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CURATIVE EFFECTS OF MALOTILATE ON ETHANOL - INDUCED HEPATIC DYSFUNCTION IN RATS

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ABSTRACT: Background: In chronic alcoholics, continuous heavy drinking leads to progression of alcoholic liver disease (ALD) from alcoholic fatty liver, hepatitis to cirrhosis. Strict abstinence from alcohol has its own limitations and there is paucity of promising drugs available to treat ALD. Malotilate showed promising hepatocyte regenerating capacity and ability to prevent such damage. **Aims:** The objective of this study was to evaluate curative effect of malotilate on ALD in rats. **Methods:** The study was done using Sprague Dawley rats. 5 groups were treated as: 1. Control: ethanol 40% v/v (orally 1ml / 100 g / d), 2. Vehicle control: Methyl Cellulose and 3, 4, 5: malotilate, three doses 25 (Low), 50 (Moderate), 100 (High) mg /kg / d orally, respectively. In all groups: ethanol was given initially for 21 days. Then vehicle or malotilate was administered for next 21 days. The liver damage was assessed bio-chemically on day-1, day-22, day-43 by measuring serum AST, ALT, ALP, GGT, total bilirubin, direct bilirubin, total protein, albumin and prothrombin time. The histo-pathological assessment of liver damage was done on day-43. **Results:** This study demonstrated that malotilate favorably altered all the biochemical parameters and the histopathology scores of liver damage in malotilate treated groups. **Conclusions**: malotilate showed hepatic structure regenerative and hepatic function restorative effect in ALD. This study indicated that malotilate may be useful in treating established ALD in alcoholics.

INTRODUCTION: Alcohol is the commonest substance of abuse. Alcoholism is one of the most dreadful self-made diseases of mankind. Alcohol induced Liver Disease (ALD) is the important cause of disability and death amongst the abusers throughout the world. Total abstinence from alcohol is the primary step for reversal and to stop progression of ALD. Mortality of continuous heavy drinkers is much higher than mortality of abstainers.

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But usually strict abstinence is very difficult for a binge drinker because of ravages of alcohol. ALD starts with fatty liver formation and progresses into hepatitis, fibrosis, necrosis and cirrhosis. Further, the risk of hepatocellular cancer highly increases in patients with cirrhosis, especially in alcoholics. Ultimately complications of cirrhosis lead to death[1]. The current remedies available to manage ALD are very few with limited proof, questionable efficacy and compromised adverse effects. Therefore, still there is a need to invent newer treatment strategies for prevention and cure of alcohol induced liver damage.

Malotilate is a new drug suggested for use in chronic liver diseases. It has been studied in animal models of different hepatotoxic chemicals induced liver damage. It is found to be effective in

preventing liver damage in these models by hampering the process of liver fibrosis [2]. In our previous study malotilate was found to prevent ethanol induced liver damage [3]. Hence the objective of the present research was to assess the restorative effect of malotilate on hepatic structure and function on ethanol induced liver disease in rats.

MATERIALS AND METHODS:

Study design: Sprague Dawley rats, purchased from National Toxicology Center, Pune (Maharashtra State Lic No. P-D-T-L- 7) of either sex, weighing 200-250g were used. The animals were housed in plastic cages under controlled conditions of 12-h light/12-h dark cycle. They all received a standard pelleted diet (Mfg. by Pranav Agro Industries Ltd., Pune, Maharashtra, India) and water *ad libitum*. After obtaining the approval of Institutional Animal Ethics Committee (IAEC/BVUMC/8/2010-11; Date-27/08/2010) the study was conducted as follows:

The rats were divided into 5 groups (n=6) as follows, to study the hepato-restorative / regenerative effect of malotilate on ALD:

Group-1: (Control -E): Ethanol 21 days (d1-21)

Group-2: (E ➔ MC): Ethanol for initial 21 days (d1-21) + next 21 days (d22-42) only Methyl Cellulose 0.5%

Group-3: (E➔Malotilate 25): Ethanol for initial 21 days (d1-21) + Next 21 days (d22-42) only Malotilate 25 mg / kg

