

EFFECT OF EXOGENOUS LECITHIN ON ETHANOL-INDUCED TESTICULAR INJURIES IN WISTAR RATS

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Abstract : Infertility is well-established harmful effect in chronic alcoholism and so far, there is no effective treatment for this condition. The study was conducted to determine the effects of lecithin, a known hepatoprotective on ethanol induced testicular injuries in male albino rats of Wistar strain. Five groups (n=6) of animals were used. Group I served as control. Group II received daily 1.6 g ethanol/kg body weight/day for 4 weeks orally. Group III received 1.6 g ethanol + 500 mg lecithin/kg body weight/day for four weeks orally. Group IV received 1.6 g ethanol/kg body weight for/day 4 weeks and followed by 500 mg lecithin/kg body weight/day for four weeks orally. Group V received 1.6 g ethanol/kg body weight/day orally for 4 weeks, followed by 4 weeks abstinence. Twenty-four hours after the last treatment the rats were sacrificed using anesthetic ether. Testes were removed and used for the estimation of extent of lipid peroxidation and tissue levels of antioxidants and steroidogenic enzymes. Lecithin protected testes from ethanol induced oxidative stress. However, the drug did not show any considerable effect on the activities of testicular Δ^5 , 3β -HSD and 17β -HSD. In conclusion, ethanol induced oxidative stress can be reversed by treatment with lecithin. However the effect of lecithin on steroidogenesis was not promising.

Key words : ethanol lecithin oxidative stress steroids testes

INTRODUCTION

It is well known that alcohol abuse impairs reproductive performance in experimental animals and in human (1).

Alcoholics are often found having fertility abnormalities with low sperm count and impaired sperm motility (2). Numerous studies have indicated that chronic alcohol intake in men can cause impaired testosterone

production and shrinkage of the testes (i.e., testicular atrophy) (3). It is reported that ethanol significantly augmented lipid peroxidation in the testis (4) and inhibits the conversion of both dehydroepiandrosterone and androstenedione to testosterone (5) by decreasing the activities of 3β hydroxy steroid dehydrogenase (3β -HSD) and 17β hydroxy steroid dehydrogenase (17β -HSD). Mitochondrial enriched extracts obtained from the testes of alcohol treated rats showed significant increase in the malondialdehyde formation; moreover there was a significant decrease in glutathione (6), superoxide dismutase (7), glutathione peroxidase (8) levels in the testes of alcohol treated rats.

Impotence was prominent in the majority of alcoholic subjects and it occurred more frequently among with greater liver damage (9). The liver is exposed to the higher concentrations of alcohol in blood and is responsible for more than 95% of its metabolism (10). Seventy-five percentages of the men with advanced alcoholic cirrhosis have been reported to have testicular atrophy (11). Essential-L, containing essential phospholipids, a purified fraction of phosphatidyl choline molecule from soya beans has been reported to be safe and effective in the treatment of liver disease (12). Essential phospholipids have multifactorial action (13). Because of its antioxidant property it has protective effect on the cell membrane (14). It stabilizes the structure and function of mitochondria (15), decreases membrane rigidity, inflammation and fat deposition and reduces the action cytokines and inhibits the peroxidation of polyunsaturated fatty acids in microsomal

membranes (16). With the understanding of the role of oxidative stress in alcohol induced testicular injury, association of the functional status of liver and impotence in alcoholic subjects, and the protective effects of Essential L the present study was conducted to evaluate the role of oral lecithin in experimental alcohol induced testicular injury.

MATERIAL AND METHODS

Chemicals – Fine chemicals were purchased from Sisco Research Laboratory, India and Sigma Chemical Co., St. Louis, USA. All other chemicals used were of analytical grade and were purchased from Merck Ltd., India and Sisco Research Laboratories Ltd., India. Drug: Essential-L (Nattermann)-350 mg/cap (lecithin USP 350 mg equiv. to 175 mg vital phospholipids)

Animals – Male wistar rats (10–12 weeks of age) weighing 100–120 g were used for following experiments. 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 $22 \pm 2^\circ\text{C}$ with 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 five groups of 6 each

Group I: (Control): 1 g double distilled water/kg body weight/day for 4 weeks orally.

