Effect of chronic ethanol administration on testicular antioxidant system and steroidogenic enzyme activity in rats

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In order to find out the effect of chronic ethanol administration on testicular antioxidant system and steroidogenic enzyme activity, male rats fed with ethanol 1.6g/kg body weight per day for four weeks were studied. Besides a drastic reduction in body and testis weight, there was decrease in ascorbic acid, reduced glutathione and activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase in the testicular tissue of the treated animals. Simultaneously, there was increase in lipid peroxidation and glutathione S-transferase activity. Activities of 3β-hydroxy steroid dehydrogenase and 17β-hydroxy steroid dehydrogenase were also found decreased in the treated animals. The results indicate that chronic ethanol administration resulted in increase in oxidative stress and decrease in the activities of steroidogenic enzymes in the rat testes.

Keywords: Antioxidants, Ethanol, Rats, Steroids, Testes

Ethanol is the most commonly used and abused chemical substance. It contributes to 15 to 30% of all hospital admissions and as per World Health Organization (WHO) estimates, alcohol accounts for 3.5% of the total days lost due to death and disability.

Alcohol abuse in men causes impaired testosterone production, shrinkage of the testes, reduced sperm counts, abnormal sperm shapes and altered sperm motility. Alcohol reduces serum testosterone in men with and without alcoholic liver diseases. Free radicals are formed in both physiological and pathological conditions in mammalian tissues. Lipid peroxidation mediated by free radicals is considered to be a mechanism of cell membrane destruction and cell damage. Reactive oxygen species (ROS) have been shown to induce defective sperm function and infertility. Since human-sperm plasma membranes have a high content of polyunsaturated fatty acids, the membranes are sensitive to damage by free radicals.

The cytochrome P450 enzymes of the steroidogenic pathway use molecular oxygen and electrons transferred from NADPH to hydroxylate the substrate and in this process, superoxide anion or other oxygen free radicals are produced as a result of electron leakage in normal reactions or due to interaction of steroid products or other pseudo substrates with the enzymes.

However, the cellular mechanisms contributing to alcohol-induced infertility have not been fully understood. Hence, the present study has been undertaken to evaluate the effect of chronic ethanol administration on the testicular antioxidant system and steroidogenic enzyme activity in rat testes.

Materials and Methods

Chemicals—Chemicals used were of analytical grade and purchased from Merck Ltd. India, Sisco Research Laboratories Ltd. India and Sigma Chemicals, USA.

Animals—Male Albino rats of Wistar strain (10-12 weeks of age), weighing 100-120 g were housed in plastic cages of size 14"x9"x8" (6 rats in each cage) in a well-ventilated room at 22±2°C with a 12 hr light/dark cycle. All rats had free access to a standard diet and tap water. The Animal Ethics Committee, Sikkim Manipal Institute of Medical Sciences, Gangtok approved all the procedures used.

Treatment—Animals were divided into two groups of six rats each, control and ethanol treated. Ethanol treated rats received ethanol (Ethanol 99.9-100% "absolute", Merck Ltd. India) orally by gastric intubation
at a dosage of 1.6 g ethanol/kg body weight/day for 4 weeks. Ethanol was diluted with double distilled water to get the desired concentration. The control animals received a similar volume of double distilled water alone for 4 weeks.

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 4 weeks. Ethanol orally at a dosage of 1.6g/kg body weight/day for 4 weeks produced features of liver injury comparable to that observed in clinical situations of moderate alcoholic liver disease. Therefore, the dose of 1.6g/kg body weight/day for 4 weeks was chosen for this study.

Rats were weighed and killed by cervical dislocation under light ether anesthesia; testes were removed, cleaned of the adhering tissues and weighed. Tissues were immediately rinsed and perfused with ice cold normal saline, trimmed and stored in pre-cooled (-4°C) containers. Tissues were thawed on ice before analysis.

Total protein, extent of lipid peroxidation, ascorbic acid, reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, glutathione S-transferase, 3β-hydroxy steroid dehydrogenase and 17β-hydroxy steroid dehydrogenase were estimated. Statistical analyses were performed by Student's t test and significance of difference was set at P<0.05.

**Results**

Alcohol exposed animals showed lower level of increment in body weight (2.3% increase after 1 week, 8.3% increase after 2 weeks, 11.2% increase after 3 weeks and 17% increase after 4 weeks) than the control group (15% increase after 1 week, 24% increase after 2 weeks, 35% increase after 3 weeks and 42% increase after 4 weeks). However, these differences in body weights among different groups were not statistically significant (Fig. 1). Ethanol fed rats showed significant decrease in testicular weight compared to controls. However, when normalized against body weight, the difference was found nonsignificant (Fig. 2).

Ethanol fed rats demonstrated increased lipid peroxidation (36%) in testes and decreased testicular levels of ascorbic acid (9%) and reduced glutathione (28%) and in the activities of superoxide dismutase (27%), catalase (21%), glutathione reductase (17%) and glutathione peroxidase (27%) (Table 1). However, testicular activity of glutathione S-transferase was significantly (P<0.05) enhanced (19%) by ethanol treatment (Table 1). Ethanol has significantly (P<0.05) reduced activities of Δ5, 3β-HSD (22%) and 17β-HSD (21%) in the testes (Table 2).

**Discussion**

Chronic ethanol administration not only resulted in the reduction of body and testis weight, but also in ascorbic acid, reduced glutathione and activities of SOD, catalase, glutathione reductase and glutathione peroxidase in the testicular tissue of the treated animals. Simultaneously, increased lipid peroxidation and glutathione-S-transferase activity were observed. On the other hand, activities of steroidogenic enzymes (3β-hydroxy steroid dehydrogenase and 17β-hydroxy steroid dehydrogenase) were declined in the treated animals.

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**Fig. 1**—Percentage increase in body weights of control and ethanol treated rats after 1st, 2nd, 3rd and 4th weeks. Data are represented as mean ± SD from 6 observations.

**Fig. 2**—Testicular weights of control and ethanol treated rats in grams and grams per body weights. Data are represented as mean ± SD from 6 observations.
The testis has been shown to be highly susceptible to ethanol as it percolates through the 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. 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. It is reported that excessive ROS production beyond critical levels overwhelms antioxidant defense strategies of spermatozoa in seminal plasma resulting in an increased oxidative stress. Elevated lipid peroxidation results in sperm immobilization, reduced acrosomal reaction and membrane fluidity, DNA damages and also causes high frequencies of single and double DNA strand breaks 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.

ROS are regularly formed during the process of normal respiration. However, the production is kept at physiologically low levels by intracellular free radical scavengers. The major sources of ROS in semen are from the spermatozoa and infiltrating leucocytes. Spermatozoa and seminal plasma have their own antioxidant defense 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 antioxidant defense processes. Conditions that perturb intracellular levels of glutathione have been shown to result in significant alteration in cellular metabolism.

Glutathione reductase is concerned with the maintenance of cellular level of GSH by effecting fast reduction of oxidized glutathione (GSSG) to reduced form. Glutathione peroxidase plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes, spermatogenesis, sperm morphology and sperm motility. 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. 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.

It is noteworthy that in the present study the activities of two important steroidogenic enzymes, i.e. 17β HSD and 3 β 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. The decreased activities of 17β HSD and 3β HSD are indicative of reduced steiodogenesis and the nonavailability of testosterone ultimately affects spermatogenesis.

In conclusion, the present findings indicate that chronic ethanol administration increases oxidative stress and down-regulates steroidogenic activity leading to altered testicular function.

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