Group-4: (E➔Malotilate 50): Ethanol for initial 21 days (d1-21) + Next 21(d22-42) days only Malotilate 50mg/kg

Group-5: (E➔Malotilate 100): Ethanol for initial 21 days (d1-21) + Next 21(d22-42) days only Malotilate 100 mg/kg

Material:

Malotilate was purchased from Bosche Scientific, 100 Jersey Ave, New Brunswick, NJ 08901, USA. 1gm in 100 ml malotilate suspension in 0.5 % methyl cellulose was prepared with a concentration of 10mg malotilate /ml. ethanol (Absolute alcohol) GR grade, 99.8 % was obtained from E. Merck, Germany. Ketamine (Aneket) 50mg/0.01%/10ml. Manufactured by Neon Laboratories Limited., Mumbai, India was used to anesthetize the rat in the dose of 100 mg/kg intraperitoneal.

Methodology:

First ethanol 40% v/v was administered orally in the dose of 1ml/100gm/day [4] to all the groups every day for 21 days to induce liver damage in all animals. Then from day-22 onwards till day-42 methyl cellulose or malotilate was administered as per the groups respectively, to evaluate curative effect of malotilate on hepatic damage. In anaesthetized animals, the blood samples were withdrawn and assessed for the biochemical parameters on day-1 and day-22. On day-43, the blood was withdrawn after anesthesia and then the animals were sacrificed by cervical dislocation to isolate liver for histopathological evaluation.

The rats were anesthetized by Ketamine 100 mg/kg IP and blood was withdrawn by retro-orbital vein puncture technique. All the biochemical parameters were measured using commercial kits (ERBA diagnostic Mannheim GmbH, Germany) on an auto analyzer machine (Fully Automated Clinical Chemistry Analyzer - EM360-Mftr: Transasia Bio-Medicals Limited, Mumbai, Maharashtra, India). The serum levels of Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline Phosphatase (ALP) and Gama Glutamyl Transferase (GGT), Total Bilirubin(TB), Direct Bilirubin (DB), Total Protein (TP), Albumin (ALB) and Prothrombin Time (PT) were measured on day-1, day-22 and day-43.

At the end of study the livers were dissected and fixed in 10% formalin solution for 24 h. The fixed tissues were embedded in paraffin, sectioned to 3-5 µm thickness, deparaffinized, 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 & eosin and Masson's trichrome using standard techniques [5]. Liver sections were graded numerically to assess the degree of histological changes in hepatic injury.

- Numerical scoring of liver damage was carried out as follows [6]:
  - Portal fibrosis (PF 0-6),
  - Lobular infiltration and necrosis (LIN 0-3),
  - Mallory bodies (MB 0-3),
  - Hepatocytes ballooning (HB 0-3)
  - Perisinusoidal Fibrosis (PSF 0-3)
  - Fatty changes (FC 0-3).
- The first parameter of portal fibrosis was graded from score 0 to 6, with 0 indicating no abnormality, 1 to 2 mild injury, 3 to 4 moderate injury and 5 to 6 with severe liver injury. The other parameters were graded from score 0 to 3 with 0 indicating no abnormality, 1 mild injury, 2 moderate injury and 3 severe liver injury.
- Total score of liver damage for each rat was calculated by addition of all scores.

Statistical analysis:
All the data were expressed as Mean ± SD. The Statistical analysis of results was done by applying Wilcoxon Signed Ranked test and Kruskal Wallis with post-hoc Dunn's test using the Graph Pad Prism -5 software.

RESULTS: In this study the AST, ALT, ALP and GGT levels measured on day-22 were significantly ($p< 0.001$, $p<0.05$) raised in all groups because of ethanol exposure for 21 days. Further, these levels of AST, ALT, ALP and GGT in ethanol (E), and methyl cellulose (MC) groups were significantly higher on day-43 than their day-22 values (**Tables 1, 2**). The treatment with only vehicle; methyl cellulose (Group-2) showed no significant difference in the enzyme levels than those in the ethanol pretreated group-1. Furthermore, in all malotilate treated groups, (group-3-low, group-4-moderate and group-5-high dose) the elevated hepatic marker enzyme levels were significantly reduced to normal baseline levels on day-43 (**Tables 1, 2**). The trend of AST levels and effects of various treatments over study period was seen in **Graph 1.** Similar trend was observed with other biochemical markers which were elevated by ethanol.

