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PREVENTIVE EFFECT OF MALOTILATE ON ETHANOL INDUCED HEPATIC DYSFUNCTION IN RATS

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ABSTRACT

The liver damage of variable severity is well known in chronic alcoholics. There are no promising drugs available to prevent or treat the Alcohol Induced Liver Disease (ALD). Malotilate, a sulphur containing compound, was discovered in Japan, which shows hepatocyte regenerating activity by enhancement of RNA and protein synthesis in liver. The objective of the study was to evaluate hepatoprotective effect of malotilate on ALD in rats. Sprague Dawley rats were divided into 5 groups, and treated as: 1. Control: ethanol40% v/v (orally 1ml / 100 g Day), 2. Vehicle control: ethanol+ methyl cellulose and 3, 4, 5: ethanol+ malotilate, at three dose levels viz. 25 (Low), 50 (Moderate), 100 (High) mg/kg/day orally, respectively, for 21 days. The liver function was assessed biochemically on day -1 and day-21. The histopathological assessment was done on day-21. The results demonstrated that, ethanol induced significant hepatic dysfunction and structural damage. Malotilate showed hepatocyte protective effect in ALD in a dose dependent manner. This study indicated that malotilate prevented ALD in alcoholic rats. However, clinical studies are needed to establish the efficacy of Malotilate on ALD in chronic alcoholics.

KEY WORDS: Ethanol, Liver Disease, Malotilate, LFTs, Histopathology.

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INTRODUCTION

Alcoholic liver Disease (ALD) is a life style associated disease similar to diabetes, hypertension, atherosclerosis etc., which are still among the chief causes of morbidity and mortality in the world[1,2]. Fat accumulation and inflammation in the liver are the classic pathological features of ALD which advance in to cirrhosis and hepatocellular carcinoma if alcohol consumption is not stopped. The development of steato-hepatitis plays a major step in the progression to cirrhosis[3]. Chronic and excessive Ethanol consumption impairs fat oxidation and stimulates lipogenesis, thus leading to steatosis[2]. Fat accumulation in the liver increases the sensitivity of the liver to the second hit that leads to inflammatory liver cell damage[4]. Oxidative stress, endotoxins, and cytokines are considered to be the causes of the second hit and are related to the pathogenesis of ALD. In spite of the vast studies conducted regarding pathophysiology and mechanism of ALD, a validated therapeutic tool to attenuate or reverse ALD is still in obscure. Although, abstinence from alcohol can be achieved by general supportive and symptomatic care, relapse is prevalent in most of the addicts. Multiple treatment strategies for both the short and long term mortality and morbidity of this disease have been proposed, but strong disagreement exists**.** Phytotherapeutic approaches are being used with limited proof for their efficacy. Hence, search for an effective moiety in attenuating ALD still exists. Nowadays, Sulphur and Thiol containing compounds are gaining much attention since they exert various biological activities in the oxidative stress related diseases.[5,6] Malotilate**,** a Sulphur-containing drug, has been reported to exert a number of favorable effects on hepatic functions in experimental animals. Malotilate has shown to stimulate hepatic blood flow and bile secretion and protect against the liver damage induced by agents such as allyl alcohol, bromobenzene, thioacetamide, carbon tetrachloride, paracetamol and D-galactosamine[7,8,9]. Hence, the objective of the study was to assess the preventive effect of Malotilate on Ethanol induced hepatic dysfunction in experimental model in rats.

MATERIALS AND METHODS

Adult Sprague Dawley rats of either sex, weighing 200-250g were housed in plastic cages under controlled conditions of 12-h light/12-h dark cycle, 50% humidity and at 25°C. They all received a standard pelleted diet (Pranav Agro Industries Ltd., Pune, Maharashtra, India) and water ad libitum. [22]
They rats were divided into 5 groups (n=6) as follows:

Group-1(E): Control: Ethanol 40 % v/v 1ml / 100gm/d

Group-2(MC): Vehicle Control: Ethanol& Methyl Cellulose 0.5% 1ml/kg/d

Group-3(Mal-1): Malotilate low dose:Ethanol & Malotilate 25 mg / kg/d

Group-4(Mal-2); Malotilate Moderate Dose : Ethanol & Malotilate 50 mg / kg /d

Group-5 (Mal-3): Malotilate High Dose: Ethanol & Malotilate 100 mg / kg/d

Ethanol and Malotilate administration was startedsimultaneously. Ethanol 40% was administered orally in the dose of 1ml/100gm/day to all the groups every day in the evening at 4.30PM. for 21 days[21]. From Group-2 onwards the respective drugs (Vehicle Methyl Cellulose and Malotilate) were administered orally every day in the morning at 11.30 AM, for 21 days. On day- 1 before drug administration, and on day-21 after the final doses of drugs, the animals were anaesthetized with Pentobarbitone Sodium and the blood was collected by retro-orbital puncture to assess various biochemical functions. Later, on day-21 the animals were sacrificed by cervical dislocation. The livers were removed and preserved for the histopathological assessments. Sprague Dawley Albino Rats were purchased from National Toxicology Center, Pune (Maharashtra State Lic No. P-D-T-L-7). Malotilate was purchased from Bosche Scientific, 100 Jersey Ave, New Brunswick, NJ 08901, USA. Its suspension (1gm in 100 ml) was prepared in 0.5% Methyl Cellulose to obtain final concentration 10mg Malotilate /ml, used for administration. The other chemicals viz. Absolute alcohol (GR grade, 99.8% Mfg by E. Merck, Germany), Pentobarbitone

Sodium (LR grade Loba-Cheme, Indo Astranal Co. Mumbai 4000 005) were purchased from respective suppliers. The study was done after IAECapproval, following the CPCSEA guidelines. The liver function was assessed by biochemical parameters. The levels of aspartate transaminase (AST), alanine transaminase (ALT) alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) in serum were estimated by using commercial kits (ERBA diagnostic Mannheim GmbH, Germany). Total bilirubin, direct bilirubin, total protein and albumin were estimated by using commercially available kits (ERBA diagnostic Mannheim GmbH, Germany) and prothrombin time (PT) by using auto analyzer.(Fully Automated Clinical Chemistry Analyser - EM360-Mftr: Transasia Bio-Medicals Limited, Mumbai, Maharashtra, India). Small portions of the liver was dissected and fixed in 10% formalin solution for 24 h. The fixed tissues were embedded in paraffin, sectioned to 3-5 µm thickness, de-paraffinized, and rehydrated using standard techniques.The extent of alcohol-induced necrosis and steatosis was evaluated by assessing morphological changes in liver sections stained with hematoxylin and eosin using standard techniques. Liver sections were graded numerically to assess the degree of The parameters were graded from score 0-6 with 0 indicating no abnormality, 1-2 mild, 3-4 moderate and 5-6 sever damage in form of portal fibrosis and 0-3 with 0 indicating no abnormality, 1-mild, 2- moderate and 3-severe damage for the rest of the histopathological parameters. Total score of liver damage for each rat was calculated.

The scoring of liver damage was done as follows[10,11,12].

- Portal fibrosis (PF): score 0-6,
- Lobular infiltration and Necrosis (LIN): score 0-3,
- Mallory bodies (MB) 0-3,
- Hepatocytes ballooning (HB) : score 0-3
- Perisinusoidal Fibrosis (PSF) : score 0-3
- Fatty changes (FC): score 0-3.

All the data is expressed as Mean ± SD. The results were evaluated using a paired 't' test and analysis of variance (ANOVA) using the Graph Pad Prism -5 software.

RESULTS

In this study, Ethanol alone (Group-1; E) and Ethanol with Methyl Cellulose (Group-2; E+MC) administered rats displayed a significant elevation in the levels of AST, ALT, ALP and GGT on day-21 when compared to day-1 and thus reveal derangement of hepatic function on day-21 (Table-1). This induction of hepatic dysfunction is also manifested by elevation in the level of total bilirubin, direct bilirubin and and prolongation of prothrombin time (Table-2), and significant decrease in total protein and albumin. Malotilate treated rats [moderate dose 50mg/kg (Group-4; Mal-2) and high dose 100 mg /kg (Group-5; Mal-3)] did not show any significant alteration in the levels of hepatic marker enzymes when compared to day-1 (Table-1). However, this is not seen with Malotilate low dose 25mg/kg (Group-3; Mal-1). Malotilate (low, moderate and high doses) has also significantly attenuated the ethanol induced changes in of total bilirubin, direct bilirubin, prothrombin time, total protein and albumin (Table-2).