Group II: Ethanol treated rats (1.6 g ethanol/kg body weight/day for 4 weeks orally).

Group III: Ethanol + lecithin treated rats (1.6 g ethanol + 500 mg lecithin/kg body weight/day for 4 weeks orally).

Group IV: Ethanol followed by lecithin treated rats (1.6 g ethanol/kg body weight/day for 4 weeks, followed by 500 mg lecithin/kg body weight/day for next 4 weeks orally)

Group V: Ethanol treatment (1.6 g ethanol/kg body weight/day orally) for 4 weeks, followed by 4 weeks abstinence.

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 body weight/day for four weeks. Ethanol orally at a dosage of 1.6 g/kg body weight/day for four weeks produced features of liver injury comparable to that observed in clinical situations of moderate alcoholic liver disease. Therefore the dose of 1.6 g/kg body weight/day for four weeks was chosen for this study. Ethanol and lecithin were freshly dissolved in double distilled water to get desired concentration.

After the experimental period rats were weighed and sacrificed by cervical dislocation under light ether anesthesia. Testes were removed, cleared of the adhering tissues and weighed. Tissues were immediately rinsed, perfused with ice cold normal saline, trimmed and stored in pre-cooled (-4°C) containers. Tissues were thawed on ice before analysis.

Tissue protein (17), extent of lipid peroxidation (18), ascorbic acid (19), reduced glutathione (20), superoxide dismutase (21), catalase (22), glutathione peroxidase (23), glutathione reductase (24), glutathione S-transferase (25), 3β -hydroxy steroid dehydrogenase (26) and 17β -hydroxy steroid dehydrogenase (27) were estimated. Statistical analysis were performed by Student's 't' test and significance of difference were set at $P < 0.05$.

RESULTS

The present study was undertaken to evaluate the effect of exogenous lecithin on ethanol induced testicular oxidative stress and decreased steroidogenesis.

Changes in body weights – Alcohol exposed animals showed lower level of increment (17%) in case of body weight after four weeks of treatment than control group (42%). In the follow up treatments 22.9% and 21.38% increase in body weight were observed in group III and IV, while abstained animals had 26% increase in body weight. However, these differences in body weights were not statistically significant (Table I).

TABLE I: Effect of exogenous lecithin on mean body weight (\pm SD) and mean weight (\pm SD) of the testis expressed in grams and grams per body weight. Mean \pm SD. (n=6). *P<0.05 compared with control group. @P<0.05 compared with ethanol treated group. #P<0.05 compared with abstained group.

Parameters Experiment	% increase in body weight	Weight of testis	
		In grams	In grams per body weight
Group I	42	0.781 \pm 0.073	0.512 \pm 0.003
Group II	17	0.623 \pm 0.063*	0.508 \pm 0.004
Group III	22.9	0.765 \pm 0.063@#	0.507 \pm 0.003
Group IV	21.38	0.762 \pm 0.069@#	0.506 \pm 0.003
Group V	26	0.750 \pm 0.068*	0.507 \pm 0.003

Changes in testicular weight – Treatment with lecithin significantly protected testes form ethanol induced weight reduction. Changes in the testicular weights were well correlated with changes in body weights (Table I).

TABLE II: Effect of exogenous lecithin on tissue levels of protein, ascorbic acid, thiobarbitric acid reactive substances (TBARS) and reduced glutathione (GSH) Mean \pm SD. (n=6). *P<0.05 compared with control group. @P<0.05 compared with ethanol treated group. #P<0.05 compared with abstained group.

	Protein ¹	Ascorbic acid ²	TBARS ³	GSH ⁴
Group I	21.89 \pm 0.83	1.86 \pm 0.159	15.27 \pm 0.30	2.14 \pm 0.04
Group II	19.27 \pm 0.30*	1.69 \pm 0.058*	20.82 \pm 0.13*	1.55 \pm 0.04*
Group III	19.94 \pm 0.20*@#	1.72 \pm 0.031	18.95 \pm 0.30*@#	1.76 \pm 0.06*@#
Group IV	20.77 \pm 0.31*@#	1.71 \pm 0.013	19.10 \pm 0.11*@#	1.75 \pm 0.08*@#
Group V	19.31 \pm 0.11*	1.71 \pm 0.037	20.27 \pm 0.56*	1.56 \pm 0.06*

¹mg/100 mg tissue ²mg/gm tissue ³nmol H₂O₂ consumed/mg protein/min ⁴μg/mg tissue.