TABLE 1: EFFECT OF MALOTILATE ADMINISTRATION ON SERUM ASPARTATE TRANSAMINASE (AST) AND ALANINE TRANSAMINASE (ALT) LEVELS OF ETHANOL-INDUCED HEPATIC DYSFUNCTION IN RATS.

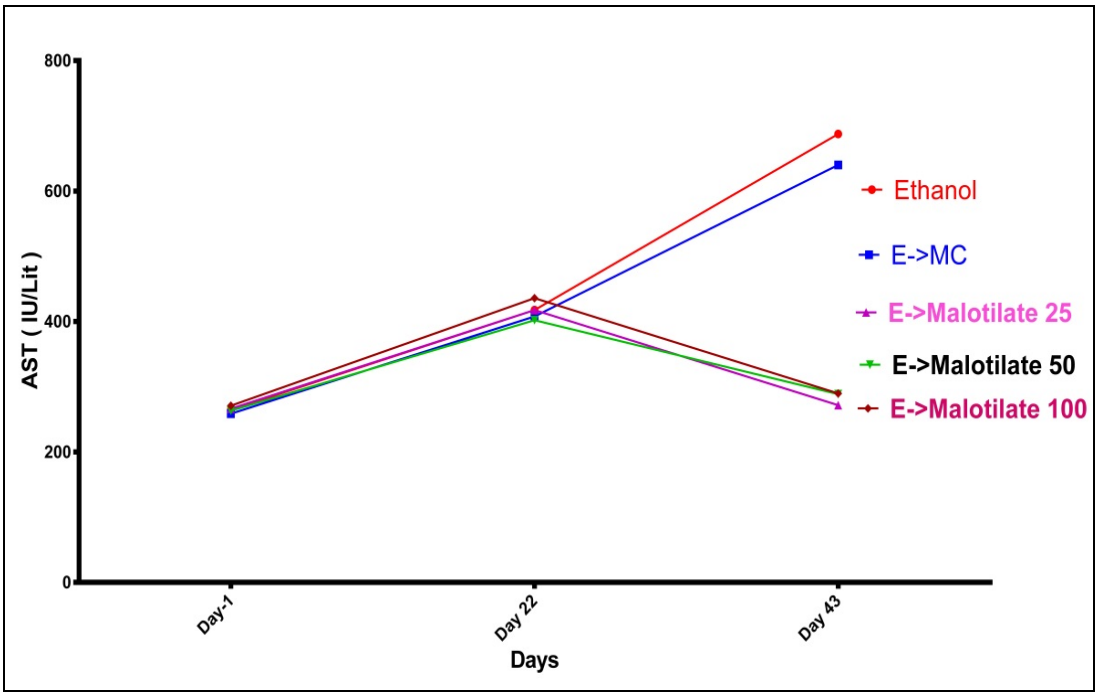
Parameter→ Groups↓ (n=6)	AST (IU/L)			ALT (IU/L)		
	Day-1	Day-22	Day-43	Day-1	Day-22	Day-43
Group-1: (Control -E)	264.7± 30.77	417.7± 38.54 ***	687.5 ± 20.92 *** ###	41.17± 6.34	61.17± 10.11 ***	84.8 ± 5.08 ***
Group-2: (E ➔ MC)	258.7± 33.96	407.8± 58.03 ***	640.2 ± 44.35 ***	38.50± 6.90	61.5 ± 9.61 ***	83.33 ± 6.22 ***
Group-3: (E➔Malotilate 25)	266.2± 33.29	417.7± 64.57 ***	271.± 42.86 ### $$$@@@	42.17± 8.61	57.83 ± 15.47 ***	44.67 ± 7.99 ### $$$@@@
Group-4 : (E➔Malotilate 50)	263.2± 28.05	402± 59.9 ***	288.± 32.597 ### $$$@@@	41.33± 6.25	60.67 ± 12.64 **	44.67 ± 6.59 # $$$@@@
Group-5: (E➔Malotilate 100)	270.8± 27.77	435.8± 41.12 ***	289.8 ± 18.0 ###$$$@@@	38.17± 5.53	57 ± 12.62 **	43.67 ± 6.47 #$$$ @@@

