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EFFECT OF ASCORBIC ACID, α-TOCOPHEROL, LECITHIN AND L-ORNITHINE-L-ASPARTATE ON ETHANOL INDUCED HYPOPROTEINEMIA AND HYPERLIPIDEMIA IN RATS

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Abstract : We studied effect of exogenous ascorbic acid, α-tocopherol, lecithin and L-ornithine-L-aspartate on serum lipids and proteins in experimental hepatotoxic Wistar rats. Eleven groups (n = 6) of animals were used. Hepatotoxicity was induced by administering ethanol (1.6 g/kg/day) for 28 days. Both preventive and curative options were studied. Percentage increase in body weight was significantly lower in ethanol treated rats. Ethanol significantly ($P<0.05$) increased cholesterol, triglycerides and LDL, and decreased protein, albumin and A: G ratio in serum. Ascorbic acid, α-tocopherol, lecithin and L-ornithine-L-aspartate exhibited an ability to counteract the alcohol-induced changes in the body weight and biochemical parameters in preventive and therapeutic models in varying degree. Antioxidants showed better effect.

Key words : ethanol hyperlipidemia hypoproteinemia lecithin ascorbic acid alpha tocopherol L-ornithine-L-aspartate

INTRODUCTION

Alcohol abuse is a significant public health problem. Ethanol is known to have profound effect on lipid, lipoproteins and protein metabolism. Hyperlipidemia (1) and hypoalbuminemia (2) are the striking initial manifestation of alcohol induced liver disease. An effective, economical, simple treatment form reversal of systemic changes when patients stop alcohol consumption could have significant clinical impact. This study was designed to explore therapeutical manipulation of ethanol-induced hyperlipidemia with ascorbic acid, α-tocopherol, lecithin and L-ornithine-L-aspartate. Ascorbic acid is a terminal water-soluble small antioxidant that protects lipids against peroxidation and it regenerates alpha tocopherol from tocopheroxyl radical, which keeps its steady state concentration in the host (3). Lecithin and L-ornithine-L-aspartate are effective in treatment of liver disease and have antioxidant activity. Lecithin decreases the steatosis, membrane rigidity, inflammation and fat deposition (4). L-ornithine-L-aspartate reduces ammonia and improved psychosomatic function (5).

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MATERIAL AND METHODS

Animals – Male Wistar rats (10–12 weeks of age) weighing 100–120 g were used. The animals were housed in plastic cages of size 14"×9"×8" (6 rats in each cage) in side a well-ventilated room. The room temperature was maintained at 22 ± 2°C with a 12–12 hr L:D cycle. All rats had free access to a standard diet and tap water. Food and water were given *ad libitum*. The experimental study protocol was approved by the Institutional Animal Ethics Committee, SMIMS, Gangtok and National Institutes of Health (NIH), Bethesda, MD, USA. Guidelines were followed for maintenance, handling, experimentation, sacrifice and disposal of animals.

Experimental design : The animals were divided in to following groups of 6 each :

Group I (control) : 1 g double distilled water/kg/day for 4 weeks, orally.

Group II : 1.6 g ethanol/kg/day for 4 weeks, orally.

Group III : 1.6 g ethanol + 200 mg ascorbic acid/kg/day for 4 weeks, orally.

Group IV : 1.6 g ethanol/kg/day for 4 weeks, followed by 200 mg ascorbic acid/kg/day for next 4 weeks, orally.

Group V : 1.6 g ethanol + 80 mg α-tocopherol/kg/day for 4 weeks, orally.

Group VI : 1.6 g ethanol/kg/day for 4 weeks, followed by 80 mg α-tocopherol/kg/day for next 4 weeks, orally.

Group VII : 1.6 g ethanol + 500 mg lecithin/kg/day for 4 weeks, orally.

Group VIII : 1.6 g ethanol/kg/day for 4 weeks, followed by 500 mg lecithin/kg/day for next 4 weeks, orally.

