



# Ovarian oxidative stress response due to aluminium exposure in Wistar rats

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## ABSTRACT

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**Introduction:** Aluminium is a known toxicant. The female reproductive system is highly susceptible to the deleterious effects of aluminium as it is also a metalloestrogen. It is claimed that aluminium is capable of creating a state of oxidative stress in tissues exposed to it. A delicate balance between generation of pro oxidant and antioxidant enzymes is needed for proper functioning of ovaries, which in turn is essential for the maintenance of general and reproductive health in females.

**Aim:** The work aims to find how dose, duration of exposure to aluminium influences the oxidative status in female Wistar rats' ovary.

**Materials and methods:** 48 female Wistar rats were divided into 2 sets and exposed to aluminium chloride at doses of 0, 50, 100, 200 mg/Kg bw daily via oral route for 4 and 8 weeks. Levels of enzymes indicative of oxidative status like GSH, LPO, SOD, Catalase, GPx & GR were measured in ovary. Statistics were done using Kruskal-Wallis test and Mann-Whitney test and p value <0.05 was considered significant.

**Results:** In rat ovary, aluminium intoxication increased the rate of lipid peroxidation, whereas a decrease in antioxidants like GSH, SOD, GPx was observed. However, these changes were significant only in aluminium doses of 100, 200 mg/Kg bw in both 4 & 8 weeks study. Only, GPx showed a



significant decrease even at aluminium dose of 50 mg/Kg bw when exposed to 8 weeks. Changes in levels of ovarian Catalase and GR were insignificant in all three aluminium treated groups when compared to the controls.

**Conclusion:** Aluminium stimulated the production of reactive oxygen species (ROS) in rat ovary, as evidenced by enhanced rate of lipid peroxidation, but this was not compensated by increased production of antioxidants. Moreover, aluminium exposure handicapped the antioxidant defense system significantly in doses of 100mg/Kg bw and above. Hence, we should exert caution and minimize our daily exposure to the sources of aluminium.

**Keywords:** Aluminium, GSH, lipid peroxidation, SOD, catalase, GPx, glutathione Reductase, oxidative stress, ovary.

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## INTRODUCTION

Aluminium is found in substantial amount throughout the surroundings.<sup>1</sup> It is contributed to the earth's atmosphere by human industrial activities, and its exposure generates hazardous effects leading to aluminium intoxication.<sup>2,3</sup> Accumulation of aluminium in the excretory, nervous, and hepatic system enhances the degeneracy of the renal, neural, and hepatic cells, respectively.<sup>4,5</sup> Exposure to aluminium is also reported the formation of free radicals<sup>6</sup> due to lipid peroxidation.<sup>7</sup> This in turn burdens the body by loading oxidative stress<sup>8</sup> as evidenced by enhanced level of malondialdehyde (MDA),<sup>9</sup> a well-accepted and a consistent marker of lipid peroxidation (LPO).

Free radical threats are managed by the body with the help of antioxidant which are enzyme related such as reduced glutathione (GSH), ascorbic acid, tocopherol or non-enzymatic like, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione reductase (GR).<sup>10</sup> Free radicals are scavenged by the antioxidants and the above-mentioned enzymes<sup>11</sup> while some enzymes are directly or indirectly involved in the replenishment of used antioxidants.

Exposure to aluminium is unavoidable. Moreover, aluminium being a metalloestrogen has the potential to be an endocrine disruptor.<sup>12</sup> Thus, ovary become a vulnerable target organ for aluminium toxicity. Therefore, the current investigation is planned to assess the oxidative stress responses of ovary in terms of endogenous enzymes as biomarkers when animals are exposed to aluminium.

## MATERIALS AND METHODS

48 female Wistar rats, weighing between 120-150g, procured from NIN, Hyderabad, and were nested under hygienic conditions in the Central Animal House of NRI Medical College and General Hospital. Premium quality reagents were purchased from Sigma, SRI, SDS, Merck and Himedia.