Values: mean ± SD Kruskal Wallis test, Post-hoc Dunns test
Comparisons of Mean values – * P <0.05, ** P < 0.01, *** P < 0.001
* Day-1 Vs Day-22/Day-43; # Day-22 Vs Day-43;
$ Group-1 (ethanol) Vs Other Groups;
@ Group-2 (MC-Vehicle) Vs Other Groups (malotilate)

TABLE 2: EFFECT OF MALOTILATE ADMINISTRATION ON SERUM ALKALINE PHOSPHATASE (ALP) AND GAMMA GLUTAMYL TRANSFERASE (GGT) LEVELS OF ETHANOL- INDUCED HEPATIC DYSFUNCTION IN RATS.

Parameter→ Groups↓ (n=6)	ALP (IU/L)			GGT (IU/L)		
	Day-1	Day-22	Day-43	Day-1	Day-22	Day-43
Group-1: (Control -E)	238.2± 34.85	317.± 42.46 *	414.5± 42.23 *** ##	29.8±4.0 7	49± 7.7 **	69.33± 11.96 *** ##
Group-2: (E ➔ MC)	224.3± 29.45	320.3±47.5 **	427.3± 48.8 ***	29± 6.51	42.83±12.6 7*	77.17± 6.85 **
Group-3: (E➔Malotilate 25)	239.7± 41.14	317.7±38.7 *	242.8± 38.73 # $$$@@@	31± 3.58	48.5± 11.59**	32.67± 4.08 ## $$$@@@
Group-4 : (E➔Malotilate 50)	228.5± 41.49	317± 47.24 **	238.2± 44.91 # $$$@@@	31± 5.06	47.33±9.85 **	34 ± 5.06 #$$$@@@
Group-5: (E➔Malotilate 100)	245.5± 44.95	333± 38.99 **	260± 45.72 #$$$@@@	32± 6.29	53.33±5.57 ***	33.83± 4.67 ###$$$@@@

Values: mean ± SD Kruskal Wallis test, Post-hoc Dunns test
Comparisons of Mean values – * P <0.05, ** P < 0.01, *** P < 0.001
* Day-1 Vs Day-22/Day-43; # Day-22 Vs Day-43;
$ Group-1 (ethanol) Vs Other Groups;
@ Group-2 (MC-Vehicle) Vs Other Groups (malotilate)



GRAPH 1: EFFECT OF MALOTILATE (DAY 22TO 42) ON ETHANOL (DAY 1 TO 21) INDUCE SERUM ASPARTATE TRANSMINASE (AST) LEVELS ON VARIOUS DAYS IN CURATIVE STUDY

Ethanol administration to all rats for 21 days resulted in significant ($p<0.05$ & $p<0.001$) elevation in the values of total bilirubin and direct bilirubin on day-22 when compared to day-1. Further, in ethanol treated group-1(E) and methyl cellulose treated group-2 (MC), the total bilirubin and direct bilirubin levels on day-43 were significantly ($p<0.001$) elevated when compared to day- 22 (**Table 2**). While, in all the malotilate treated groups (group-3 Mal-1, group-4 Mal-2 and group-5 Mal-3), the levels of total bilirubin and direct bilirubin, were significantly reduced to their base line levels ($p<0.001$) when compared with day-22 (**Table 3**).

TABLE 3: EFFECT OF MALOTILATE ADMINISTRATION ON SERUM TOTAL BILIRUBIN (TB) AND DIRECT/CONJUGATED BILIRUBIN (DB) LEVELS OF ETHANOL-INDUCED HEPATIC DYSFUNCTION IN RATS.