Group IX : 1.6 g ethanol + 200 mg L-ornithine-L-aspartate/kg/day for 4 weeks, orally.

Group X : 1.6 g ethanol/kg/day for 4 weeks, followed by 200 mg L-ornithine-L-aspartate/kg/day for next 4 weeks, orally.

Group XI : 1.6 g ethanol/kg/day, orally for 4 weeks and followed by 4 weeks abstination.

The dose of ethanol was determined from serial dose response studies in rats with doses of 0.8, 1.2, 1.6 and 2 g/kg/day for 4 weeks. Ethanol orally at a dosage of 1.6 g/kg/day for 4 weeks produced features of liver injury comparable to those observed in clinical situations of moderate alcoholic liver disease. Therefore the dose of 1.6 g/kg/day for 4 weeks was chosen for this study. Ethanol and drugs were freshly dissolved in double distilled water to get desired concentration.

After the experimental period, blood was collected by decapitation in heparinised, chilled tubes and quickly placed under ice-cold solution. Plasma was separated at 3000 rpm for 10 min in cold centrifuge and kept in deep freezer at –30°C until use.

Total protein, albumin, total cholesterol, triglyceride and HDL-cholesterol were estimated using commercial kits (Monozyme, India Ltd.)

Statistical analysis : The data were presented as means ± SD. Statistical analysis was performed using Student's 't' test for unpaired data. Significance of difference was set at $P<0.05$.

RESULTS

Therapeutic and prophylactic effects of ascorbic acid, α-tocopherol, lecithin and L-ornithine-L-aspartate on body weights as percentage change in relation to initial body weight are given in Table I. Ethanol treated rats showed lower increment (16.8%) in case of body weight while control rats showed 42% increase after 4 weeks. In the prophylactic treatment, 24.44%, 25.9%, 21.38% and 21.38% increase in body weight were found when rats were administered with ascorbic acid, α-tocopherol, lecithine and L-ornithine-L-aspartate respectively while the rats kept on abstinence after ethanol treatment showed 19.8% increase in body weight. In the therapeutic treatment, 24.44% increase in body weight was found when rats were treated with ascorbic acid and α-tocopherol and 22.9% increase in body weight was found when rats were treated with lecithin and L-ornithine-L-aspartate.

Ethanol caused significant ($P<0.05$) elevation in cholesterol, triglycerides and LDL and reduction in protein, albumin and A:G ratio in serum. In both preventive and curative options α-tocopherol showed better protective effect followed by ascorbic acid. Preventive option was better than curative option. The effects of lecithin and L-ornithine-L-aspartate were not promising (Table I & II).

TABLE I: Percentage increase in body weight and serum levels of proteins (in gm/dl).

	% Increase in body weight	*Protein*	*Albumin*	*Globulin*	*A:G ratio*
Group I	42	7.4±0.26	4.6±0.25	2.59±0.50	1.88±0.46
Group II	16.8	5.21±0.36*	2.46±0.28*	2.73±0.39	0.91±0.27*
Group III	24.44	6.46±0.18*@	3.43±0.23*#	2.99±0.30	1.14±0.17
Group IV	24.44	6.36±0.18*@	3.29±0.23*	3.07±0.18	1.08±0.11*
Group V	25.9	6.71±0.27@	3.64±0.31*@#	3.06±0.38	1.21±0.21
Group VI	24.44	6.61±0.27@	3.46±0.31*#	3.22±0.20	1.06±0.04*
Group VII	21.38	6.14+0.35@	3.07+0.26*	3.08+0.26	0.99+0.07*
Group VIII	22.9	6.10±0.35*@	3.0±0.26*	3.13±0.20	0.98±0.07*
Group IX	21.38	5.89±0.41*	2.98±0.19*	3.17±0.50	0.96±0.12*
Group X	22.9	5.81±0.41*	2.78±0.19*	3.08±0.11	0.90±0.03*
Group XI	19.8	5.86±0.34*	2.64±0.23*	3.16±0.25	0.98±0.19*

Mean ± SD. (n=6). *$P<0.05$ compared with control group. @$P<0.05$ compared with ethanol treated group. #$P<0.05$ compared with abstinated group.

