

## Influence of Aluminium Chloride on Antioxidant System in the Testis and Epididymis of Rats

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### ABSTRACT

**Background:** In recent years, the use of chemicals in agriculture, industry, and public health has become so common that the environment is continuously contaminated by the toxic substance-like metals. Aluminum released due to anthropogenic activities such as mining and industrial uses. Aluminium has several industrial uses. The present study was designed to investigate the effect of aluminium chloride (AlCl<sub>3</sub>) on enzymatic and non-enzymatic antioxidants in the testis and epididymis of rats.

**Methods:** Adult male rats were administered with aluminium chloride at two different doses, 50 mg and 100 mg/kg body weight, orally, daily for 45 days. At the end of the experimental period, the animals were sacrificed and their testis and the epididymis were removed. Antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-s-transferase (GST) were assayed. Lipid peroxidation (LPO), vitamin C, and vitamin E levels were also determined.

**Results:** Aluminium chloride administration had no effect on the bodyweight of the animals but the weight of the testis and epididymis was decreased. Almost all the antioxidant enzymes studied markedly diminished in the testis and epididymis of aluminium chloride treated animals. The non-enzymatic antioxidants, vitamin C and vitamin E, also declined. Lipid peroxidation, on the other hand, significantly increased. The influence was found to be more in 100 mg treated rats when compared to 50 mg treated rats.

**Conclusions:** The present study suggests the reproductive toxicity of aluminium by inducing the oxidative stress in the testis and epididymis and possible interference in sperm production and further maturational processes.

**Keywords:** Aluminium Chloride, Antioxidant Enzymes, Epididymis, Rat, Testis.

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### INTRODUCTION

Aluminum (Al) is one of the highly abundant elements in the environment and the most common metal and the third most elements in the earth crust [1]. The wide distribution of this element ensures the potential for causing human exposure and harm [2]. Al compounds are widely used in medicines as antacid, vaccines, anti-diarrhoeals, phosphate binders and allergen injections [3], food additives and tooth paste [4], and water purification agents [5]. It is still a metal of choice in making various kinds of household cookware and storage utensils.

Al contributes to a variety of cognitive impairments in mice, rabbits, and rat pups [6,

7]. Epidemiological studies have indicated a link between Al in drinking water and Alzheimer's disease and a variety of human and animal studies have implicated learning and memory deficits after Al exposure [8, 9]. Aluminium chloride is able to generate reactive oxygen species (ROS) [10]. Chinoy *et al.* [11] reported Al induced toxicity in epididymis, vas deferens, seminal vesicle, and ventral prostate of mice. AlCl<sub>3</sub>-induced free radicals and inhibited antioxidant enzymes in blood and seminal plasma, liver, testes, kidney, lung, and brain of rabbits [10, 12].

Oxidative stress has been shown to play an important role in causing male infertility by inducing defects in sperm functions. Excessive production of ROS causes

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oxidative stress in spermatozoa [13]. ROS are central to a host of pathologies, including inflammation, toxicity, and endocrine disruption by environmental chemicals. ROS damage almost all macromolecules of the cell causing impairment of cellular functions.

ROS, such as hydrogen peroxide ( $H_2O_2$ ), appear to be a key agents causing cytotoxicity in spermatozoa to produce oxidative stress by decreasing the enzymatic defenses [14]. ROS are degraded by the organized system of antioxidants. Antioxidants have been described as substances that either directly or indirectly protect cells against adverse effects of xenobiotics, carcinogens, drugs, and toxic agents. Since both spermatogenesis and leydig steroidogenesis are vulnerable to oxidative stress, the low oxygen tension that characterizes this issue may be an important component of the mechanisms by which the testis protects itself from free radical mediated damage [15].

The present study was done to delineate the influence of aluminium chloride on enzymatic antioxidants, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-s-transferase, and non-enzymatic antioxidants, vitamin C and vitamin E, as well as lipid peroxidation in the testis and epididymis of adult rats.

## MATERIALS AND METHODS

### Animals

Healthy adult male rats (90 days) of Wistar strain (*Rattus norvegicus*) obtained from the Central Animal House, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, weighing 190-200g were used for the present investigation. The animals were maintained and handled as per the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and approval was obtained from Institutional Animal Ethical Committee (IAEC-845/GO/ac/04/CPCSEA).

The animals were housed in polypropylene cages lined with paddy husk, in a room with controlled temperature (25°C

$\pm 1^\circ\text{C}$ ), humidity (50%  $\pm$  5%) and lighting (12 hrs light and 12 hrs dark). The rats were fed with standard rat pellet diet (Raghavendra Enterprises, India) and drinking water *ad libitum*. After 15 days of adaptation period, the rats were divided into three groups of 10 animals each.

### Animal Groups

Group I- Control group: The rats were given distilled water as vehicle orally, daily for 45 days.

Group II- Experimental group I: The rats were given aluminum chloride 50 mg/kg body weight (in 0.5 ml distilled water) orally, daily for 45 days.