Parameter→ Groups↓ (n=6)	TB (mg%)			DB (mg%)		
	Day-1	Day-22	Day-43	Day-1	Day-22	Day-43
Group-1: (Control -E)	0.28±0.06	0.94±0.22 *	1.86 ± 0.64 *** ##	0.23±0.05	0.83±0.2 *	1.58 ± 0.5 ***##
Group-2: (E ➔ MC)	0.29±0.06	0.8± 0.12 ***	1.13 ± 0.19 *** #	0.23±0.06	0.73±0.11* **	0.98 ± 0.12 *** #
Group-3: (E➔Malotilate 25)	0.3± 0.06	0.87±0.07 ***	0.39 ± 0.1 ###$$$@@	0.24±0.04	0.77±0.1 ***	0.34± 0.07 ###$$$@@
Group-4 : (E➔Malotilate 50)	0.29±0.06	0.85±0.08* **	0.39 ± 0.09 ### $$$@@	0.21±0.07	0.77±0.04* **	0.29 ± 0.07 ###$$$@@@
Group-5: (E➔Malotilate 100)	0.2± 0.06	0.92±0.1 ***	0.33 ± 0.11 ### $$$ @	0.22±0.06	0.8± 0.06***	0.26 ± 0.1 ###$$$ @@

Values: mean ± SD Kruskal Wallis test, Post-hoc Dunns test
Comparisons of Mean values – * P <0.05, ** P < 0.01, *** P < 0.001
* Day-1 Vs Day-22/Day-43; # Day-22 Vs Day-43;
$ Group-1 (ethanol) Vs Other Groups;
@ Group-2 (MC-Vehicle) Vs Other Groups (malotilate)

The total protein and albumin levels were significantly decreased in all groups after ethanol administration for 21 days. Further, the total protein and albumin levels were significantly lower on day-43 in ethanol (E) and methyl cellulose (MC) groups, than their day-1 and day-22 values. In all malotilate treatment groups the total protein and albumin levels on day-43 were increased significantly as compared to their day-22 values, and were comparable with their day-1 values. The prothrombin time was significantly increased in all groups after ethanol administration for 21 days. Further, the Prothrombin time was significantly higher on day-43 in ethanol (E) and methyl cellulose (MC) groups, than its day-1 and day-22 values. In all malotilate treatment groups the Prothrombin time on day-43 was decreased significantly as compared to its day-22 values, and was comparable with its day-1 values (**Tables 4, 5**).

TABLE 4: EFFECT OF MALOTILATE ADMINISTRATION ON TOTAL PROTEIN (TP) AND ALBUMIN (ALB) LEVELS OF ETHANOL-INDUCED HEPATIC DYSFUNCTION IN RATS

Parameter→ Groups↓ (n=6)	Total Protein (gm%)			Albumin (gm%)		
	Day-1	Day-22	Day-43	Day-1	Day-22	Day-43
Group-1: (Control -E)	8.62 ±0.43	6.68 ±0.3 ***	4.65 ±0.47 *** ###	5.22 ±0.43	3.97 ±0.45 ***	2.67 ±0.27 *** ###
Group-2: (E ➔ MC)	8.28 ±0.48	6.78 ±0.44 ***	4.8 ±0.28 *** ###	5.57 ±0.54	3.85 ±0.46 ***	2.88 ±0.42 ***#
Group-3: (E➔Malotilate 25)	8.12 ±0.43	6.03 ±0.36 ***	7.58 ±0.29 ### $$@	5.48 ±0.82	3.85 ±0.35 **	4.83 ±0.63 #$$$@@@
Group-4 : (E➔Malotilate 50)	8.23 ±0.39	5.98 ±0.49 ***	7.75 ±0.38 ###$$$@@@	5.6 ±0.79	3.95 ±0.83 **	5.15 ±0.7 #$$$@@@
Group-5: (E➔Malotilate 100)	8.17 ±0.43	6.18 ±0.61 ***	7.6 ±0.47 ###$$$@@	5.55 ±0.75	4.05 ±0.27 **	5.22 ±0.69 #$$$@@@

Values: mean ± SD Kruskal Wallis test, Post-hoc Dunns test
Comparisons of Mean values – * P <0.05, ** P < 0.01, *** P < 0.001
* Day-1 Vs Day-22/Day-43; # Day-22 Vs Day-43;
$ Group-1 (ethanol) Vs Other Groups;
@ Group-2 (MC-Vehicle) Vs Other Groups (malotilate)

TABLE 5: EFFECT OF MALOTILATE ADMINISTRATION ON PROTHROMBIN TIME (PT) OF ETHANOL-INDUCED HEPATIC DYSFUNCTION IN RATS.