The experimental protocol was approved by the Institutional Animal Ethics Committee (Lt. No. 47/Chairman-IAEC, NRI Medical College and GH, Chinakakani), all procedures were done following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India). Rats were placed in polypropylene cages with unrestricted access to food and water, maintaining 12 h light/dark cycles, 22±2 °C temperature and were given a week time for acclimatization, and checked for vaginal opening and regularity of estrus cycle before starting the experiment. The study was conducted in two sets for different durations of aluminium exposures (4 and 8 weeks). Four groups of rats were created in each set, receiving aluminium at a dose of 0, 50, 100 and 200 mg/Kg body weight through daily orogastric gavage. Body weights, food intake, and water intake were noted on regular basis. Animals were sacrificed by cervical dislocation, ovaries were collected, washed in ice-cold saline, weighed and stored securely until used for analysis.

Using phosphate buffer ovaries were homogenized and centrifuged at 1000rpm in cold (4°C) for 5 minutes. The resultant supernatant was used for estimation of various biochemical assays indicative of their

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oxidative status like GSH, LPO, SOD, Catalase, GPx, GR as described by Nayak.<sup>13</sup>The protein levels of serum and ovary were measured by Lowry method.<sup>14</sup>

All the collected data are presented as Mean ± SEM of 6 observations. Analysis of variance of the grouped data were carried out by Kruskal-Wallis test, Mann-Whitney test was used to find the difference among groups. All the statistical test was done using PAST statistical software (ver. 4.03; Copyright: Ø. Hammer) and significance was considered at the p<0.05.<sup>15</sup>

### RESULTS

As expected, it is observed that higher the exposure time, higher was the effect of toxicity in terms of body weight gain. Gain in body weight was also impacted by the doses of aluminium exposure (data not presented). Neither the body weight gain nor the oxidative stress parameters in the least dose of aluminium varied significantly from control group in both the 4 weeks and 8 weeks duration studies (Table 1).

Ovarian GSH contents were found to be reduced significantly in response to higher doses (100 and 200 mg) of aluminium exposures in both durations. LPO levels of both 4 weeks and 8 weeks studies were significantly increased in the higher doses of aluminium exposure (Table 1). Ovarian SOD activities significantly decreased in those groups receiving higher aluminium doses at both durations. No change in catalase and GR activities were noticed in response to aluminium exposure irrespective of used doses and durations (Table 1). Interestingly, ovarian GPx activity was found to be significantly reduced even in 50 mg dose of aluminium exposure, along with higher doses, for 8 weeks of duration study. However, in case of 4 weeks duration study, the GPx activities of ovary were found to be reduced in 100 mg and 200 mg dose of aluminium exposure having only insignificant alterations in GPx of 50 mg dose study (Table 1).

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Table 1: Oxidative stress parameters of ovary in female rats with aluminium exposure of different doses and durations

4 weeks						
Animal groups	GSH	LPO	SOD	Catalase	GR	GPx
Al-0	82.91±4.10	58.73±4.65	34.25±1.55	59.45±5.29	2.33±0.26	3.69±0.36
Al-50	83.72±4.12	64.10±3.26	33.0±1.26	56.54±3.49	2.05±0.15	3.82±0.23
Al-100	44.95±2.33*	79.11±4.18*	26.39±1.48*	75.67±6.24	1.17±0.06*	3.31±0.30
Al-200	34.49±1.51*	108.19±5.00*	20.41±1.43*	73.75±6.07	0.66±0.03*	3.24±0.09
8 weeks						
Animal groups	GSH	LPO	SOD	Catalase	GR	GPx
Al-0	72.60±6.71	52.65±3.77	27.44±2.54	60.22±5.02	2.30±0.13	4.25±0.39
Al-50	67.49±3.61	63.85±3.11	25.56±1.35	58.76±4.97	1.70±0.18*	4.01±0.28
Al-100	40.92±4.28*	89.40±9.25*	17.03±2.37*	64.20±10.59	0.77±0.09*	4.18±0.50
Al-200	82.91±4.10*	58.73±4.65*	34.25±1.55*	59.45±5.29	2.33±0.26*	3.69±0.36

