central nervous system (CNS) hy-

per excitability emerge in a time dependent fashion after cessation of drinking (Xu A et al., 2003). Most alcoholics recognize the negative effects of drug abuse on health and would prefer to quit, but despite many attempts, very few succeed (You M et al., 2005; Song Z et al., 2008). Multiple mechanisms of cell death, including apoptosis and necrosis, are activated during the progression of alcoholic liver disease (Thakur V et al., 2006; Chen X et al., 2007). Chronic ethanol exposure decreases the serum adiponectin concentration in mice and rats (Poirier LA et al., 2001; Kang L et al., 2007; Kang L et al., 2007; Xiaocong Chen et al., 2009; Kumar V et al., 2003). Chronic ethanol impairs insulin-stimulated glucose uptake (Saravanan N and Nalini N, 2007) and disrupts the hormonal regulation of lipolysis (Ronis MJ et al., 2004).

MATERIALS AND METHODS

Chemicals: Ethanol was obtained from Sigma-Aldrich, Chemical, U.S.A, and Enzyme kits were obtained from Span Diagnostics Ltd. Surat, India. All other chemicals were of analytical grade procured from reputed Indian manufacturers

Experimental animals

Male Wister rats, weighing 135-160 g were selected from an inbred group maintained under standard condition of temperature (25 ± 5°C) and humidity. Animals were provided with food and water ad libitum. All experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly adhering to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental induction of hepatotoxicity

Experimental hepatotoxicity was developed by the chronic administration of ethanol. A total of eighteen animals were equally divided into 3 groups of 6 each. Group I served as normal control without any treatment. Animals of groups II and III were administered with ethanol treatment for 20% v/v and 10% v/v respectively by oral route on a daily basis and continuously for 28 days. After the end of the study, all the rats were sacrificed by cervical dislocation after overnight fasting and before sacrifice, rats blood was collected from the retro-orbital sinus plexus under mild ether anesthesia and blood sample collected in heparinized tubes and serum was separated. Tissue was separated for the histopathology investigations. All the experimental activity conducted as per the animal ethical committee's recommendations.

Histopathological studies

Liver slices were fixed in 10% formalin and embedded in paraffin wax. Sections of 5 micron thickness were made using a microtome and stained with haematoxylin- eosin and observed under microscope. Photographs of each of the slides were taken at 40× magnification.

Statistical analysis: All the data were expressed as means ± standard error mean (SEM). The measurement data of multiple groups were compared with one-way ANOVA, the comparison between normal control versus other groups, and a value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Chronic alcohol consumption produces a variety of pathological conditions varying from simple intoxication to severe life-threatening pathological states (Rajagopal SK et al., 2003; Bin Gao and Ramon Bataller, 2011; Jacob M. Kneeman et al., 2012; Suprakash Chaudhury, 2010). Liver injury transport function of the hepatocytes gets disturbed, resulting in the leakage of plasma membrane and thereby causing an increased enzyme level in serum (Lieber CS, 2003). The elevated activity of AST and ALT indicates cellular leakage and the functional integrity of the cell membranes in the liver. AST and ALP were found to be in higher concentrations in cytoplasm, and AST exists in mitochondria. ALP is excreted by the liver via bile in the liver injury due to hepatotoxins, which results in a defective excretion of bile from the liver and is reflected in their increased levels in serum. In ethanol-induced liver toxicity, the level of lactate dehydrogenase (LDH), total bilirubin (TB) and DB get elevated. The present study was found ethanol induced changes of serum marker enzymes. The histological observations supported the results obtained on induction of hepatotoxicity by ethanol.

As shown in table1, decrease in the rat body weight is a sensitive parameter for chronic administration of ethanol toxicity. These results expressed different percentage (20%v/v and 10%v/v) of chronic ethanol administration that affects the intake of normal food and relatively body weights were significantly different (190.16±1.70 and 197.16±1.42; group-II and group-II) from normal control (224.33±2.17).