Parameter→ Groups↓ (n=6)	Prothrombin Time (Sec)		
	Day-1	Day-22	Day-43
Group-1: (Control -E)	11.83 ±0.75	16.83 ±1.72 ***	20.5 ±1.52 *** #
Group-2: (E ➔ MC)	12.33 ±0.82	18.17 ±1.84 ***	21.5 ±2.43 ***#
Group-3: (E➔Malotilate 25)	12.67 ±1.21	19.67 ±1.37 ***	14.0 ±1.41 ###$$$@@@
Group-4 : (E➔Malotilate 50)	12.5 ±1.05	18.17 ±2.86 ***	13.17 ±1.6 ## $$$@@@
Group-5: (E➔Malotilate 100)	12.33 ±0.82	18.83 ±2.4 ***	12.83 ±1.47 ### $$$@@@

Values: mean ± SD Kruskal Wallis test, Post-hoc Dunns test
Comparisons of Mean values – * P <0.05, ** P < 0.01, *** P < 0.001
* Day-1 Vs Day-22/Day-43; # Day-22 Vs Day-43;
$ Group-1 (ethanol) Vs Other Groups;
@ Group-2 (MC-Vehicle) Vs Other Groups (malotilate)

On day- 43 the total histopathological scores of group-1; ethanol (E) and group-2; methyl cellulose (MC) were significantly high indicating severe hepatic damage. Whereas, treatment with malotilate (low, moderate and high doses) significantly (p<0.001) reduced the toxic effects of ethanol and restored the hepatic architecture to normalcy when compared with group-1 and group-2 (**Table 6).**

TABLE 6: EFFECT OF MALOTILATE ADMINISTRATION ON DAY-43 LIVER HISTOPATHOLOGY SCORE (HPS) OF ETHANOL-INDUCED HEPATIC DYSFUNCTION IN RATS

Parameters→ Groups↓ (n=6)	1 Portal Fibrosis (PF): 0-6	2 Lobular Inflammation & Necrosis : (LIN): 0-3	3 Mallory Bodies (MB) : 0-3	4 Hepatocyte Balooning (HB) : 0-3	5 Perisinusoidal Fibrosis (PSF) : 0-3	6 Fatty Change (FC): 0-3	Total score (HPS) 0-21
Group-1: (Control -E)	2.33 ±1.03	2.0 ±0.63	2.0 ±0.89	2.5 ±0.55	0.67 ±0.82	2.0 ±0.89	10.83 ±1.17
Group-2: (E ➔ MC)	3.5 ±0.84	2.5 ±0.55	1.67 ±0.82	2.17 ±0.75	1.0 ±0.0	2.33 ±0.82	12.17 ±2.14
Group-3: (E➔Malotilate 25)	2.0 ±0.63	1.67 ±0.52	0.5 ±0.55	0.33 ±0.52	0.5 ±0.55	1.67 ±0.82	6.17 ±1.17 $$$@@@
Group-4 : (E➔Malotilate 50)	1.67 ±0.52	1.5 ±0.55	0.83 ±0.41	0.67 ±0.52	0.5 ±0.55	1.5 ±0.55	6.17 ±1.17 $$$@@@
Group-5: (E➔Malotilate 100)	2.0 ±0.63	1.17 ±0.41	0.17 ±0.41	0.0 ±0.0	0.17 ±0.41	1.5 ±0.55	4.83 ±0.75 $$$@@@

