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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC FRUIT EXTRACT OF *TERMINALIA CHEBULA* ON ETHANOL INDUCED HEPATOTOXICITY IN RATS.

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ABSTRACT

The purpose of this study was to assess the effect of ethanolic Fruit extract of *Terminalia chebula* in ethanol induced Hepatotoxicity in rats. Rats were divided into six different groups each having six. Group 1 served as a control, Group 2 received 40%Ethanol (2ml/100g, oral), in sterile water, group 3,4 and 5 served as extract treatment groups and received 50, 100 & 200 mg/kg, orally, ethanolic fruit extract of *Terminalia chebula*(TCE) and group 6 served as standard group and received Silymarin 25 mg/kg orally. All the treatment protocols followed 21 days and after which rats were sacrificed, blood samples were taken for biochemical studies. The Ethanol treated group rats (G2) showed variable increase in serum AST, ALT, ALP, total protein and total bilirubin levels. Administration of ethanolic extracts of *Terminalia chebula* significantly prevented Ethanol-induced elevation in the levels of serum diagnostic liver marker enzymes aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) levels and decreased antioxidant parameters in experimental groups of rats. Moreover, total protein and total bilirubin levels were significantly increased in treatment groups. The effect of extract was compared with a standard drug, Silymarin. It is concluded that the ethanolic fruit extract of *Terminalia chebula* protects against Ethanol-induced oxidative liver injury in rats.

KEYWORDS: *Terminalia chebula*; Ethanol; Hepatotoxicity; Silymarin; TCE.

INTRODUCTION

Alcoholic liver disease (ALD) develops in patients consuming excessive amounts of alcohol. Alcohol dependency is not always a pre-requirement for ALD development. In fact, some patients develop ALD and, in particular, cirrhosis, without a history of dependence. Moreover, the severity of disease does not always correlate with the amount of alcohol intake, and environmental and genetic factors likely play a crucial role in ALD development. Although a dose–effect relationship between alcohol intake and alcohol-induced hepatic damage has been reported, there is no set amount of alcohol consumption that could surely predict the development of ALD.[1]

In fact, the majority of long-term heavy drinkers develop fatty liver, but only 10–35% develop hepatitis and only 8–20% will progress to cirrhosis, Daily ethanol consumption exceeding 40–80 g/day for males and 20–40 g/day for females for 10–12 years will almost certainly lead to ALD. In a large survey conducted in Northern Europe, the relative risk of ALD significantly increased above a threshold of 7–13 drinks/week for women and 14–27 drinks/week in men.[2]

Alcohol leads to increased liver oxidative stress via generation of highly reactive oxygen species (ROS) and adducts. ADH generates acetaldehyde, which is subsequently oxidized to acetate by ALDH. Acetaldehyde can form hybrid-adducts with reactive residues (e.g. malondialdehyde adduct) acting on proteins or small molecules (e.g. cysteines), mediating lipid peroxidation and nucleic acid oxidation. Further oxidations in alcohol metabolism are accompanied by an excessive reduction of nicotinamide adenine dinucleotide (NAD), with a shift in the NADH/NAD ratio. Under normal circumstances, reduction of NAD (NAD/ NADH) is finely regulated by the cell Krebs cycle.[3] The shift caused by excessive alcohol consumption is thought to impair carbohydrate and lipid metabolism, finally causing impairment of gluconeogenesis and diversion of metabolism to ketogenesis and fatty acid synthesis. The increased amount of reducing equivalents, such as NADH, leads to their shuttering into mitochondria, which induces the electron transport chain components to assume a reduced state. This facilitates the transfer of an electron to molecular oxygen to generate reactive species as superoxide anion. Mitochondrial ROS generation can also derive from the alterations produced in mitochondrial complexes I and III, which have been

discussed above. In fact, such alteration can also promote superoxide anion generation within the mitochondria.[4]