Group III- Experimental group II: The rats were given aluminium chloride 100 mg/kg body weight (in 0.5 ml distilled water) orally, daily for 45 days.

Before starting the treatment, the food intake was measured daily and the body weight was recorded. In the treatment period, the food consumed every day was recorded. The body weight was measured every five days and the percent changes were calculated.

The animals were treated between 8 AM and 9 AM and 24 hours after the last treatment the rats were weighed and sacrificed by decapitation. Testis and epididymis were removed from the adhering connective tissues, washed in cold physiological saline and weighed accurately.

### Biochemical Estimations

Testis and epididymis tissues were homogenized in 0.1 mmol/L Tris-Hcl buffer, pH 7.4 and used for the biochemical estimations.

Total protein [16], superoxide dismutase activity [17], catalase [18], glutathione peroxidase [19], glutathione reductase [20], glutathione-s-transferase activity [21], vitamin C [22], vitamin E [23], and lipid peroxidation [24] were measured by the standard spectrophotometric methods.

### Statistical Analysis

All the data were analyzed using one way ANOVA and the data were expressed as mean  $\pm$  SEM. The *P*- value of  $<0.05$  was considered as significant against control.

## RESULTS

In testis (Table 1) the specific activities of superoxide dismutase, catalase, and glutathione peroxidase significantly decreased in 50 mg as well as 100 mg aluminium chloride treated rats. However, the decrease was more significant in 100 mg treated animals. The activities of glutathione reductase did not change significantly in 50 mg treated animals, but significantly decreased in animals treated with 100 mg aluminium chloride. The activities of glutathione-s-transferase were not altered significantly in both experimental groups.

In epididymis (Table 2), the specific activities of superoxide dismutase, catalase,

and glutathione peroxidase significantly decreased in 50 mg as well as 100 mg aluminium chloride treated rats. The decrease was more significant in 100 mg treated animals. The activities of glutathione reductase did not change significantly in animals treated with 50 mg of aluminum chloride, but significantly decreased in animals treated with 100 mg aluminum chloride. However, the activity of glutathione-s-transferase was not altered significantly in both experimental groups. In 100 mg treated group, the activity of glutathione-s-transferase slightly decreased but it was not significant.

**Table 1.** Effect of aluminum chloride on antioxidant system in the testis of rats.

E2	E1	Control	Parameter
4.12 ± 0.284 **	5.15 ± 0.348 *	6.82 ± 0.467	SOD
6.24 ± 0.487***	8.39 ± 0.548 *	10.85 ± 0.609	CAT
8.47 ± 0.649 **	10.15 ± 0.729 *	13.45 ± 0.894	GPX
7.49 ± 0.548* *	10.29 ± 0.714 NS	10.67 ± 0.674	GR
7.84 ± 0.624 NS	8.95 ± 0.614 NS	9.43 ± 0.584	GST
1.15 ± 0.082 **	1.34 ± 0.068 **	1.82 ± 0.081	V-C
0.61 ± 0.041 ***	0.72 ± 0.057 ***	1.24 ± 0.052	V-E
24.54 ± 1.175***	21.15 ± 0.947 *	16.72 ± 0.984	LPO

**Table 2.** Effect of aluminum chloride on antioxidant system in the epididymis of rats<sup>1</sup>.

E2	E1	Control	Parameter
13.48 ± 0.927 **	16.31 ± 1.074 NS	19.35 ± 1.243	SOD
13.23 ± 0.927***	16.65 ± 0.714 *	20.15 ± 0.947	CAT
9.28 ± 0.698** *	12.15 ± 0.846 *	16.24 ± 1.014	GPX
8.17 ± 0.697 ***	11.85 ± 0.846 NS	12.54 ± 0.082	GR
9.87 ± 0.514 NS	10.56 ± 0.642 NS	11.19 ± 0.638	GST
1.56 ± 0.092 **	1.68 ± 0.087 **	2.17 ± 0.094	V-C
1.56 ± 0.092 ***	1.68 ± 0.087 ***	2.76 ± 0.079	V-E
21.42 ± 0.874 ***	19.65 ± 0.979 * *	14.24 ± 0.867	LPO

1. The results are expressed as Mean ± SEM (n = 10) per treatment and respective control groups. Levels of significance values are

\* $P < 0.05$ ,

\*\* $P < 0.01$ ,

\*\*\* $P < 0.001$

In both organs, vitamin C and vitamin E levels significantly decreased while the level of lipid peroxidation significantly increased. This change was observed in both experimental groups in the testis as well as in the epididymis.

## DISCUSSION

Cells are equipped with antioxidant defense system to counter the effect of ROS. Environmental contaminants are known to induce reproductive toxicity by perturbing the pro-oxidant and antioxidant balance leading to oxidative stress [25].

In the present study, aluminium chloride treatment decreased the activities of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-s-transferase as well as the non-enzymatic antioxidants, vitamin C and Vitamin E. At the same time, the level of lipid peroxidation was increased by the aluminium chloride administration. This clearly indicates an imbalance between pro-oxidant and antioxidant system, which could induce oxidative stress. The reduction in the activities of antioxidant enzymes could reflect the adverse effect of aluminium chloride on antioxidant system in the testis and epididymis of rats.