TABLE II: Serum levels of lipids and lipoproteins (in mg/dl).

	Cholesterol	*Triglyceride*	*HDL-C*	*LDL-C*
Group I	66.87±3.61	49.44±1.14	32.26±0.70	24.42±0.33
Group II	82.46±3.76*	64.28±0.88*	35.06±0.77	32.48±2.41*
Group III	74.71±1.13*@	59.47±0.94*	33.43±0.26	28.45±1.08
Group IV	76.74±1.32*	60.13±0.64*	34.65±0.64	29.47±1.24
Group V	72.85±1.04*@	56.59±0.37*@#	34.53±0.46	25.85±0.94@#
Group VI	74.89±1.34*@	60.39±0.65*	34.67±0.45	27.74±0.48
Group VII	76.27±1.48*	63.23±0.74*	33.46±0.70	31.47±0.68*
Group VIII	76.31±0.83*	63.42±1.24*	33.25±0.83	31.79±0.53*
Group IX	77.36±1.12*	62.56±0.43*	33.28±0.89	31.24±0.67*
Group X	77.39±1.28*	62.67±0.73*	34.57±0.82	31.48±0.57*
Group XI	74.43±2.19*	63.47±0.86*	34.94±0.63*	32.45±0.61*

Values are mean ± SD, (n=6). *P<0.05 compared with control group. @P<0.05 compared with ethanol treated group. #P<0.05 compared with abstinated group.

DISCUSSION

In addition to lower increment in body weight ethanol also caused significant elevation in cholesterol, triglycerides and LDL and reduction in protein, albumin and A:G ratio in serum. Ascorbic acid, α-tocopherol, lecithin and L-ornithine-L-aspartate exhibited an ability to counteract the alcohol-induced changes in the body weight and biochemical parameters in preventive and therapeutic models in varying degree.

Decrease in the body weight in ethanol treated rats is essentially due to fat mass reduction (6), reduced adipose tissue and inadequate nutritional intake (7). Although ethanol can supply >50% of dietary energy, the calories provided by ethanol cannot be stored (7) i.e. energy from ethanol may not be utilized to maintain body weight. Ethanol slows down the rate of hepatic protein metabolism (8) and albumin synthesis (9). Hypoalbuminemia, a common feature of alcohol abuse (10) is attributed to nutritional status of the subjects (11). Differences in digestion and adsorption that are common in alcoholics contribute to protein deficiency. Albumin forms adducts with acetaldehyde, which can stimulate the formation of immunoglobulins (12). Treatment with ascorbic acid and alpha tocopherol significantly increased serum total protein, albumin and A:G ratio and decreased serum globulin in both preventive and curative options. Lecithin showed significant prophylactic effect on serum proteins while its therapeutic effects.

Hyperlipidemia is mainly due to the hepatic oxidation of ethanol and associated reduction of nicotinamide adenine dinucleotide (NAD) to reduced nicotinamide adenine dinucleotide (NADH). Reducing equivalents inhibit tricarboxylic acid cycle activity and fatty acid oxidation. Ethanol also inhibits lipoprotein export and increases fatty acid uptake (13). Treatment with ascorbic acid and alpha tocopherol significantly lowered serum triglycerides in both preventive and curative options. Antioxidants affect the catabolism of

cholesterol to bile acids and ascorbic acid is an important factor in cholesterol homeostasis (14). It is necessary in the rate-limiting step in the conversion of cholesterol to bile acids (15). We, however, were unable to detect any significant effect of exogenous ascorbic acid and alpha tocopherol on serum cholesterol (except group III), HDL-C and LDL-C (except group III, V and VI).

In conclusion, alpha tocopherol has better effect followed by ascorbic acid on ethanol induced hypoproteinemia and hyperlipidemia. Preventive option was better than curative option. Effects of lecithin and L-ornithine-L-aspartate were disappointing.

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