Thus, mitochondria represent a main site where huge amount of ROS are generated, leading, in turn, to cell damage and necrosis. Finally, the NADH-induced inhibition of mitochondrial b-oxidation leads to accumulation of intracellular lipids, thus promoting steatosis. Excessive alcohol consumption is also associated with the enzymatic induction of CYP2E1 pathway of alcohol metabolism. The recruitment of this pathway may indirectly contribute to ALD development by excess production of superoxide radicals via the interaction of CYP2E1 with cytochrome reductase, which leads to electron leaks in the respiratory chain and ROS production. The species produced in this cascade can interact with iron (Fenton reaction) generating even more potent hydroxyl, ferryl and perferryl radicals which perpetuate liver damage.[4, 5]

Terminalia chebula fruits contain wide range of Tannins, Triterpinoids, Glycosides, Anthraquinones, Flavanoids. Due to its anti-oxidant activity it protects the body's cells and DNA from free radical damage.[6] The present investigation was undertaken to study the effect of *T.chebula* fruit extract on liver anti-oxidant enzymes in ethanol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Collection and authentication of plant

Terminalia chebula fruits were freshly collected from the medicinal plants farm, Mulugu, Warangal district surroundings Telangana state. The plant material was taxonomically identified and authenticated by Mr.P.V.Prasanna, Scientist 'E'/Officer In-charge, BSI, Govt.of India and the voucher specimen No.BSI/DRC/2014-2015/Tech/746.

Chemicals

Total bilirubin, Total protein, Aspartate transaminase (AST), Alanine Transaminase (ALT) and Alkaline phosphatase (ALP) were assayed by using kits. All the drugs, chemicals and reagents used for biochemical estimation were purchased from Sigma-Aldrich, USA**.**

Animals

Male Wistar albino rats, weighing about 150 - 200 g were obtained from National Institute of Nutrition, Hyderabad and used in the experiments. The protocol was approved by the Institutional Animal Ethical Committee (Reg no: MRCP/CPCSEA/IAEC/2013-14/MPCOL/11). Animals were kept in the animal house at an ambient temperature of 25°C and 45-55% relative humidity, with 12 h each of dark and light cycles. Animals were fed pellet diet and water *ad-libitum*.

Acute toxicity study was conducted for both the extracts by stair case method[7]. The LD_{50} of ethanol and aqueous leaf extracts were found to be 500 mg/kg p.o. and 1000 mg/kg p.o. and 2000 mg/kg One tenth of this was selected as maximum dose for the evaluation of anti hepatotoxic activity[8] i.e., 50 mg/kg p.o., 100 mg/kg p.o. and 200 mg/kg.

Induction of experimental Hepatotoxicity

Rats were treated with 40%Ethanol (2ml/100g, orally) for 21 days. In order to study the effect of ethanolic Fruit extract of Terminalia chebula (TCE) in rats, 50, 100 and 200 mg/kg, orally were used. Silymarin (25 mg/kg orally.) was used as a standard drug in this study.

Treatment protocol

Thirty six Wistar Albino male rats of weight 150gms-250gms were selected for this study. Animals were divided into six groups of six animals each.

Group 1: Control group (distilled water) for 21 days.
Group 2: Inducer (Ethanol 2ml/100g body weight, p.o) for 21 days.
Group 3: *T.chebula* fruit extract 50 mg/kg body weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.
Group 4: *T.chebula* fruit extract 100 mg/kg body weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.
Group 5: *T.chebula* fruit extract 200 mg/kg body weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.
Group 6: Silymarin 25 mg/kg body weight p.o + Ethanol 2ml/100g body weight, p.o for 21 days.

Biochemical estimation

At the end of the study, animals were sacrificed by cervical dislocation with ether anesthesia; blood was obtained from carotid artery. The blood samples were allowed to clot for 45min at room temperature. Serum was separated by centrifugation using Remi cool centrifuge at 4000 rpm for 15 min and utilized for the estimation of various biochemical parameters like AST.[9], ALT.[10], ALP.[11], Total proteins[12] and Total bilirubin.[13]

STATISTICAL ANALYSIS

The results are expressed as mean ± SEM. The evaluation of the data was done using one way ANOVA followed by Dunnets multiple comparisons tests. P values <0.01 were considered statistically significant.