Table1

Effect of Malotilate and Ethanol (21 days) on serum hepatic marker enzymes

Parameters		AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
Group 1 Ethanol (40 % v/v) 1ml / 100gm	**Day 1**	272.8± 47.58	45.5±10.31	288.7±113.7	37.83±6.55
	Day21	491.7±165.3*	62.67±15.62*	316.5±117.6***	269.7±24.01***
Group 2 Ethanol + MC (0.5% suspension 1ml/kg)	**Day 1**	351.8±58.61	83.83±11.25	215±16.7	45.3±10.3
	Day21	595.8±64.79***	106.5±15.44*	316±67.5**	135±36.1***
Group 3 Ethanol + Malotilate (25mg/kg)	**Day 1**	359.2±23.22	81.83±10.98	200±38.5	44.7±6.12
	Day21	388.7±17.8*	95.33±8.066*	248±30.7*	53.3±9.58 NS
Group 4 Ethanol + Malotilate (50mg/kg)	**Day 1**	319.5±32.33	84±15.91	152±38.7	47.17±6.46
	Day21	329.2±33.08 NS	92±10.64 NS	185±57.3 NS	48.33±7.11 NS
Group 5 Ethanol + Malotilate (100 mg/kg)	**Day 1**	308.2±51.81	67±8.60	120±32.2	40.7±3.78
	Day21	311.2±54.66 NS	71.17±12.69 NS	140±32.7 NS	42.2±3.97 NS

Values are Mean ± SD; n = 6 animals in each group: Comparisons were made between Day1 Vs Day 21, within group
**** P <0.05, ** P < 0.01, *** P < 0.001*** NS= Not Significant

Table 2

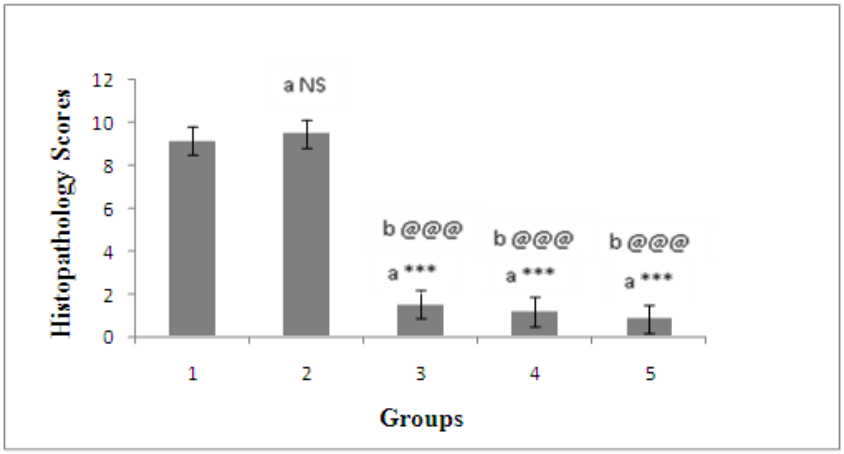
Effect of Malotilate and Ethanol (21 days) on total bilirubin, direct bilirubin, total protein, albumin and prothrombin time

Parameters		Total Bilirubin (mg %)	Direct Bilirubin (mg %)	Total Protein (mg %)	Albumin (mg %)	Prothrombin Time (Sec)
Group 1 Ethanol (40 % v/v) 1ml / 100gm	Day 1	0.3±0.1587	0.245±0.13	8.58±0.30	4.46±0.67	11.33±0.5164
	Day21	1.212±0.40***	1.002±0.22***	6.6±0.40***	2.73±0.35***	19.83±4.262**
Group 2 Ethanol (40 % v/v) 1ml / 100gm+ MC	Day 1	0.472±0.11	0.4±0.10	8.36±0.24	3.8±0.42	11.5±1.049
	Day21	0.727±0.09**	0.64±0.11**	6.85±0.36***	2.81±0.46***	19.5±1.643***
Group 3 Ethanol + Malotilate (25mg/kg)	Day 1	0.462±0.05	0.405±0.03	8.31±0.31	3.63±0.23	11.33±0.8165
	Day21	0.484±0.05 NS	0.406±0.02 NS	8.11±0.28 NS	3.4±0.36 NS	12.17±0.7528*
Group 4 Ethanol + Malotilate (50mg/kg)	Day 1	0.578±0.13	0.53±0.13	8.71±0.33	4.31±0.21	11.5±0.8367
	Day21	0.61±0.14 NS	0.55±0.13 NS	8.63±0.22 NS	3.35±1.45 NS	11.17±0.4082 NS
Group 5 Ethanol + Malotilate (100 mg/kg)	Day 1	0.27±0.14	0.24±0.13	8.2±0.36	4.25±0.25	10.67±0.8165
	Day21	0.28±0.13 NS	0.25±0.12 NS	8.08±0.34 NS	4.05±0.25 NS	11±0.8944 NS

Values are Mean ± SD; n = 6 animals in each group: Comparisons were made between Day1 Vs Day 21, within group
**** P <0.05, ** P < 0.01, *** P < 0.001 NS=Not Significant***

Figure 1

Effect of Malotilate and Ethanol (21 days) on liver histopathology



Values are Mean ± SD; n = 6 animals in each group: Comparisons were made between:
a :Group 1 vs Group 2,3,4 & 5; b: Group 2 vs Group 3,4 & 5. *** P < 0.001,@@@P<0.001 & NS-Non Significant

The histopathological scores of Ethanol treated rats (Group-1) and Ethanol + MC treated rats (Group-2) were high indicating severe hepatic damage. Co-administration of Malotilate (low, moderate and high doses) has significantly prevented these toxic effects of Ethanol and maintained the hepatic architecture to normalcy on day -21 (Fig.1).