Table 1 showed the results of AST and ALT when, the liver cell is damaged, AST and ALT in the liver will be released into serum. Therefore, levels of GOT and GPT are the most commonly used biochemical indexes for evaluating the damage of liver (Molina P et al., 2002). As the previous evidence, the present experimental study was found that the ethanol is a confirmative agent for damage of liver tissues and changes in liver function (Ki Tae Suk et al., 2014).

According to the biochemical indexes (Table 1), the administration of ethanol caused the AST by liver-damaged group (Group II and III) 190.16±1.70 and 197.16±1.42 ($p < 0.0001$) respectively. In chronic administration of ethanol caused the ALT liver-damaged group (Group II and III) 190.16±1.70 and 197.16±1.42

Table 1: Effect of ethanol induced changes in body weight and serum SGOT, SGPT, LDH for assessing hepatotoxicity in albino Wistar rats

Groups	Body weight changes (gm)	SGOT (IU/L)	SGPT (IU/L)
Normal control	224.33±2.17	61.28±1.06	27.36±0.88
Treated 20%v/v of ethanol	190.16±1.70□□□	155.61±2.76□□□	71.22±3.98□□□
Treated 10%v/v of ethanol	197.16±1.42□□□	135.07±3.62□□□	60.56±3.83□□□

Values are expressed as mean ± SEM (n = 6). Data were analyzed using One-way analysis of variance followed by Dunnett's multiple comparison tests. P values: □□□< 0.0001; P value: □ < 0.05 considered as significant; all groups are compared with normal control

Table 2: Effect of ethanol induced changes in serum ALP, TB and DB for assessing hepatotoxicity in albino Wistar rats

Groups	LDH (IU/L)	ALP (IU/L)	TB (IU/L)	DB (IU/L)
Normal control	1128±16.53	171.28±1.29	0.121±0.002	0.089±0.004
Treated 20%v/v of ethanol	2114.83±12.21□□□	275.89±2.76□□□	0.451±0.012□□□	0.291±0.004□□□
Treated 10%v/v of ethanol	1950.16±19.70□□□	262.16±2.66□□□	0.361±0.006□□□	0.227±0.005□□□

Values are expressed as mean ± SEM (n = 6). Data were analyzed using One-way analysis of variance followed by Dunnett's multiple comparison tests. P values: □□□< 0.0001; P value: □ < 0.05 considered as significant; all groups are compared with normal control

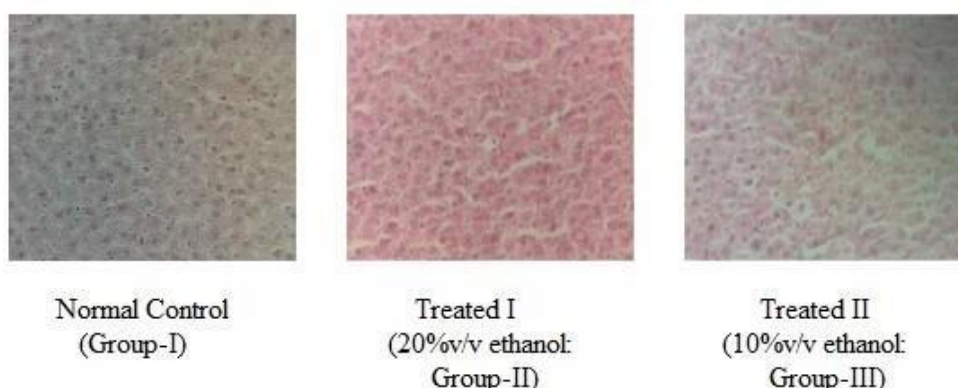

Figure 1: Histopathological analysis of liver tissue of rats treated with different percentage of ethanol 20%v/v and 10%v/v respectively. Group-I served as normal control, Group-II and III treated with ethanol

($p < 0.0001$) respectively as higher than control group (Group I).