The reduction in the activity of SOD causes a rise in the level of superoxide anion, which inactivates CAT activity [26]. SOD is considered as the first line of defense against deleterious effects of oxyradicals in the cell by catalyzing the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen [27].

The decreased activity of CAT in the testis and epididymis of aluminium chloride treated animals observed in the present study may reflect the inability of these organs to eliminate  $H_2O_2$  produced by the influence of aluminium chloride. The antioxidant enzymes CAT and peroxidase protect SOD against inactivation by hydrogen peroxide. In turn, SOD protects the CAT and peroxidase against inhibition by superoxide anion [27]. Significant decreases were observed in the plasma and testicular CAT activities in animals treated with lead. The decreased CAT

activity results in the augmentation of  $H_2O_2$  generation [15].

Decreased activity of CAT could be associated with the oxidative stress in testis and epididymis. CAT is the main scavenger of hydrogen peroxide at high concentrations [28]. CAT activity is also linked to SOD activity. The decrease in SOD activity in animals exposed to high dose of metals may result in more accumulation of  $O_2^-$  which has been shown to inhibit CAT [26]. Along with CAT, GPx is also involved in the scavenging of hydrogen peroxide [29]. It is evident that ROS induced the tissue damage by initiating the self propagating lipid peroxidation reaction [30]. The increase in lipid peroxidation in the testis and epididymis, as observed in the present study, could be due to the concomitant increase in the generation of free radicals like hydrogen peroxide and hydroxyl radicals in these organs of aluminium chloride treated rats.

GPx is involved in catalyzing the reduction of  $H_2O_2$  at the expense of reduced GSH [31]. GR can interact directly with certain ROS (hydroxyl radical) to detoxify them, as well as performing other critical activities in the cell. Balasai Chaitanya *et al.* reported that the significant decrease in GSH level was observed in liver of aluminum-exposed rats [32].

Increased lipid peroxidation in biological membrane can lead to impairment of membrane functions. This is evident from the previous study in our laboratory showing the adverse effect of mercuric chloride on plasma membrane enzymes which may have an impact on the physico-chemical properties of testicular membranes [33]. It has been reported that ROS, such as hydrogen peroxide, appear to be a key agent causing cytotoxicity in spermatozoa to produce oxidative stress by decreasing enzymatic defenses [14]. An increase in  $H_2O_2$  generation reflects on the ROS produced by metal-like mercury is not eliminated by the antioxidant enzymes which could induce lipid peroxidation [34]. Other studies have reported that ROS induce lipid peroxidation and the toxicity of lipid peroxides play a key role in the inhibition of sperm functions and the pathophysiology of male infertility [35]. The

reduction in the activities of antioxidant enzymes in the testis and epididymis could reflect the adverse effects of aluminum chloride on the antioxidant system of spermatozoa as well.

It is shown that Al reduces antioxidants and increases lipid peroxidation [36]. Khattab [37] reported that the Al-induced oxidative damage and the ability of Al to cross the blood-testis barrier after inducing oxidative stress and lipid peroxidation that damages the biological membrane of the testes.

The observed reduction in vitamin C and vitamin E indicates the subnormal scavenging of lipid peroxidation in the testis and epididymis of aluminium chloride treated rats. The reduction in vitamin E concentration, as a chain breaking antioxidant, in these organs of aluminium-treated rats indicates a state of uncontrolled lipid peroxidation.

Many enzymatic functions of Vitamin C are essential for the normal integrity and function of the testis, i.e. the synthesis, development, and maintenance. Low or deficient ascorbate levels have been associated with low sperm counts, increased number of abnormal sperm, reduced motility, and agglutination [38]. Castellini *et al.* found that Vitamin C inhibited the oxidative processes and improved the characteristics of fresh and stored rabbit semen [39]. Vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect [40].

Metal toxicity is considered to be one of the pro-oxidants that induce oxidative stress. ROS are important mediators of normal sperm function and are involved in the induction and development of sperm hyperactivation, capacitation, and acrosome reaction [41]. However, excessive production of ROS above normal levels results in lipid peroxidation and membrane damage leading to loss of motility [42], damage to the acrosomal membranes and DNA oxidation, which render the sperm cell unable to fertilize the oocyte [43].

From the results obtained in the present study, it is evident that the toxic impact of AlCl<sub>3</sub> over the antioxidant system is more in

the epididymis than the testis. This clearly shows that the adverse effect of Al is not only affecting the testis impairing spermatogenesis, but it may also have severe impacts on sperm maturation and capacitation. It may also be assumed that though the spermatogenesis may be normal, the maturational events may be affected if the observed influence of Al is more in epididymis. However, it needs further studies at the level of sperm morphology and physiology.

## CONCLUSION

The present study suggests that the exposure to aluminum chloride induces the depletion of defense systems differentially in the testis and epididymis. This effect may lead to disruption in the functional integrity of these organs and thus adverse effects on the male reproduction.

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