RESEARCH ARTICLE

Effects of Ethanol on Testis: A Histopathological Study in Adult Rats

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Abstract

Chronic alcohol consumption leads to many dysfunctions in the reproductive system. The pathological changes in the testis is depends on the level and duration of alcohol consumption. The purpose of the present study was to find out the histopathological changes in the testis in chronic ethanol exposure. Adult rats were divided into control (C) and ethanol treated (E) groups eight animal each. The rats in group E were exposed to ethanol 1-gm/kg body weight for three months after due approval from the institutional animal ethics committee. After the exposure period, testes were processed for light microscopic examination. Ethanol treated rats showed loss of normal distribution of spermatogenic cells in the seminiferous tubules and few fragmented sperms in the lumen. Most of the tubules showed that the germ layers were detached from the basal lamina. It has been suggested that ethanol induced the degeneration of spermatogenic cells in the testis.

Keywords: Ethanol, Histopathology, Seminiferous tubules, Spermatozoa, Testis.

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Introduction

Addiction to alcohol abuse led to increased risk of major depression.[1] Alcohol has impacts on central nervous system. It increases oxidative stress, and impairs cerebral vasoreactivity, and thus may result in an increase in ischemic stroke induce brain damage.[2] Alcohol consumption harm fetus during pregnancy. It also may toxic effects in the reproductive process through miscarriage, aneuploidy, anomaly, disordered fetal growth, developmental delay, perinatal death.[3] The effects of alcohol on the reproductive system become very interesting for researcher. The most important endocrine consequences of long-term alcohol use are its effects on the gonads. Chronic alcohol consumption leads to disorder of spermatogenesis in human.[4] Alcohol toxicity in male induces erectile dysfunction and infertility and it is explained by alcohol induced reduction in testosterone.[5] Chronic ethanol ingestion confirms gonadal dysfunction and it's suggested that alcohol is a testicular toxin.[6] Testicular atrophy and impaired testosterone production are due to alcohol consumption in men and result in impotence, infertility, and reduced male secondary sexual characteristics.[7] Ethanol-treated animals enhanced testicular DNA fragmentation and increased the number of apoptotic spermatogonia as well as spermatocytes.[8,9] Alcohol also adversely affects on the secretory function of Sertoli cells. It is also noted that moderate alcohol consumption increases the HDL level, reduces the cholesterol level in the blood, and reduces the risk of stroke and stress, anxiety and tension, and AD.[10] Moderate alcohol consumption and risk of coronary heart disease among women with type 2 diabetes mellitus.[11] It is also noted that moderate alcohol consumption lowers the risk of type 2 diabetes.[12] Consuming large amounts of alcohol can result in acute and delayed impairments in cognitive and executive functions, spatial learning and memory impairment. These impairments lead to medical and social problems, including dementia, violence, and decreased work productivity.[13] Consumption of alcohol is suggested to increase the susceptibility of rats to certain effects of aluminum (Al) but it is also noted that consumption of beer may afford a protect against the toxic effect of Al.[14-16] The study aimed to identify the effects of ethanol administration on the microscopic structure of testis.

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Materials and Methods

This study used six wistar rats to include an equal number of male and female rats of an average weight of 200 gm and an average age of 120 days. Animals were kept individually in plastic cages in noise free, air-conditioned animal house with temperature maintained at 75°F and on a light dark cycle of 12:12 hours. Humidity was maintained with a minimum of 50%. Rats were fed on diet pellets, tap water ad libitum and treated with utmost humane care. The Institute Animal Ethics Committee approved the experimental protocol and the procedures were performed according to the guidelines

of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India). After one week of acclimatization, rats were randomly divided (with the help of Random Allocation Software Version 1.0, May 2004) into 2 groups namely-

Group C (Control group, 8 animals) received the normal saline water (Sodium chloride) for 3 months.

Group E (Experimental group, 8 animals) received 10% ethanol v/v in plain water ad libitum for 3 months. On average, each rat of group E consumed 10 mL (equivalent to 1 gm/kg body weight) of the ethanol solution daily. The treatments were carried out through oral feeding gavage. Their weights were recorded daily. After 3 months, the animals were anesthetized with pentobarbitone (i.p) and intracardiac perfusion of normal saline followed by 10% formol saline was performed. The testis of both groups of animals was dissected out and blotted. The testis was separated from the epididymis. After transverse sectioning, the testis of all animals was processed for routine paraffin embedding.