DISCUSSION

The present study shows that ethanol causes significant hepatic dysfunction and architectural derangement. Slew of literature had shown that biotransformation of Ethanol in hepatocytes generates various noxious metabolites, free radicals and provokes oxidative insult which is the mainstay in the pathogenesis of ALD. Further, oxidation of Ethanol through the cytochrome P450 2E1 enzyme (CYP 2E1) elicits rapid generation of superoxide anion radical and hydrogen peroxide[6,13,14]. Interestingly, these free radicals have a potential to damage the cellular structures such as nucleic acids, proteins and lipids. In the present communication, preventive efficacy of Malotilate, a sulphur containing drug, on Ethanol induced hepatic injury was evaluated. Hepatocytes play a vital role in various metabolic reactions and contain a wide network of enzymes. In tissues, AST and ALT are found in higher concentration in cytoplasm and AST in particular also exists in mitochondria[15]. During hepatic injury the transport function of the hepatocytes get disturbed, resulting in the increased permeability of plasma membrane[(16)], thereby causing an increased enzyme level in the serum[17]. If injury involves organelles such as mitochondria, soluble enzymes like AST normally located there, will also be similarly released. The elevated activities of AST and ALT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver. ALP is excreted normally via bile by the liver. In hepatic injury due to hepatotoxins, there is a defective excretion of the bile by the liver, which is reflected in its increased levels in serum. Gamma-Glutamyl Transferase (GGT) is located in a variety of organs including plasma membranes of the hepatocytes. This enzyme is a component of the gamma-glutamyl cycle and plays a significant role for the transport of extracellular amino acids through the outer membrane of the cells. During the course of hepatic damage, hepatic amino acid metabolism is altered leading to the elevation of serum GGT level. In the present study, Ethanol (40 % v/v) 1ml / 100gm/d intoxication for 21 days displayed a marked increase in the level of hepatic marker enzymes (AST, ALT, ALP and GGT) in the serum which is lined with previous reports .Treatment with Malotilate at moderate (50mg/kg/d) and high dose (100mg/kg/d) significantly prevented the elevation in marker enzyme levels and thus helped to maintain the structural integrity of hepatic membrane. This action is produced by neutralizing the free radicals generated in the event of Ethanol induced oxidative hepatic damage. However, Malotilate low dose (25mg/kg/d) did not show effective protection which might be due to the low concentration of the drug to produce the desirable effect. Hyperbilirubinaemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis. It increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degradation rate. In the present study, Ethanol intoxication revealed a significant increase in the level of total and direct bilirubin[18]. Malotilate treatment (50 &100mg/kg) significantly prevented elevation in the levels of these marker. Furthermore, in Ethanol provoked hepatotoxicity, a depression in total proteins as well as albumin was observed due to the defect in protein biosynthesis which might be due to the disruption and dissociation of polyribosomes from endoplasmic reticulum(ER). Co-administration of Malotilate (25, 50 & 100 mg/kg) significantly maintained the levels to normalcy which indicates the ability of the drug to stimulate the hepatic protein synthesis[19]. In the present study, Ethanol treated rats displayed a significant increase in the prothrombin time (PT) which clearly indicates the disability of the liver to synthesize the clotting factors. Treatment with Malotilate (25, 50 & 100 mg/kg) significantly normalized the PT and thus restored the hepatic functions of liver[20]. Histopathological analysis of hepatic tissues intoxicated with Ethanol displayed a severe necrosis and treatment with Malotilate significantly preserved the hepatic architecture to normal (Fig.1)The protective effect of Malotilate in ethanol induced hepatic disease may be through free radical scavenging mediated and

stimulation of protein synthesis. But, studies towards elucidating precise mechanism are highly warranted. In conclusion, the present study confirms the preventive effect of Malotilate in Ethanol induced hepatic damage in a dose dependant manner, which is evident from prevention of elevation in the marker enzymes, total and direct bilirubin levels and prolongation of Prothrombin time. Further, the levels of total protein and albumin were maintained to normal. The histopathological analysis reveals maintenance of the normal architecture of liver with Malotilate co-administration. However further studies are needed to establish its role in alcoholic liver disease.

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