Toxic effects of aluminium on testis in presence of ethanol coexposure

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SUMMARY

Both aluminium and ethanol are pro-oxidants and toxic. Uncontrolled use of aluminium and increasing trends of ethanol consumption in India increased the chance of co-exposure to aluminium and ethanol. There are possibilities, that both of them follow common mechanisms to produce reproductive toxicity. The present study was planned to identify the effects of aluminium administration on the microscopic structure of testis in presence of ethanol.

Sixteen male rats were divided into one vehicle control and three experimental groups and exposed to aluminium (4.2 mg/kg body weight) and ethanol (1 g/kg body weight) for 3 months. After the exposure period, testes were processed for light microscopic examination. Aluminium-treated and ethanol-treated rats showed loss of normal distribution of spermatogenic cells in the seminiferous tubules and few fragmented sperms in the lumen. Combined treatment of aluminium and ethanol showed acute degeneration of the spermatogenic epithelium. Vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium and Sertoli cells. Abnormal distribution of spermatozoa was seen in the lumen. Most of the tubules showed that the germ layers were detached from the basal lamina. It has been suggested that the ethanol induced augmentation of impacts of aluminium on the testis.

Key words: Aluminium – Ethanol – Testis – Seminiferous tubule – Histopathology

INTRODUCTION

Aluminium (Al) is the 3rd most common element found in nature after silicon and oxygen, comprising approximately 8% of the earth crust (Elif et al., 2018). Al is a ubiquitous element with known toxicity in the human body, mainly in the central nervous system, and may act as foetal teratogen (Dominigo, 1995; Nayak, 2010). In the 70s of the last century, the medical fraternity considered the toxicity of the so-called 'biologically inert' metal, aluminium. A large volume of research, since then, has been conducted to get insight of and combat Al toxicity. While the awareness about the toxic effects is bringing down the daily and avoidable uses of the Al in developed countries, Al wares are the commonly used cooking utensils and containers in India, as they are the cheapest. In 1989, a joint FAO/WHO Expert committee on food additives (JECFA) recommended a provisional tolerable weekly intake (PTWI) of 7.0 mg / kg body weight aluminium. This was changed in 2007 to 1.0mg /

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kg body weight because of potential effects on the reproductive system and the developing nervous system (Kawahara et al., 2011). Al toxicity causes Alzheimer disease (AD), dialysis dementia, parkinsonism, and amyotrophic lateral sclerosis. It also affects skeletal system brain tissue, bone, blood cells, liver and kidney (Berihu, 2015). It has been shown that Al has severe toxic effects on the mouse embryo/foetus, which leads to significant increase in resorption rate, decreased body weight and major anomalies, foetal death, and skeletal anomalies (Karimpour, 2005). Transplacental passage of Al from pregnant mice to the foetus's organs is noted after maternal transcutaneous exposure (Anane et al., 1997). It has been reported that oral Al exposure increases the incidence of foetal abnormalities in rats and mice (Agrawal, 1996). Exposure to Al is inevitable because of its abundance in the earth's crust, use in cookware, foods and drinks, medications, etc. (Nayak, 2010). The nervous system is the most sensitive to Al toxicity, as it induces cognitive deficiency and dementia in brain. Accumulation of excessive amounts of Al leads to testicular dysfunction in both humans and animals. High Al contents in human testes, spermatozoa, seminal plasma, Leydig cells, blood and urine, were associated with impaired sperm quality and viability (Hovatta et al., 1998; Reusche et al., 1994). Alteration in the microscopy of testis (Kutlubay et al., 2007), deterioration in spermatogenesis and sperm quality and alterations in antioxidant enzymes (Yousef et al., 2005, 2007), interruption in sex hormone secretion (Guo et al., 2005) are several of the aspects suggested that Al exposure causes adverse impact on male reproduction. Addiction to alcohol abuse led to increased risk of major depression (Fergusson et al., 2009). Alcohol has impacts on the central nervous system. It increases oxidative stress, and impairs cerebral vasoreactivity, and thus may result in an increase in ischemic stroke and induce brain damage (Sun et al., 2008). Alcohol consumption can harm the foetus during pregnancy. It also may have toxic effects in the reproductive process through miscarriage, aneuploidy, anomaly, disordered foetal growth, developmental delay and perinatal death (Fraser, 2006). The effects of alcohol on the reproductive system become very interesting for the 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 (Pajarinen, 1996). Alcohol toxicity in male induces erectile dysfunction and infertility, and it is explained by alcohol induced reduction in testosterone (Bannister et al., 1987). Chronic ethanol ingestion confirms gonadal dysfunction and it is suggested that alcohol is a testicular toxin (Van Thiel et al., 1975). Testicular atrophy and impaired testosterone production are due to alcohol consumption in men, and result in impotence, infertility, and reduced male secondary sexual characteristics (Adler, 1992). Ethanol-treated animals enhanced testicular DNA fragmentation, and increased the number of apoptotic spermatogonia as well as spermatocytes (Zhu et al., 2000; Bamac et al., 2005). Alcohol also causes an adverse effect on the secretory function of Sertoli cells. It is also noted that moderate alcohol consumption increases the HDL level and reduces the cholesterol level in the blood; it also reduces the risk of stroke and stress, anxiety and tension and AD (Elizabeth et al., 2000). Moderate alcohol consumption and risk of coronary heart disease among women with type 2 diabetes mellitus (Regan, 2000). It is also noted that moderate alcohol consumption lowers the risk of type 2 diabetes (Koppes, 2005). Consumption of 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 (Kuzmin, 2013). However, the Al load caused by Al exposure may be influenced by ethanol co-exposure (Nayak, 2006; Nayak et al., 2013). Consumption of alcohol is suggested to increase susceptibility of rats to certain effects of Al, but it is also noted that consumption of beer may supply a protective for the toxic effect of Al (Flora, 1991; Pena, 2007; Gonzalez-Munoz, 2008).