Values: mean ± SD Kruskal Wallis test, Post-hoc Dunns test
Comparisons of Mean values – * P <0.05, ** P < 0.01, *** P < 0.001
* Day-1 Vs Day-22/Day-43; # Day-22 Vs Day-43;
$ Group-1 (ethanol) Vs Other Groups;
@ Group-2 (MC-Vehicle) Vs Other Groups (malotilate)

DISCUSSION: Ethanol administration for 21 days caused liver damage in all rats. The functional damage was manifested as significant increase in hepatic marker enzyme levels; AST, ALT, ALP and GGT, significant increase in total bilirubin and direct bilirubin levels, significant decrease in total protein and albumin levels and significant increase in prothrombin time. Whereas, the structural damage was observed in terms of histopathological changes such as fatty liver, portal fibrosis, lobular inflammation and necrosis, Mallory body formation, hepatocyte ballooning and perisinusoidal fibrosis. The raised levels of AST, ALT and high bilirubin are suggestive of liver cell injury and hepatitis which may be similar to viral hepatitis A, B, C.

But, raised GGT levels are highly suggestive of alcoholic liver disease. GGT levels tend to be high if there is recent alcohol consumption even in absence of liver disease because this enzyme is induced by alcohol [7]. Prothrombin time (PT) is dependent on availability of coagulation factors which are synthesized in liver. In case of liver damage deficiency of coagulation factors leads to prolongation of PT [7]. It is the warning sign of liver cirrhosis and progression of the disease to mortality. According to Douglas M Heuman [8], ethanol produces these toxic effects when it gets metabolized into acetaldehyde by CYP2E1 enzymatic pathway. There is increase in oxidative stress that triggers the inflammatory response in hepatocytes via release of inflammatory mediators and cytokines.

Further 21 days treatment with malotilate effectively restored the altered AST and ALT levels to normalcy at day-43 which were comparable to their baseline values indicating reversal of liver damage. This curative effect was observed at all doses of malotilate.

Exactly similar results were observed for ALP and GGT levels. Malotilate at all doses significantly lowered the ethanol induced elevation in ALP and GGT levels whereas; its vehicle methyl cellulose could not produce such reversal. These observations confirm the curative effect of malotilate on ethanol induced liver damage.

Although, it has been proved that strict abstinence from alcohol helps to reverse the liver damage in terms of reduction in portal pressure, improvement in histology, greater responsiveness to medical therapy and decrease progression of cirrhosis [9], in our study no such improvement was observed. As from day-22 to day-42 there was no administration of ethanol and still worsening in all these parameters is noted, it is likely that, merely abstinence from alcohol may not be sufficient to achieve improvement in ALD [10]. Also in vehicle control group-2(MC), it was observed that methyl cellulose has not shown any improvement in spite of abstinence from alcohol from day 22 onwards. Thus, it is apparent that the improvement observed in malotilate treated groups - 3, 4, 5 was due to treatment of malotilate and abstinence though important, plays little role.

Hyperbilirubinaemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis. It indicates the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degradation rate [4].

Ethanol administration leads to hepatic dysfunction accompanied by increase in total bilirubin and direct bilirubin. In the present study, ethanol administration for 21 days resulted into significant increase in the levels of total and direct bilirubin. These levels were further increased on day-43 even though ethanol administration was stopped. Also administration of methyl cellulose in group-2 (MC) did not show any favorable alteration in total and direct bilirubin levels. Glucoronide conjugated serum bilirubin (direct bilirubin) level is a marker of metabolizing capacity of liver.

Whereas, significant increase in total bilirubin shows hepatocyte damage induced by ethanol. These findings are also supported by significantly high histopathological scores of liver damage in ethanol treated groups. Treatment with malotilate (25, 50 & 100 mg/kg) significantly diminished the altered total bilirubin and direct bilirubin levels to normalcy by day-43 as the levels reached the baseline.