Histological study

The testes were fixed in 10% formaldehyde solution, passed through ascending series of ethanol baths, cleared in xylene and embedded in paraffin. Tissues were sectioned at 5 µm and stained with Haematoxylin and Eosin staining as per standard protocol.[17] Hematoxylin eosin staining slide were deparaffinized through xylene (2-3 min) and absolute alcohols (1-2 min) then dipped in 95 % alcohol followed by 70% alcohol, 50% alcohol, and 30% alcohol. Then washed thoroughly with distilled water and placed in hematoxylin for 3-5 min and then examined the section after rinse with distilled water under low magnification of microscope to confirm it is over stained. Then rinsed in distilled water and placed the slide in another jar of 30% alcohol for 3 minutes. Then placed in 50% alcohol and followed by 70% alcohol and 95% alcohol. Then the slides were counter stained in 0.5–1% eosin in 90% alcohol for 30 seconds to 1-min until the cytoplasm took a deep pink stain. Then dipped in 95% alcohol for a few seconds and placed it to absolute alcohol for 3 minutes. It was kept in the next absolute alcohol for 3 minutes to ensure full dehydration. Then the slides were transferred in xylene for 2 minutes and followed by next xylene for 2 minutes until the section appeared clear or transparent. The stained slides were labeled properly and placed under light microscope obtained with a digital camera attached to the microscope for observation.

Result

Seminiferous tubules from the vehicle control group rats showed circular or oval outlined with normal stratified germinal epithelium containing spermatogenic cells resting on the basal lamina. Spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids are identified and lumen of the tubules containing spermatozoa. Leydig cells are present in between seminiferous tubules in Figures1a and 1b.

Ethanol-treated rats showed loss of normal distribution of spermatogenic cells in the seminiferous tubules and few fragmented sperms in the lumen. Most of the tubules showed that the germ layers were detached from the basal lamina. Vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium and Sertoli cells in Figures 2a and 2b.

Discussion

The testes are male gonads responsible for testosterone hormone and generating sperm. Seminiferous tubules are present within the testis and are responsible for spermatogenesis.[18] Alcohol is well known as teratogenic and fetotoxic in human. Its effects on sperm production and sperm quality. Many studies explain that alcohol altered testosterone production and testicular atrophy due to decrease the diameter of seminiferous tubules.[3]

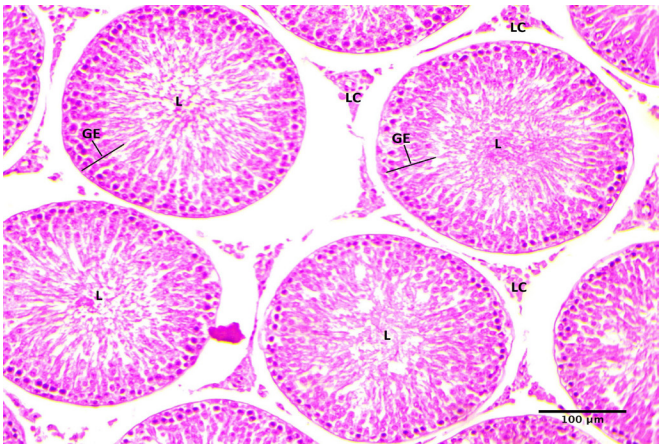

Fig.1a: Photomicrograph of testis of the control group showing cross section of the seminiferous tubules showing germinal epithelium (GE) containing cells of the spermatogenic cells with spermatozoa in the lumen (L). Interstitial Leydig cell (LC) seen between the seminiferous tubules. (Haematoxylin & Eosin stain, X 100, scale bar = 100 µm).