The brain is the primary organ affected by Al toxicity. Only a few studies have described its effect on the structure of the testis. Furthermore, the use of ethanol against Al toxicity needs to be

investigated. Many studies on the effects of alcohol on male reproductive system but combined effects of Al and ethanol has not been documented yet. The aim of the study was to identify the effects of Al administration on the microscopic structure of testis in the presence of ethanol.

MATERIALS AND METHODS

Sixteen Wistar male rats of an average weight of 200 g and an average age of 120 days were used in this study. 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 experimental protocol was approved by the Institute of Animal Ethics Committee and the procedures were performed according to the guidelines of the 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 4 groups, namely:

Group I (Vehicle Control group, 4 animals) received the normal saline water.

Group II (Experimental group, 4 animals) received aluminium chloride (4.2 mg/kg body weight) dissolved in drinking water containing sodium chloride (0.9%).

Group III (Experimental group, 4 animals) received ethanol (1g/kg body weight) dissolved in drinking water containing sodium chloride (0.9%).

Group IV (Experimental group, 4 animals) received aluminium chloride (4.2 mg/kg body weight) and also ethanol (1 g/ kg body weight).

The treatments were carried out through oral feeding gavage once daily for periods of 90 days. Their weights were recorded daily. After 3 months, the animals were anaesthetized with pentobarbitone (i.p) and an intracardiac perfusion of normal saline followed by 10% formaldehyde saline was performed. The testis of both groups of animals were dissected out and blotted. The testes were separated from the epididymis. After transverse sectioning, the testes of all animals were 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 (H&E) staining according to John D Bancroft Theory and Practice of Histological Techniques. Haematoxylin and eosin staining slides 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 haematoxylin for 3-5 minutes, and then the section examined after rinse with distilled water under low magnification of microscope to confirm its over-staining; then rinsed in distilled water and the slide placed in another jar of 30% alcohol for 3 minutes, then placed in 50% alcohol, 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 minute until the cytoplasm took a deep pink stain, and then dipped in 95% alcohol for a few second and placed into absolute alcohol for 3 minutes. To ensure full dehydration, it was next kept in absolute alcohol for 3 minutes. Then the slides were transferred in xylene for 2 minutes and followed by next xylene for 2 minutes until the section appeared absolutely clear or transparent. The stained slides were labelled properly and placed under light microscope, obtained with a digital camera attached to the microscope for observation.

RESULTS

The general health status of all the rat groups was good and water and fodder intakes were adequate during the experimental time. No significant behavioural alteration or illnesses were found.

Seminiferous tubules from the vehicle control group rats showed circular or oval outlines with normal stratified germinal epithelium, containing spermatogenic cells resting on the basal lamina.

Spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids were identified, and the lumen of the tubules contained spermatozoa. Leydig cells were present in between seminiferous tubules. (Figs. 1a, 1b).

Aluminium-treated rats showed loss of normal distribution of spermatogenic cells in the seminiferous tubules and few fragmented sperms in the lumen (Figs. 2a, 2b). Ethanol-treated rats also showed degeneration of spermatogenic epithelium and fragmented sperm in the lumen. Few seminiferous tubules showed a single layer of basal spermatogonia (Figs. 3a, 3b). A significantly acute degeneration of the spermatogenic epithelium was observed in the combined treatment group compared to the other groups. Vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium and Sertoli cells. Abnormal distribution of spermatozoa were seen in the lumen. Most of the tubules showed that the germ layers were detached from the basal lamina (Figs. 4a, 4b).

DISCUSSION

The testes are male gonads which are responsible for testosterone hormone and for generating sperm. Seminiferous tubules are present within the testis and are responsible for spermatogenesis (Elaine, 2004; Scott, 2000). Microscopic examination of rats orally treated with Al induced

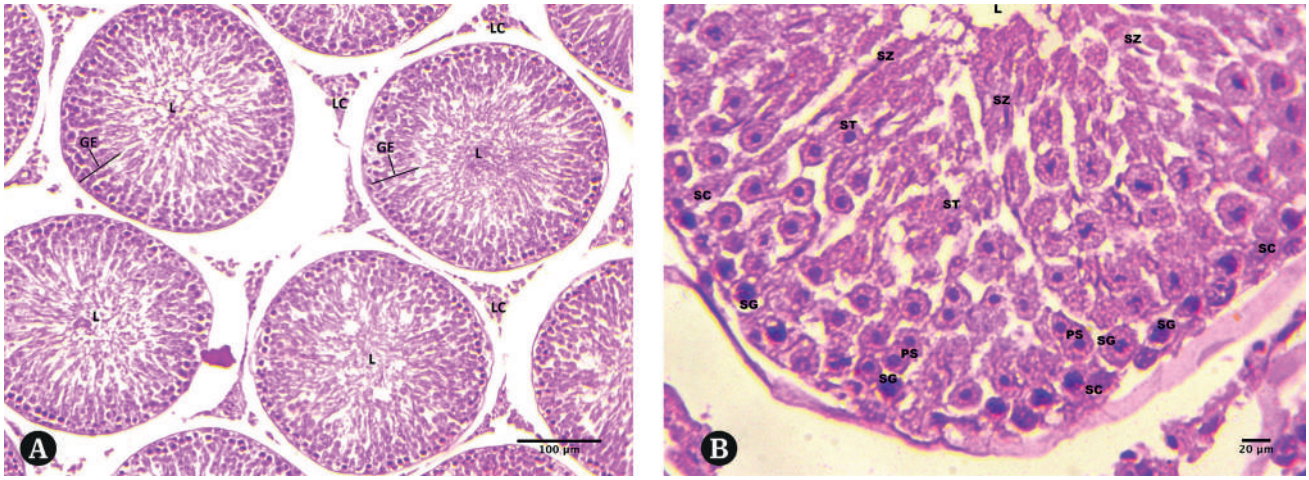

Fig. 1.- Testis of the control group I (Haematoxylin & Eosin staining). **1a:** cross section of the seminiferous tubules showing germinal epithelium (GE) containing cells of the spermatogenic cells with spermatozoa in the lumen (L). Interstitial Leydig cell (LC) seen between the seminiferous tubules. (x100, scale bar = 100 µm). **1b:** 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). (x400, scale bar = 20 µm).