RESULTS

Table 1: Effect of TCE on serum enzymes in ethanol induced hepatotoxicity in rats

Groups	Treatment	ALT(IU/L)	ALP(IU/L)	AST(IU/L)	Total proteins (mg/dl)	Totalbilirubin (mg/dl)
1	Normal control	159.1±5.774	185.1±5.76	26.49±0.09	69.74±0.27	1.3±0.32
2	Inducer (ethanol40%)	560.5±5.77***	674±5.77***	68.8±0.052***	26.77±0.14***	2.12±0.49***
3	TCE (50mg/kg)	410.2±5.77*	556±5.77**	60.80±0.03**	32.91±0.10*	1.92±0.37*

	+ethanol (40%)					
4	TCE (100mg/kg) + ethanol (40%)	290.1±5.768**	431.3±5.768*	47.06±0.239**	41.22±0.13**	1.74±0.35*
5	TCE (200mg/kg) +ethanol (40%)	183.9±5.77**	223.3±5.74**	37.7±0.096**	56.8±0.19**	1.41±0.29**
6	Silymarin(25mg/kg)+ethanol40%	213.8±5.80**	198.8±5.74**	30.29±0.033**	59.28±0.25**	1.37±0.38**

Values are expressed as mean ± SEM, n=6. The values were find out by using ONEWAY ANOVA followed by DUNNETS test.

***p<0.001 as compared with normal control
*p<0.05, **p<0.01 as compared with inducer ethanol

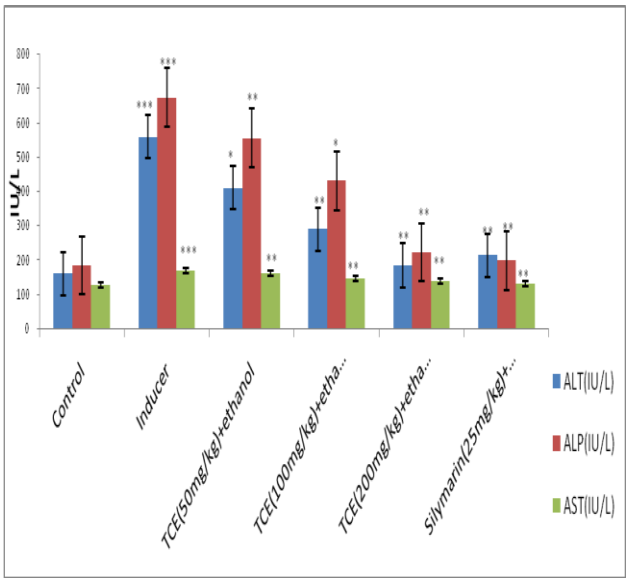

Figure 1: Effect of TCE on serum parameters (ALT, ALP, AST) in ethanol induced hepatotoxicity in rats

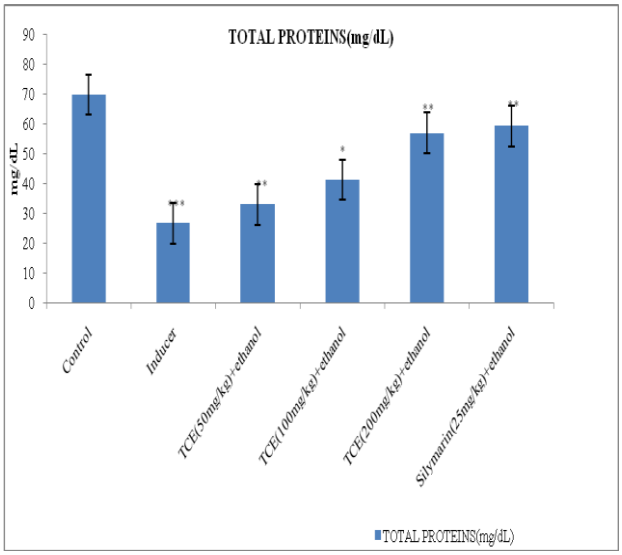

Figure 2: Effect of TCE on total proteins in ethanol induced hepatotoxicity in rats

