



RESEARCH ARTICLE

ASSESSMENT OF ALUMINIUM INDUCED IMPACT ON LIPID PEROXIDATION, ANTIOXIDANTS AND BIOCHEMICAL STATUS OF TESTIS IN WISTAR RATS

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ABSTRACT

The present study was designed to evaluate adverse effects on biochemical and antioxidant parameters of testis in rats treated with aluminium chloride (AlCl₃). Male Wistar rats were randomly divided into four groups. Rats of Group I received vehicle treatment while rats of Group II, III and IV were orally administered with AlCl₃ at 30, 60 and 90 mg/kg b.wt/day respectively for 60 days. Half of the rats of Group IV were left untreated to observe reversibility of the adverse effects. Biochemical analysis showed a significant decline in the concentration of protein, sialic acid and in the activity of acid and alkaline phosphatase while the levels of total cholesterol and glycogen increased in a significant dose dependent manner in testis. AlCl₃ treatment resulted in an increased level of lipid peroxidation (TBARs) whereas the activity of marker antioxidant parameters decreased as compared to control rats. In recovery group, after 60 days of treatment cessation, biochemical and antioxidant parameters restored which shows partial recovery. Results indicate that altered biochemistry as well as enhanced levels of free radicals and decreased antioxidants level may lead to deteriorative testicular function.

Key words: Aluminium; rats; biochemical; antioxidant; recovery

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INTRODUCTION

Metals are naturally occurring elements that are found throughout the earth's crust. Metals enter the environment by natural and anthropogenic means. Any metal may be considered as "contaminant" if it occurs in a concentration that causes a negative effect on human or environment. Humans are primarily exposed to these contaminants in the workplace or in the living place through the consumption of contaminated food and water which may result in a series of adverse health issues including cardiovascular disorders, neuronal damage, renal injuries and risk of cancer and diabetes. The general mechanism involved in heavy metal-induced toxicity is recognized to be production of reactive oxygen species resulting oxidative damage and related health adverse effects (Rehman *et al.*, 2017). Aluminium is one of the most abundant elements in the environment. Human beings are widely exposed to aluminium through pharmaceuticals, utensils, food additives and consumer products (Newairy *et al.*, 2009). Once enter into the body, it can be absorbed and reach to various organs through blood. It can cause nerve cell degeneration if cross the blood-brain barrier and accumulate in the brain. Aluminium can provoke cellular damage in different tissues,

including the liver and brain by enhancing the level of oxidative stress. A number of studies have reported toxic effects in humans or laboratory animals induced by aluminium exposure (Pandey and Jain, 2013). It is well known that aluminum overload can cause severe neurodegeneration by enhancing oxidative stress and inflammation. Although a number of studies have also been reported decreased epididymal sperm count, viability and motility (Exley, 2016; Ige and Akhigbe, 2012) as well as disrupted steroid genesis (Pandey *et al.*, 2014; Guo *et al.*, 2001) in aluminium exposed organisms. However, there are only a few studies reporting the effects of aluminium on biochemical and antioxidant parameters as well as reversibility after treatment withdrawal so the present study is conducted to estimate the rate of lipid peroxidation and the status of antioxidants and biochemical parameters of testis in aluminium chloride treated rats as well as recovery of toxic effects after 60 days of treatment withdrawal in highest dose group.

MATERIAL AND METHODS

Experimental animals

Colony bred adult, healthy male albino rats (Wistar strain) were used for experimental purpose. A colony was maintained under standard laboratory condition of light-dark cycle (14hr-10hr) and temperature (22±3°C).

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Table 1. Tissue biochemistry in testis of rats treated with various doses of aluminium chloride

Treatments	Total protein (mg/g)	Glycogen (mg/g)	Sialic acid (mg/g)	Total cholesterol (mg/g)	Acid phosphatase (KA unit)	Alkaline phosphatase (KA unit)	Fructose (mg/g) Seminal vesicle
Group-I Control (vehicle)	216.37 ±5.81	3.69 ±0.09	5.06 ±0.14	6.59 ±0.18	12.96 ±0.49	54.27 ±2.04	5.79 ±0.22
Group-II Aluminium chloride (30 mg/kg b.wt./day)	195.87 ±4.80 ^a	4.08 ±0.08 ^a	4.50 ±0.19 ^a	7.21 ±0.19 ^a	11.84 ±0.31 ^a	47.25 ±1.36 ^b	4.96 ±0.23 ^a
Group-III Aluminium chloride (60 mg/kg b.wt./day)	178.75 ±5.35 ^c	4.24 ±0.15 ^b	4.18 ±0.17 ^b	8.05 ±0.23 ^b	10.25 ±0.36 ^c	43.72 ±1.72 ^c	4.68 ±0.19 ^b
Group-IV Aluminium chloride (90 mg/kg b.wt./day)	156.62 ±4.76 ^c	4.77 ±0.17 ^c	3.64 ±0.13 ^c	8.87 ±0.20 ^c	7.06 ±0.42 ^c	35.92 ±2.13 ^c	3.90 ±0.18 ^c
Recovery-Group (after 60 days of treatment withdrawal)	187.87 ±5.87 ^{b**}	4.13 ±0.15 ^{a**}	4.36 ±0.23 ^{b**}	7.43 ±0.18 ^{b**}	11.14 ±0.28 ^{b***}	45.21 ±1.58 ^{b**}	4.76 ±0.24 ^{b**}