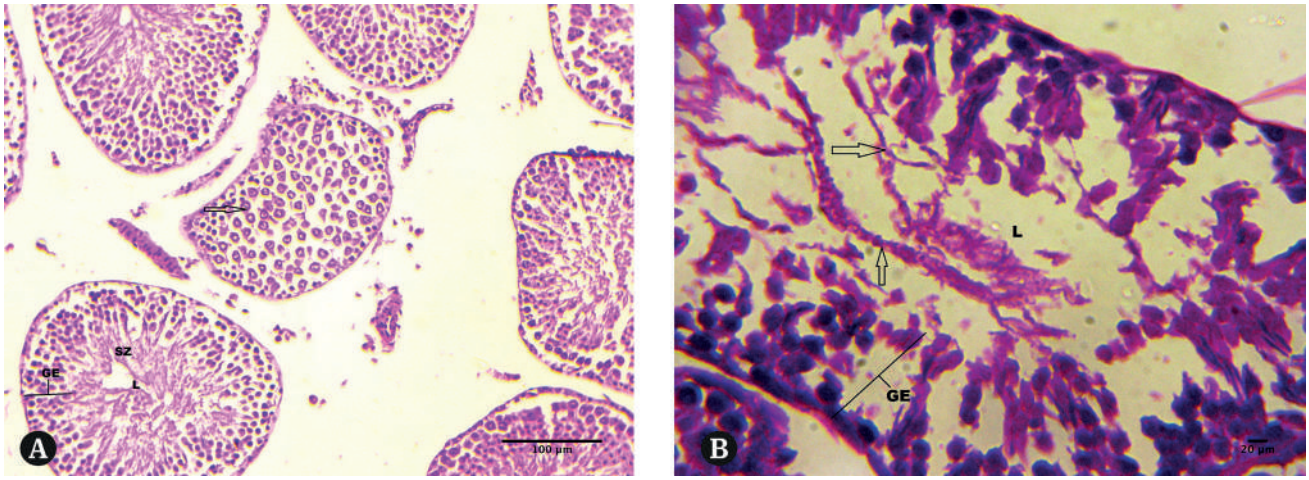

Fig. 2.- Testis of the aluminium treated group II (Haematoxylin & Eosin staining). **2a:** cross section of the seminiferous tubules showing loss of normal distribution of germinal epithelium (GE). Spermatozoa (SZ) in the lumen (L). Vacuolar cytoplasm (arrow) with loss of normal distribution of the spermatogenic cells seen. (x100, scale bar = 100 µm). **2b:** cross section of the seminiferous tubules showing loss of normal distribution of germinal epithelium (GE). Fragmented sperms (arrow) in the lumen (L). (x400, scale bar = 20 µm).

marked degeneration and necrosis of germ cells lining seminiferous tubules, as well as interstitial edema with complete absence of germ cells (Khattab, 2007). Deleterious effects of Al treatment for 2 to 5 weeks and its histopathological changes in testicular tissues showed spermatogenetic loss as necrosis in the spermatids and spermatozoa (Guo et al., 2005). Al exposures of experimental animals induce coiling tail in rats' sperm, cytoplasmic droplet in rats, interstitial edema, Leydig cell proliferation and sperm hyperemic blood vessel (Aitken et al., 2008). The present study showed morphological changes in the seminiferous tubule with vacuolar cytoplasm, with loss of normal distribution of the epithelial lining with few fragmented sperms in the lumen.

It is also reported that aluminium chloride (AlCl3) induced histological and ultrastructural changes in the testis with severe damage of germ cells, and the electron microscopy observations include atrophy of the tubular membrane, mitochondria, endoplasmic reticulum, golgi apparatus and nucleus (Khattab, 2007). Histological and ultrastructural studies on the testis of rats after treatment with aluminium chloride showed that administration of sodium fluoride together with AlCl3 to mice for 30 days caused inhibition of spermatogenesis and formation of giant cells (Chinoy et al., 2005). Apoptosis has been detected in spermatogonia and primary spermatocytes after AlCl3 administration (Abdel Moneim, 2013) and primarily results from microtubule targeting