However, there was a significant difference among group II and III as compared to normal control (group-I), this result was indicating that the ethanol is highly toxic to the liver.

As shown in table 2 assessment of liver function is made by estimating the activity of serum ALP and bilirubin which are present higher concentration in cytoplasm. When there is hepatopathy, these molecules leak into the blood stream in compliance with the extent of liver damage (R Nagalekshmi et al., 2011).

The present study reflected different percentage (20%v/v and 10%v/v) of chronic administration of ethanol significantly increased alkaline phosphatase (275.89±2.76 and 262.16±2.66) , total bilirubin (0.451±0.012 and 0.361±0.006), direct bilirubin (0.291±0.004 and 0.227±0.005) as compared to the normal control (171.28±1.29, 0.121±0.002 and 0.089±0.004) respectively. This result was significantly higher than the normal control (group-I).

Table 1 shows the serum and hepatic activities of LDH in treated and non- treated rats. Ethanol treated rats showed a significant (P<0.0001) rise in serum activities comparable to non- treated rats. Increase and decrease in the serum and hepatic activities of these enzymes may be attributed to the damaged structural integrity of the liver, which results in the leakage of these enzymes from the cytosol into the blood stream (Wu D and Cederbaum AI, 2007). In this study proven different percentage of chronic administration of ethanol markedly increased lactate dehydrogenase level (2114.83±12.21 and1950.16±19.70) when, compared to the group-I (1128±16.53).

Long-term alcohol consumption does not only activate free radical generation and also alters the levels of both enzymatic and non-enzymatic endogenous antioxidant systems. This results in oxidative stress (Lien Ai Pham-Huy et al., 2008; R John Aitken and Shaun D Roman, 2008) with cascade of effects leads to affecting both functional and structural integrity of cell and organelle membranes (Salvador Manzo-Avalos and Alfredo Saavedra-Molina, 2010).

As shown in Figure 1, the hepatic cell plate from the control group (group- I) have intact structure and the boundary between cells is clear. The cell structures are clean without impurities and droplets. This result confirmed the both cell plate and sinusoid are centripetal from the central vein and does not see in infiltration of inflammatory cells in the central venous area. However, the different percentage of ethanol treated groups (II and III) has shown obvious pathological structure changes. The cells near the central venous area are full of ballooning degeneration and fatty droplets, and look like shiny droplet. Previous study also reported these findings (Puja Sakhuja, 2014).

The most boundaries between cells are blurred and some even disappeared to become homogenized. The inflammatory reaction of lymphoid infiltration was observed in the central venous area. The condition of hyperplasia of kupffer cells, metaplasia of hepatic tissue structures and discontinuity of sinusoid structures are also observed.

The nuclei of some liver cells are inflamed. The experimental study showed multiple nuclei and over-stains are also observed and few cells are necrotic condition. Ethanol-treated group revealed that the intense distortion of the hepatic architecture. The hepatic cells, intralobular veins and the endothelium were found to be damaged in the ethanol treated rats. So, this report has been evidenced as ethanol influence normal defense mechanism. The group treated with a higher percentage of ethanol (20%v/v) showed a highly significant difference when, compare with normal group as well as a low percentage of ethanol treated group (10% v/v) also expressed significant result. However, from the histological results showed fatty degeneration, necrosis and changes of the normal hepatic cell structure.

CONCLUSION

The results of serum AST, ALT, ALP, LDH, TB, DB and histopathological examination illustrated that chronic ethanol administration leading to severe cause of liver-toxic. Further research related to the effects of ethanol induced liver damage will be continued. On the basis of the available data in this report, it can be suggested that chronic ethanol consumption induced hepatic and oxidative damage in rats. Histopathological examination reflected that the ethanol treated rats showed massive fatty changes, necrosis, and broad infiltration of the lymphocytes. So, the present work analyzed the chronic administration of ethanol induced liver damage.

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