Levels of significance: ns- Non significant; a- P<0.05; b- P<0.01; c- P<0.001, aluminium chloride treated groups compared with control group.

+/- non significant; *- P<0.05; **- P<0.01; ***- P<0.001, recovery group compared with group-IV.

(One way ANOVA followed by LSD multiple comparison test)

Table 2. Lipid peroxidation and antioxidant defense system markers in testis of rats treated with various doses of aluminium chloride

Treatments	Lipid peroxidation (TBARS) (n mol MDA/mg tissue)	SOD (unit/mg protein)	Glutathione (n mol/g tissue)	Ascorbic acid (mg/g tissue)
Group-I Control (vehicle)	1.59±0.13	8.54±0.17	2.78±0.09	1.36±0.06
Group-II Aluminium chloride (30 mg/kg b.wt./day)	2.06±0.10 ^b	7.68±0.26 ^a	2.38±0.13 ^a	1.07±0.05 ^b
Group-III Aluminium chloride (60 mg/kg b.wt./day)	2.27 ±0.12 ^c	7.27±0.21 ^c	2.19±0.11 ^b	0.86±0.05 ^c
Group-IV Aluminium chloride (90 mg/kg b.wt./day)	2.86±0.11 ^c	6.34±0.21 ^c	1.66±0.09 ^c	0.65±0.06 ^c
Recovery-Group (after 60 days of treatment withdrawal)	2.18±0.13 ^{b**}	7.49±0.25 ^{b**}	2.29±0.09 ^{b**}	1.06±0.04 ^{b***}

Levels of significance: Values represent mean ± SEM (n=8); ns- Non significant; a- P<0.05; b- P<0.01; c- P<0.001, aluminium chloride treated groups compared with control group.

+/- non significant; *- P<0.05; **- P<0.01; ***- P<0.001, recovery group compared with group-IV.

(One way ANOVA followed by LSD multiple comparison test)

Rats were provided with water and a nutritionally adequate pellet diet *ad libitum* in the animal house facility. The animal care and handling were done as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) regulations. The study was permitted by the Institutional Animal Ethical Committee of the Department of Zoology, University of Rajasthan, Jaipur.

Experiment design

Wistar male rats were divided into four groups and were treated for 60 days as follows: Group I served as control and received vehicle (distilled water, 0.5 ml/rat/day), while rats of Group II, III and IV were treated orally with aluminium chloride (30, 60 and 90mg/kg b.wt./day dissolved in distilled water; respectively). Each group has 8 rats except group IV which has 16 rats. Half of the rats of group IV sacrificed on day 60th and half were left untreated for next 60 days to observe recovery of the adverse effects in highest dose group. The rats were sacrificed at a fasting state twenty four hours after administration of the last dose under ether anesthesia. Testes were dissected out and cleaned off from adherent fat and blood clot and were kept frozen at -20° \-70° C for biochemical estimations. Total protein (Mayyas *et al.*, 2005), Glycogen (Lowry *et al.*, 1951), Sialic acid (Montgomery, 1957), Total Cholesterol (Warren, 1959), Acid phosphatase Zlatkis *et al.*, 1953, Alkaline phosphatase Zlatkis *et al.*, 1953, Lipid peroxidation (TBARS) (Kind *et al.*, 1954),

Superoxide dismutase (Ohkawa *et al.*, 1979), Glutathione (Marklund and Marklund, 1979) and Ascorbic acid (Zlatkis *et al.*, 1953) were analyzed in testis tissue.