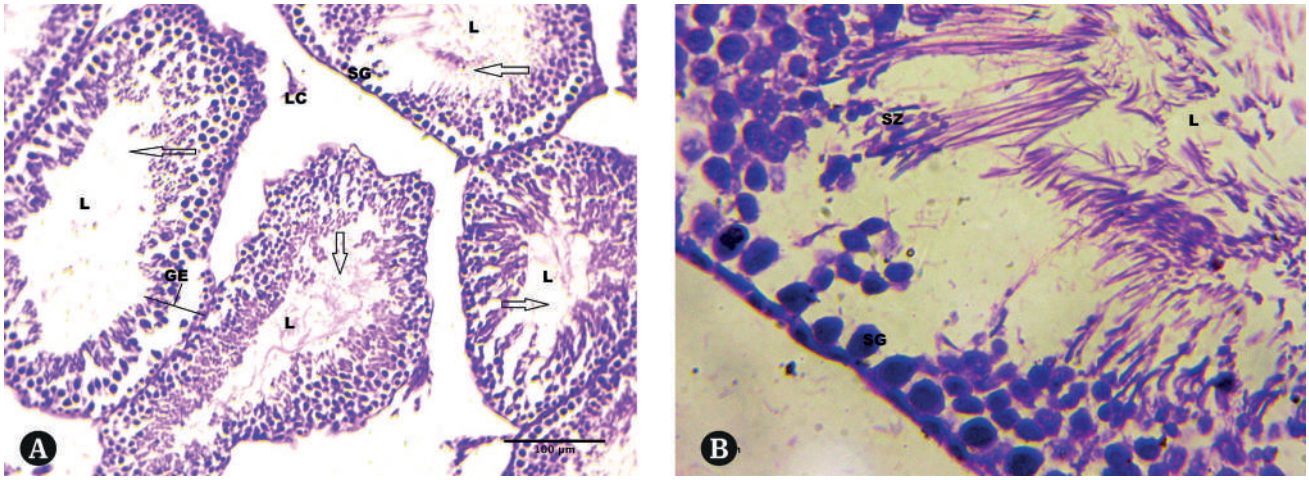

Fig. 3.- Testis of the ethanol treated group III (Haematoxylin & Eosin staining). **3a:** cross section of the seminiferous tubules which attained different shapes and hypocellularity reduction in cells of the spermatogenic series of germinal epithelium (GE) and widened empty (arrow) lumen (L). Few interstitial Leydig cell (LC) seen between the seminiferous tubules. (x100, scale bar = 100 µm). **3b:** cross section of the seminiferous tubules. Most of the spermatocyte appeared with dark stained pyknotic nuclei. Spermatogonia (SG), Spermatozoa (SZ) in the lumen (L). (x400, scale bar = 20 µm).

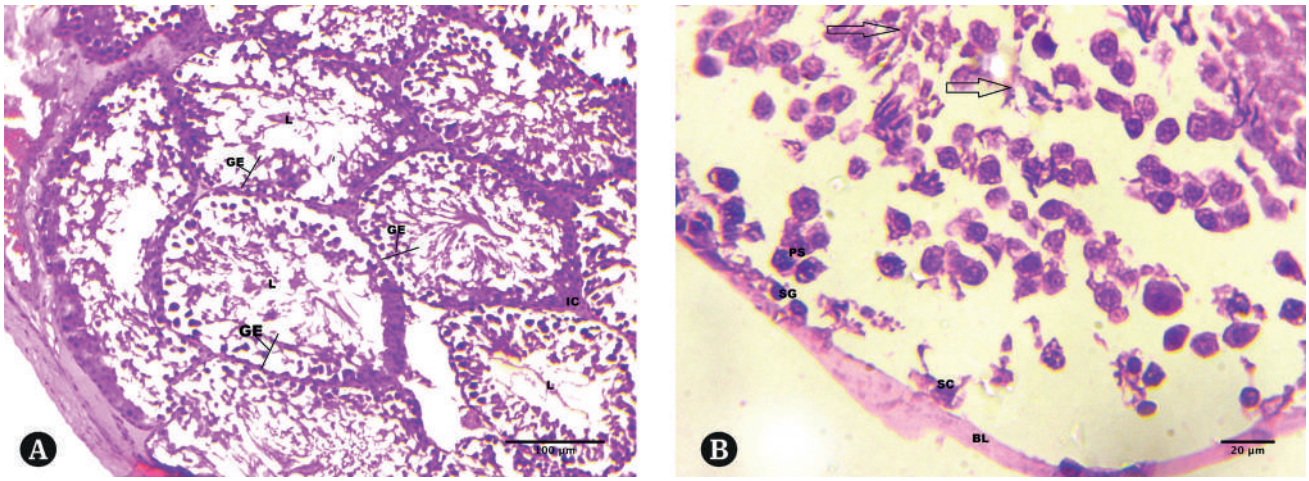

Fig. 4.- Testis of the aluminium and ethanol treated group IV (Haematoxylin & Eosin staining). **4a:** many distorted seminiferous tubules. Most of the tubules show marked reduction in the thickness of the germinal epithelium (GE). Fragmented sperms in the lumen (L). Few interstitial Leydig cell (LC) seen between the seminiferous tubules. (x100, scale bar = 100 µm). **4b:** acute degeneration of the spermatogenic cells. Spermatogonia (SG), Primary spermatocyte (PS), most of the tubules showed that the germ layers were detached from the basal lamina (BL), disintegrated Sertoli cell (SC) and abnormal distribution of spermatozoa (arrow) seen. (x400, scale bar = 20 µm).