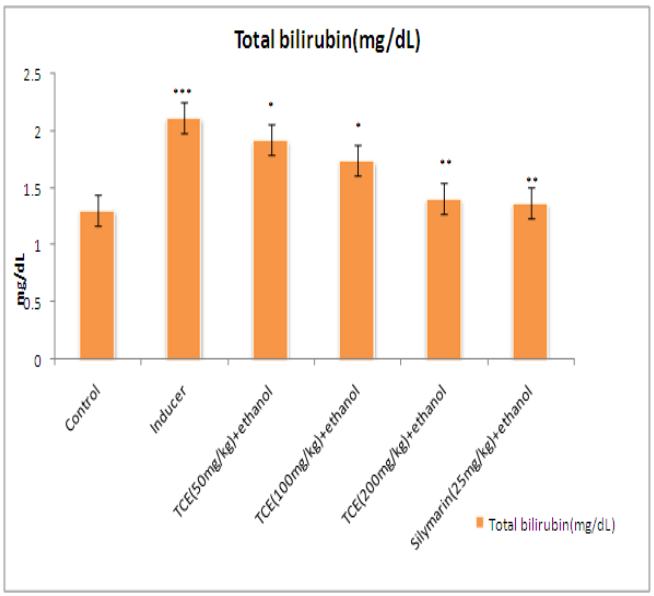

Figure 3: Effect of TCE on total bilirubin in ethanol induced hepatotoxicity in rats

DISCUSSION

Alcohol is one of the most important and commonly used hepatotoxic agents in the experimental study of liver related disorders. The hepatotoxic effects of alcohol are largely due to its active metabolite trichloromethyl radical. This activated radical bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids (PUFA). This process leads to excessive formation and accumulation of lipids in tissues such as liver. Lipids from peripheral adipose tissue are translocated to liver for accumulation.[14]

Oxidation of ethanol to water and carbon dioxide is mediated by three major hepatic enzyme systems: ADH in cytoplasm, microsomal ethanol oxidizing system in smooth endoplasmic reticulum of mitochondria (predominantly CYP2E1) and catalase in peroxisomal membrane. All these biochemical pathways produce acetaldehyde as their toxic by-product.[15]

Free radicals (ROS and RNS) are generated from our body by various endogenous systems, exposure to different physiochemical conditions, or pathological

states. A balance between free radicals and antioxidants is necessary for proper physiological function. If the free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Free radicals thus adversely alter lipids, proteins and DNA and trigger a number of disorders in humanbeing.[15]

Serum AST, ALT, ALP are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. The increased activities of AST, ALT, ALP and Total bilirubin level in serum manifested the ethanol induced hepatocellular damage. Treatment with TCE significantly decreased the activities of AST, ALT, ALP and Total bilirubin in serum suggesting that they offer protection by preserving the structural integrity of hepatocellular membrane against Ethanol.

In this study, we observed the hepatoprotective effect of *Terminalia chebula* in ethanol induced hepatotoxicity in rats. A significant elevation was observed in the levels of serum AST, ALT, ALP, total bilirubin and significant decrease level total protein in ethanolic group which received ethanol as compared to control group rats who received distilled water.

Elevated levels of these parameters in serum are presumptive markers of hepatotoxic lesions in the liver. Co-administration of standard Silymarin and various doses of (50mg/kg, 100mg/kg & 200mg/kg) *T.chebula* fruit ethanolic extract with ethanol in various extract groups, maintained the levels of AST, ALT, ALP, and serum total Protein and Total bilirubin towards normal as compared to ethanol induced rats. This was most likely due to the anti oxidant effect of *Terminalia chebula* constituents. On morphological examination in low dose *Terminalia chebula* showed partial inflammation in hepatic cells. While in high dose (200mg/kg) extract of *Terminalia chebula* fruit showed a highly recovery compared to normal.

The hepatoprotective role of Ethanolic extract of *Terminalia chebula* fruit might be due to its antioxidant potential mechanism suggesting that the extract of plant may be useful to prevent the ethanol induced Hepatotoxicity. More research is required in this View-point to develop a good hepatoprotective drug from fruits of *Terminalia chebula*. Purification of extract and identification of the active principle may yield active hepatoprotective ingredients.

CONCLUSION

The present study opens many new areas of research work. This work can be continued in the future to study and confirm liver protective activity in different experimental models and also to isolate, identify, characterize and standardize the active principle(s) that are responsible for this activity. The antioxidant activity of TCA might be responsible for its importance in traditional medicine for the treatment of various disorders.

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