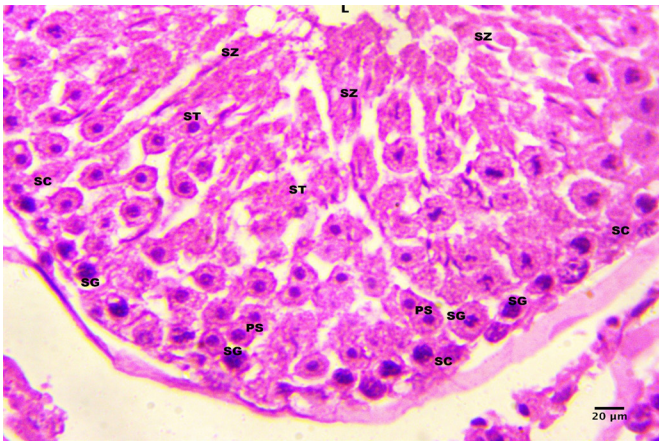

Fig.1b: Photomicrograph of testis of the control group showing cross section of the seminiferous tubules showing spermatogenic cells, Spermatogonia (SG), Primary spermatocyte (PS), Spermatids (ST), and the lumen (L) containing spermatozoa (SZ). Sertoli cell (SC). (Haematoxylin & Eosin stain, X 400, scale bar = 20 µm).

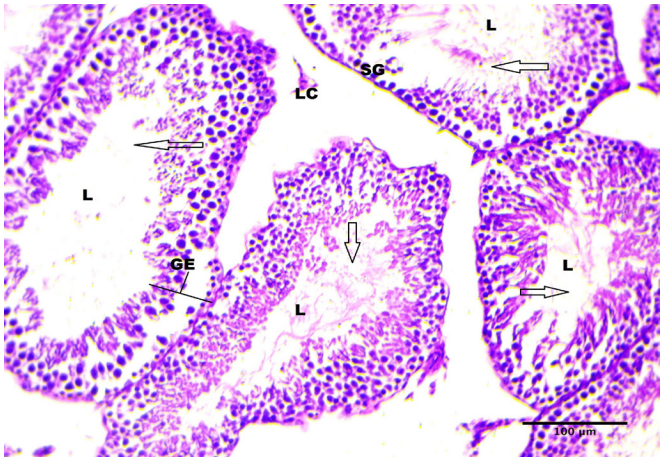

Fig.2a: Photomicrograph of testis of the ethanol treated group showing cross section of the seminiferous tubules which attained different shapes and hypocellularity reduction in cells of the spermatogenic series of germinal epithelium (GE) and widened empty (arrow) lumen (L). Few Interstitial Leydig cell (LC) seen between the seminiferous tubules. (Haematoxylin & Eosin stain, X 100, scale bar = 100 µm).

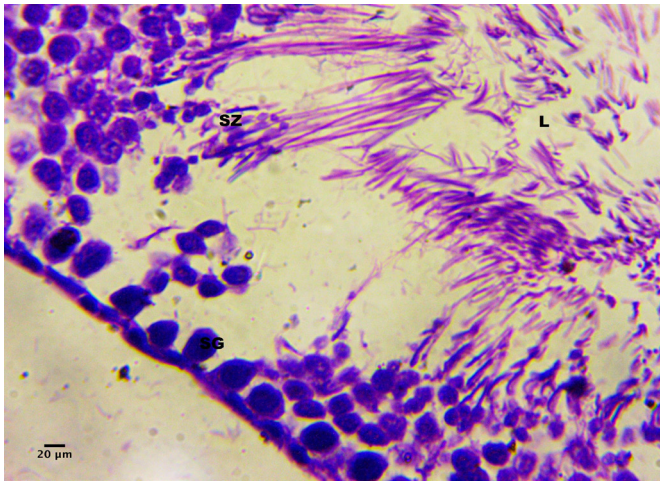

Fig.2b: Photomicrograph of testis of the ethanol treated group showing cross section of the seminiferous tubules. Most of the spermatocyte appeared with dark stained pyknotic nuclei. Spermatogonia (SG), Spermatozoa (SZ) in the lumen (L). (Haematoxylin & Eosin stain, X 400, scale bar = 20 µm).