and mitotic arrest. Saberzadeh et al. (2016) reported that Al-maltolate induced apoptosis in PC12 cells. It was reported that oxidative stress can induce male infertility and cause an increase in germ cell apoptosis and subsequent hypo spermatogenesis. Al is considered to be a non-redox active metal; it promotes biological oxidation both in vitro and in vivo because of its pro-oxidant activity (Turner and Lysiak, 2008). It is also reported that AlCl3 caused reproductive toxicity in male rats and induced oxidative stress results from the production of excess oxygen radicals (Yousef et al., 2009). Thus, the effects of AlCl3 on reproduction of male rats may be due to its generated oxidative stress. Histological study on testes of male albino rats intoxicated with Al for 90 days alone showed more exaggerated features of focal areas of spermatogenesis, arrest at the spermatid level in the form of degenerative changes in the germinal cells, together with few fragmented sperms in the lumen and acquired a thick, irregular basement membrane damage of testicular tubules and spermatogenesis, which showed histological changes in the seminiferous tubule of that testes (Thirunavukkarasu et al., 2010; Buraimoh et al., 2012). Similar results are also reported—that the effects of Al nitrite show necrosis on spermatocytes and spermatids (Libet et al., 1995).

Alcohol is well known as teratogenic and fetotoxic in humans. It has effects on sperm production and sperm quality. Many studies explain alcohol-altered testosterone production and testicular atrophy due to decrease the diameter of the seminiferous tubules (Fraser, 1992). The present study showed destruction of the seminiferous tubules and 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 is consistent with previous studies that showed 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, decrease of Leydig cell's number and the presence of degenerative germ cells in rats (Hu et al., 2003; El Sokkary, 2001). There is 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 (Wallock Montelius et al., 2007). 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 (Blanco et al., 2005). Ethanol can interfere with the function of the hypothalamic pituitary gonadal axis, thereby causing impotence, infertility, and reduced male secondary sexual characteristics (Emanuele and Emanuele, 1998). Oxidative stress in the testes due to ethanol administration along with increased extent of lipid peroxidation or due to decreased antioxidant defences, and thereby induces germ cell apoptosis leading to testicular atrophy (Ganaraja et al., 2008). Ethanol consumption disturbs epididymal spermatozoa motility, nuclear maturity and DNA integrity of spermatozoa in rats; and this may be one possible cause of infertility following ethanol consumption (Talebi et al., 2011). 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 to the neurotoxin activity of ethanol. Alteration of sperm count and semen morphology was observed in rats exposed to aluminium chloride with dose 64 mg/kg body weight (ZHU et al., 2014). It is also reported that, due to chronic alcohol consumption in the liver, it showed elongated and distorted mitochondria without normal organization (Kiessling et al., 1964). Decrease in spermatozoa viability as observed in the large number of 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, together with necrosis and suppression of cell proliferation, may contribute to testicular atrophy (Zhu et al., 2000). These effects were observed in the reduced tubular diameter and cross-sectional areas of the treated animals.

Most previous studies focused on the individual toxic effects of a single chemical; however, there is a possibility that humans and animals can be exposed to a mixture of toxic agents (Ghorbel et al., 2016). 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. An important aspect of spermatogenesis is the detachment of 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 (Chamberlain et al., 2004). 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 (He et al., 2005). From a histologic point of view, we agree with authors who reported that Al can 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 presence ethanol co-exposure in given doses. The present study explored the histopathological changes in testis by Al itself and in presence of pro-oxidants ethanol.

CONCLUSION

The result of the present study provides the evidence of adverse effects of Al on testis histology by degenerative changes in spermatogenic cells in given moderate dose. We further concluded that ethanol acts as a pro-oxidant and augments the toxic effects of Al in the testis by acute degeneration of the seminiferous tubules and spermatogenic cells.

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