OBSERVATION AND RESULTS

Table 1 show that treatment with aluminium chloride caused a dose dependent decline in the concentration of total protein and sialic acid as well as in the activity of acid and alkaline phosphatase. On the other hand, the testicular glycogen and total cholesterol concentration denoted a significant dose dependent increase in rats treated with aluminium chloride at three different doses, when compared with control group. After 60 days of cessation of treatment, the rats of recovery group showed significant elevation in sialic acid concentration and in the activity of both phosphatases while a significant decline was observed in concentration of both glycogen and total cholesterol in testis as compared to rats of Group-IV (90 mg/kg b.wt./day), although it was still low when compared to control rats. Results indicated elevation in lipid peroxidation (TBARS) while a significant decline in the activity of superoxide dismutase (SOD) as well as in the content of glutathione and ascorbic acid in a dose dependent manner in testis of rats treated with aluminium chloride at three different doses. After 60 days of treatment withdrawal, there was a significant decrease in lipid peroxidation (TBARS) and significant increase in the level of antioxidant markers in testis of

recovery group rats as compared to rats of the highest dose group. (Table 2)

DISCUSSION

Testicular secretory constituents like protein, cholesterol, sialic acid and glycogen can be used to determine the functional capacity of the testes (Roe and Kuether, 1949). Testicular protein content is considered as one of the constituent that is essential for testicular development and spermatogenesis (Aladakatti *et al.*, 2010). In present study observed reduction in protein content may be due to diminished secretory activity of the testis, disturbed protein metabolism or lessen number of germ cells in testis (Weinbauer *et al.*, 2010; Fyiad, 2007). Decreased testicular protein content in aluminium exposed rats has also reported by other researchers also (Yousef and Salama, 2009). In recovery group, improved secretory activity of testis might be responsible for restoration in the protein content of testis towards normal side. Similar recovery in testicular content was observed after treatment withdrawal in aluminium treated mice (Rawy *et al.*, 2015; Chinoy *et al.*, 2015a). Testicular glycogen provides carbohydrate reserves for spermatogenic cells in seminiferous tubules and is considered to be associated with spermatogenesis, gonadal maturation and proper functioning (Chinoy *et al.*, 2005b). In the present study, the aluminium chloride treated rats showed significant increase in the concentration of glycogen in the testis which might be due to arrested spermatogenesis or lowering of the rate of glycolysis by virtue of inhibition of enzyme activity. El Demerdash (Datta *et al.*, 1988) suggested that decreased activity of phosphorylase (an enzyme involved in glycogenolysis) in testis might be responsible for significant enhancement in the levels of glycogen in testis. The increased level of glycogen in testis showed significant recovery after 60 days of treatment withdrawal by virtue of increased utilization by spermatozoa. Sialic acid (N-acetylneuraminic acid) acts as a 'lubricant' to facilitate the movement of sperm and to reduce friction among spermatozoa in testis (El-Demerdash, 2004). A significant dose dependent decrease in the concentration of sialic acid observed in testis might be correlated with androgen level. Similar decline was observed in testicular sialic acid content due to reduced androgen level and sperm count in metal treated rats (Riar *et al.*, 1973; Pandey *et al.*, 2015). The level of sialic acid was significantly improved in aluminium chloride treated rats after 60 days of treatment withdrawal probably due to restoration of androgen level.

Testicular cells require cholesterol for membrane biogenesis, cell signaling and as a precursor required for androgen synthesis (Jain *et al.*, 2015). Guo *et al.*, 2010 reported that excessive NO compounds generated by aluminium exposure exert inhibitory action on the production of testicular adenosine 3, 5- cyclic monophosphate (cAMP) which aided in the transport of cholesterol to the inner mitochondrial membrane. So it might be suggested that increased cholesterol content in testis might be due to disturbed cholesterol transport. Abdel-Moneim, (Guo *et al.*, 201a) also observed the accumulation of multiple lipid droplets in Leydig cells which also supports the underutilization of cholesterol in aluminium treated mice. After 60 days of treatment withdrawal, increased level of cholesterol content in testis showed a significant decline probably due to its consumption in androgen biosynthesis by the Leydig cells. Acid phosphatase (ACP) is extensively distributed in lysosomes of germ cells, Sertoli cells and Leydig cells where it provide phosphate to germ cells to