In the present study, significant decrease in serum total protein and albumin levels along with significant increase in prothrombin time on day-22 in all groups after administration of ethanol for 21 days indicated ethanol induced impairment of protein synthesizing function of liver. Estimation of serum total protein levels is helpful in differentiating between a normal and damaged liver function as they are produced in the liver; albumin being the main protein in blood [11]. The parameter prothrombin time is used to differentiate between a normal and damaged liver as it evaluates the functioning of blood clotting factors that are proteins made by the liver [11]. The ethanol provoked hepatotoxicity, leading to the disruption and dis-association of polyribosomes from endoplasmic reticulum(ER) results in decreased clotting factor synthesis. 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.

Thus the synthesizing capacity of liver was hampered by ethanol. Treatment with malotilate for further 21 days effectively reversed this effect and by day-42 the total protein and albumin levels were so significantly increased that they were comparable to their baseline values whereas, no such improvement was seen in group-2 of methyl cellulose treatment. These observations confirm the restorative effect of malotilate on ethanol induced hepatic damage. Imaizumi and Kato (1981) [12] and Niwano and Katoh (1985) [13] reported restorative effect of malotilate on the liver as increase in liver weight and liver protein attributed to increase in RNA and DNA contents in the liver. Similar findings have been reported by Igarashi, Hatahara and Funaki (1983) that malotilate offers hepatotrophic action by increase in protein synthesis in hepatectomised rats [14]. These results suggest that malotilate accelerates cell proliferation, resulting in facilitation of liver regeneration in rats.

Histopathological analysis of hepatic tissues of rats exposed to ethanol for 21 days displayed significantly high scores of liver damage. Since there was no significant difference in the histopathology scores (HPS) of group-1 ethanol treated and group-2 methyl cellulose treated groups, it is clear that the vehicle has no hepatoprotective activity. Whereas, in all treatment groups of malotilate the histopathology scores were significantly low than ethanol (E) and methyl cellulose (vehicle; MC) treated groups. These results confirm the postulation that malotilate has restorative potential in ethanol induced liver damage. The cytoprotective effect of malotilate has been demonstrated in *in vitro* experiments in which the drug was found to prevent the membrane alterations of isolated hepatocytes, detected by light and electron microscopy. Malotilate prevented the reduction in DNA synthesis and the retardation of cell cycle in ethanol-induced damaged hepatocytes [15]. Malotilate accelerates the recovery of impaired protein metabolism in alcoholic liver disease. Matsuda and Takada (1988) reported that retention of transferrin, one of the secretory proteins from the liver, in the ballooned hepatocytes was inhibited by malotilate resulting into smaller number of ballooned hepatocytes and necrotic hepatocytes [15, 16].

This action of malotilate is mediated through significant lowering of transferrin contents in the Golgi fraction of the hepatocytes, significant lowering of hepatic acetaldehyde levels and limiting the process of fibrosis by reducing the availability of collagen and hydroxyproline, thereby limiting mRNA expression of marker proteins for hepatic fibrosis in livers[17]. Ryle described that malotilate offers hepatoprotective effect by mechanisms such as; stimulation RNA and protein synthesis, increase in cholesterol synthesis and thereby hepatocyte membrane stabilizing effect in damaged liver, anti-oxidant activity by increase in glutathione-S-transferase related free radical scavenging activity and anti-fibrotic property by inhibition of fibroblast proliferation and migration[18]. Such protective effect of malotilate, mediated through anti-oxidant activity, has been reported in our previous study [19].

CONCLUSIONS: Ethanol administration leads to hepatic damage and dysfunction accompanied by increase in AST, ALT, ALP and GGT, increase in total bilirubin and direct bilirubin, decrease in serum total protein and albumin and increase in prothrombin time. After administration of malotilate the levels of AST, ALT , ALP, GGT,

total bilirubin and direct bilirubin were reduced, levels of serum total protein, albumin were increased and prothrombin time was reduced and normalized to day-1. These results of malotilate were supported by favorable changes in histopathological parameters in ethanol induced liver damage. Thus, malotilate was able to reverse the changes in various biochemical as well as histopathological parameters induced by ethanol indicating hepatic structure regenerative and hepatic function restorative effect in ALD. This study indicated that malotilate may be useful in treating established ALD in alcoholics. Further, evaluation of beneficial effects of malotilate in different animal species and in clinical trials is needed.

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