TABLE III: Effect of exogenous lecithin on tissue activities of superoxide dismutase (SOD), catalase, glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione S transferase (GST) Mean \pm SD. (n=6). *P<0.05 compared with control group. @P<0.05 compared with ethanol treated group. #P<0.05 compared with abstained group.

	SOD ¹	Catalase ²	GR ³	GPx ⁴	GST ⁴
Group I	21.18 \pm 0.93	2.03 \pm 0.10	1.61 \pm 0.043	0.171 \pm 0.023	11.53 \pm 0.64
Group II	15.39 \pm 0.65*	1.60 \pm 0.54*	1.34 \pm 0.039*	0.125 \pm 0.010*	13.69 \pm 0.79*
Group III	15.74 \pm 0.35*@	1.71 \pm 0.06*@#	1.38 \pm 0.032*@#	0.144 \pm 0.010*@	12.65 \pm 0.39*@
Group IV	15.67 \pm 0.3*	1.69 \pm 0.04*@#	1.36 \pm 0.04*	0.14 \pm 0.009*@	12.87 \pm 0.31*
Group V	15.73 \pm 0.16*	1.64 \pm 0.03*	1.32 \pm 0.022*	0.139 \pm 0.006*	12.89 \pm 0.33*

¹μmol pyrogallol auto oxidized/mg protein/min ²nmol H₂O₂ decomposed/mg protein/min
³nmol NADPH oxidized/mg protein/min ⁴μmol CDNB conjugate formed/mg protein min

Extent of lipid peroxidation – Extent of lipid peroxidation in the tissue was estimated by measuring level of thiobarbitric acid reactive substances (TEARS). Exogenous lecithin had significant protective effect on tissue lipid peroxidation (Table II).

Non-enzymatic antioxidant defense system – Treatment with lecithin raised reduced glutathione (GSH) content. There was no significant effect on tissue ascorbic acid content in the drug treated groups (Table II).

Enzymatic antioxidant defense system – Lecithin treatment had significantly increased testicular catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase and decreased of glutathione S-transferase (Table III).

Steroidogenic enzyme activities – There were no significant effect for lecithin on steroidogenic enzymes activities (Table IV).

TABLE IV: Effect of exogenous lecithin on tissue activities of Δ^5 , 3 β -Hydroxy Steroid Dehydrogenase (Δ^5 , 3 β -HSD) and 17 β -Hydroxy Steroid Dehydrogenase (17 β -HSD) Mean \pm SD. (n=6). *P<0.05 compared with control group. ^aP<0.05 compared with ethanol treated group. ^bP<0.05 compared with abstinated group.

	17 β -HSD ^a	3 β -HSD ^b
Group I	0.0166 \pm 0.001	0.0198 \pm 0.002
Group II	0.0131 \pm 0.001*	0.0155 \pm 0.001*
Group III	0.0133 \pm 0.001*	0.0158 \pm 0.001*
Group IV	0.0131 \pm 0.001*	0.0157 \pm 0.001*
Group V	0.0130 \pm 0.001*	0.0156 \pm 0.001*

^aabsorbance/mg rotein/min

DISCUSSION

The study demonstrates the adverse effect of ethanol on testicular androgenic activities and its protection by lecithin administration. Attempts were also made to study the ethanol-induced testicular oxidative stress and its correction by lecithin.

The testis has been shown to be highly susceptible to ethanol as it percolates through blood testis barrier and suppresses spermatogenesis. The reduction in the testicular weight of ethanol treated rats may be due to reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis in the Leydig cells (28). As the body growth was also altered in ethanol-treated rats, the effect of ethanol on the testis may also be due to its general toxicity other than its specific toxic effect on the target organ.