meet their high energy requirements, regulate seminiferous tubule functioning. In Leydig cells, it is involved in the protein synthesis by abduction of sex hormones (Abdel-Moneim, 2013; Peruquetti *et al.*, 2010). Reduced activity of ACP in testis might be a consequence of decreased testicular steroidogenesis in the treated-rats and this may be correlated with the reduced secretion of gonadotrophins. Similar decline in the activity of testicular acid phosphatase was observed in animals treated with aluminium (Datta *et al.*, 1988). Alkaline phosphatase (ALP) plays an essential role in the transport of material from Sertoli cells to various germ cells and in differentiation and proliferation of the germinal epithelium (Porawski *et al.*, 2004). The reduction in the testicular ALP activity observed in present study might be correlated with the reduced germ cell population. Fyiad, 19 suggested that aluminium, a trivalent cation has a high affinity for negatively charged groups. Hence, it has been proposed that aluminium preferentially reacts with nucleic acid and inhibit activities of enzymes such as ACP and ALP. Alkaline phosphatase requires Mg²⁺ and Zn²⁺ for stability and catalysis. Reduction in the activity of ALP in testis could be due to incorporation or interaction of metals within the place of Mg or Zn which resulted in the alteration of steric configuration of phosphatase (Singer *et al.*, 1998). Reduced activity of ACP and ALP in testis was significantly but partially improved in rats after 60 days of treatment withdrawal suggesting partial modulation of the degenerative effects.

Antioxidant status of gonadal tissue acts as an important marker to determine male factor infertility. Estimation of end products of lipid peroxidation such as Malondialdehyde (MDA) reflects the extent of oxidative damage to cellular structures (Batra *et al.*, 2001). An increase in lipid peroxidation in the test is observed in study can be correlated with concurrent increase in the production of free radicals or reduced content/ activity of antioxidants in testis. Similar elevation in the levels of thiobarbituric acid-reactive substances (TBARs) has been observed in testis of aluminium treated rats (Fyiad, 2007). Superoxide dismutase (SOD) acts as a major protective system against free radical injury in male reproductive system (Sharma and Agarwal, 1996). Decline in the activity of SOD enzyme in testis may be due to excess consumption during the breakdown of free radicals and high level of H₂O₂. Higher intracellular concentrations of aluminium resulted in decreased synthesis of the enzyme proteins which leads to reduction in the activity of SOD (De Lamirand and Gagnon, 1993). Other metals like chromium (Nehru and Anand, 2005), cadmium (Subramanian *et al.*, 2006) and mercury (Pires *et al.*, 2013) have also been reported to inhibit SOD activity significantly in testicular tissue of various experimental animals. The SOD activity in testis was significantly but partially improved in rats after 60 days of treatment withdrawal suggesting partial recovery of the degenerative effects by virtue of partial alleviation of free radicals. Increased levels of TBARs in testis of aluminium chloride treated rats were significantly returned towards normal side after 60 days of cessation of treatment suggesting alleviation of oxidative stress to some extent. Glutathione is the most abundant non-thiol protein in mammalian cells which protects plasma membrane from lipid peroxidation, scavenges superoxide, and prevents O₂ formation (El-Desoky *et al.*, 2013). Significant dose dependent decline in GSH content in testis may be due to increased utilization in trapping free radicals generated due to toxic effect of these metals in testis. It is postulated that ROS has a greater affinity for the thiol

groups of biomolecules, thus depleting intracellular thiols inducing reduced GSH content in testicular tissue which made spermatogenic cells more vulnerable to free radicals resulting in less sperm count (Irvine, 1996). Aluminium might affect the glutathione (GSH) synthesis by decreasing the activity of glutathione-synthase (GS). Likewise, a slowing down in the conversion of oxidized- to-reduced form of GSH due to the inhibition of glutathione-reductase (GR) by aluminium could explain the increment in GSSG/GSH ratio (Fyiad, 2007; Aitken, 2013). Nehru and Anand (De Lamirande and Gagnon, 1993) suggested that aluminium exposure decrease the ATP use activity which in turn could result in glutathione synthesis alteration. Ascorbic acid, a potent water soluble antioxidant regulates the normal integrity and function of the testis by scavenging/neutralizing an array of reactive oxygen species (Yousef, 2004). Decreased concentration of ascorbic acid in present study indicates the subnormal scavenging of lipid peroxidation in testis of aluminium chloride exposed rats (Dawson *et al.*, 1990). The concentration or activity of antioxidant were partially but significantly increased in recovery group after 60 days of treatment withdrawal suggesting reduced lipid peroxidation and free radical generation concomitantly with an improvement of antioxidant defense system.

Conclusion

On the basis of present study, it can be concluded that Aluminium chloride distorted testicular biochemical milieu in a dose dependent manner which can in turn affect male fertility by disturbing spermatogenesis and steroid genesis. However, toxic effects induced by aluminium chloride can be partially recovered after treatment withdrawal.

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