The present study showed destruction of the seminiferous tubules, different shapes and hypocellularity reduction in cells of the spermatogenic series and widened empty lumen. Few seminiferous tubules show a single layer of basal spermatogonia. This observation is consistent with previous studies that showed that degenerative changes of the epithelial component of the seminiferous tubules in mice and testicular lesions including a significant decrease in the diameter of the seminiferous tubules, a decrease of Leydig cell's number and the presence of degenerative germ cells in rats.[19,20] Histological evidence of testicular lesions and incomplete progression of spermatogenesis in pigs treated with ethanol also with many basal vacuoles and great reduction in sperm density.[21] Ethanol has been shown to induce nervous system damage, including long-term reduced neurogenesis in the hippocampus and induced inflammation in the brain and widespread brain atrophy.[22] Ethanol can interfere with the function of the hypothalamic-pituitary gonadal axis; thereby causing impotence, infertility, and reduced male secondary sexual characteristics.[23] Oxidative stress in the testis due to ethanol administration and increased extent of lipid peroxidation or decreased antioxidant defenses, thereby inducing germ cell apoptosis, leading to testicular atrophy.[24] Ethanol consumption disturbs epididymal spermatozoa motility, nuclear maturity and DNA integrity of spermatozoa in rat; and this may be one possible cause of infertility following ethanol consumption.[25] The less sperm count of ethanol-treated mice in this study may be attributed to the effect of alcohol on the gonadotrophic cells of the pituitary gland and/or directly on the seminiferous tubules and Leydig cells, in addition the neurotoxin activity of ethanol. Alteration of sperm count and semen morphology was observed in rats exposed to aluminum chloride with dose of 64 mg/kg body weight.[26] It is also reported that due to chronic alcohol consumption in the liver showed elongated and distorted mitochondria without normal organization.[27] A decrease in spermatozoa viability as observed in many nonmotile/ dead spermatozoa in the ethanol-treated groups is one of the indicators that chronic ethanol consumption may compromise the structural integrity of the spermatozoa via the mitochondrial pathway. Hence, ethanol-induced elevation of germ cell apoptosis and necrosis and suppression of cell proliferation may contribute to testicular atrophy.[28] These effects were observed in the reduced tubular diameter and cross-sectional areas of the treated animals. However, when the animals were co-exposed to both ethanol and aluminium, the vacuolar-degenerative changes appeared in the cytoplasm of the spermatogenic epithelium and in the sertoli cells and abnormal distribution of spermatozoa in the Lumina. Clusters of degenerating spermatozoa and desquamated spermatogenic cells were frequently observed deep within the lumina of the seminiferous tubules. Most of the tubules showed that the germ layers were detached from the basal lamina.[29] An important aspect of spermatogenesis is detaching germ cells from the basement membrane and their subsequent migration towards the tubule lumen. Procollagen I, a precursor of type I collagen, is a trimer consisting of two α1 chains and one α2 chain whose sequences are encoded by two different genes; COL1A1 and COL1A2, respectively.[29] The distribution of procollagen I within the seminiferous tubules of immature and adult mice correlates with the process of germ cell attachment and detachment from the basement membrane. The unique distribution pattern of procollagen I in adult mouse testes implies a possible role for COL1A1, COL1A2, and procollagen I in regulating the adhesion of spermatogonia and preleptotene spermatocytes to the basement membrane and the detachment and migration of spermatocytes and spermatids towards the lumen during spermatogenesis.[30] From a histologic point of view, we agree with authors who reported that Al could produce a marked degeneration and necrosis of the germ cells lining,

interstitial edema and testicular degeneration with complete absence of germ cells in male rats treated with aluminium chloride at higher dose. The present study also found acute degeneration of seminiferous tubules epithelium in the presence of ethanol coexposure in given doses. The present study explored the histopathological changes in testis in the presence of ethanol.

Conclusion

Moreover, the present data demonstrated that chronic ethanol exposure reduces the number of spermatogenic cells in the seminiferous tubules. The dose of 1-gm alcohol / kg body weight used in this study is closer to a chronic exposure considering that it is taken everyday ad libitum. The result of the present study provides evidence of the adverse effects of ethanol on testis histology by degenerative changes in spermatogenic cells in a given dose.

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