Although the controlled generation of highly reactive oxygen species (ROS) serves as a second messenger system in many different cell types, its continuous production is detrimental to the surrounding tissue (29). It is reported that excessive ROS production beyond critical levels overwhelm antioxidant defense strategies of spermatozoa in seminal plasma results in increased oxidative stress (30). Elevated lipid peroxidation causes sperm immobilization, reduced acrosomal reaction and membrane fluidity (31), DNA damages (32) and high frequencies of single and double DNA strand breaks (33) in sperms. High levels of ROS disrupt the inner and outer mitochondrial membranes resulting in the release of cytochrome-C protein that activates caspase-induced apoptosis (34). ROS are regularly formed during the process of normal respiration. However, the production is kept at physiologically low levels by intracellular free radical scavengers (35). The major sources of ROS in semen are from the spermatozoa and infiltrating leucocytes (36). Spermatozoa and seminal plasma have their own anti-oxidative mechanisms to protect ROS-induced cellular damage. GSH is a major thiol in living organisms, which plays a central role in coordinating the body's antioxidant defense processes. Conditions that perturb intracellular levels of glutathione have been shown to result in significant alteration in cellular metabolism. The tissue glutathione concentration reflects its potential for (i) detoxification (ii) preserving the proper cellular redox balance and (iii) its role as a cellular protectant (37). GSH has a likely role in sperm nucleus decondensation and spindle microtubule formation (38). Ethanol induced depletion of glutathione supports the hypothesis that reactive oxygen intermediates generated

during the metabolism of ethanol lead to glutathione oxidation and lipid peroxidation and are responsible for the toxic effects of ethanol (39). Glutathione reductase is concerned with the maintenance of cellular level of GSH by effecting fast reduction of oxidized glutathione (GSSG) to reduced form (40). Glutathione peroxidase plays a significant role in the peroxy scavenging mechanism and in maintaining functional integration of the cell membranes, spermatogenesis, sperm morphology and sperm motility (41). It is suggested that the metabolic pathway of testosterone biosynthesis requires protection against peroxidation and will be affected by a decrease in the activity of this enzyme (40). The increased tissue glutathione S-transferase activity in ethanol treated rats might be an adaptive defense mechanism. Glutathione S-transferase plays an essential role in eliminating toxic compounds by conjugation (42). It is noteworthy that in the present study the activities of two important steroidogenic enzymes, i.e. 17β HSD and 3β HSD were measured using testosterone and dihydrotestosterone as the substrates respectively, and suppression of activities of both the enzymes occurred in ethanol treated rats. The results suggest the inhibitory role of ethanol on conversion of both dehydroepiandrosterone to androstenedione and androstenedione to testosterone (43). The decreased activities of 17β HSD and 3β HSD are indicative of reduced steroidogenesis and the nonavailability of testosterone ultimately affects spermatogenesis.

Treatment with lecithin exhibited an ability to counteract the ethanol induced changes in the testes in preventive and therapeutic models in varying degrees. In an attempt to understand the mechanism

by which lecithin prevents tissue damage caused by ethanol, investigation on tissue levels of TEARS and antioxidants were carried out in both preventive and therapeutic groups. Reduction in the tissue levels of TEARS in lecithin treated animals supports its antioxidant role (16). Raised levels of enzymatic and non enzymatic antioxidants in the drug treated animals elicit protective response against the toxic manifestations of chemicals, particularly those involving oxidative stress. Explanations against the possible mechanisms underlying the protective properties of drugs include the prevention of GSH depletion (44) and destruction of free radicals (45). Supplementation with lecithin showed a minimal effect on reversing the inhibitory effect of ethanol on steroidogenesis. The antioxidant effect of lecithin against oxidative stress induced by ethanol could have contributed some effect on these enzymes. This possibility is supported by the fact that lecithin reversed the extent of lipid peroxidation and restored the testicular scavenger enzymes against free radicals. The increase in Δ^5 , 3β -HSD and 17β -HSD activities in drug-treated rat may be the result of low testicular conjugated dienes and MDA (46). Low level of testicular free radicals in drug-treated rats was further supported by the recovery of testicular scavenger enzymes against free radicals (47). Gain in the testicular weights in drug-treated rats also supports the low TBRAS and increased activity of steroidogenic enzymes (48).

From the results it may be concluded that lecithin has a protective effect on ethanol-induced testicular oxidative stress. The treatment showed only minimal effect in restoring steroidogenesis.

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