\* p < 0.05; GSH (mole/g protein); LPO (nmol MDA/100mg protein); SOD (unit/100mg protein); Catalase (nmol H<sub>2</sub>O<sub>2</sub> decomposed/hr/100mg protein); GPx (nmol NADPH oxidized/hr/100mg protein); GR (nmol NADPH oxidized/hr/100mg protein)

### DISCUSSION

Oxidative stress is an outcome of disparity of the body's aptitude to detoxify reactive oxygen species resulting in damage at the cellular level, thus causing the building of free radicals and peroxides, which in turn

influences the normal states of body organ systems like renal, neural, hepatic as well as genetical systems at cellular level.<sup>16</sup>

The threat of oxidative stress can be resisted through the proper responses of the antioxidant system in the body.<sup>17</sup>Both ROS as



well as the antioxidants have a vital role in the ovarian metabolism and good balance between the two is ideal for its functioning.<sup>18</sup> We tried to explore the potential of aluminum in disturbing this ovarian oxidative balance. The most abundant intracellular thiol based antioxidant is GSH, which by donating electrons helps keep cell in reduced state. Therefore, GSH offers a potent defense to the body's organ systems, that are exposed to reactive oxygen species or free radicals.<sup>19</sup> In the present study, ovarian GSH was lowered in all the aluminum-treated groups. However, only in test groups with high doses of aluminium (100 and 200mg/kg), the GSH levels were significantly lowered. Nevertheless, the lowered level of GSH in ovary is an indicator of distress of systemic antioxidant capacity. It is also observed that aluminium enhanced the ovarian lipid peroxidation. In ovary, a functional concentration of ROS is essential for ovulation; however, excess ROS can have detrimental effects on oocyte. Administration of a sublethal dose of aluminium phosphide which is a pesticide, significantly increased ovarian MDA levels, with decreased activities of SOD, Catalase, GPx and GSH.<sup>20</sup> Current findings are similar to that of Olusegun *et al* regarding LPO, GSH and SOD, but we did not find any significant change in ovarian catalase activities. GR maintains GSH levels at cellular level by reducing GSSG back to GSH. Optimal levels of GSH are crucial for normal ovarian function, gamete viability and fertilization.<sup>21,22</sup> In the current study, GSH is significantly reduced, despite of GR being unaffected by aluminum exposure, this observation shows that there might be multiple aspects that influence GSH levels in ovary like levels of gonadotropins.<sup>23</sup> A study conducted by Paszkowski *et al* among patients undergoing in vitro fertilization, reported that the levels of GPx in ovarian follicles that did not get fertilized was lower compared to the follicles whose oocytes were fertilized.<sup>24</sup> This indicates the probable role played by GPx in fertilization among humans. In our study, GPx was significantly decreased even in 50 mg/Kg dose when exposed to a longer duration (8 weeks). This varied response in the

antioxidant handling capacity of ovary indicates that multiple factors modulate ovarian activity as it is also an endocrine gland and it might be more susceptible to the effects of metalloestrogenaluminium.

## CONCLUSION

The present study demonstrates that exposure to aluminium through the oral route enhances the pro-oxidant activity as evidenced by increased lipid peroxidation but decreases the antioxidant potential by inhibiting the activities of enzymes like reduced Glutathione (GSH), Lipid peroxidation (LPO), Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Glutathione Reductase (GR), in female Wistar rat's ovary in both 4 and 8 weeks study. However, the above-mentioned changes were significant only in test groups receiving 100 mg/kg and 200 mg/kg aluminium chloride daily. Hence, our study states that attention should be paid to reducing our daily exposure to sources of aluminium and a dose of 100mg/kg per day can be selected as a minimum dose for further studies.

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