



Role of aluminium in inducing changes in antioxidant enzymes and lipid peroxidation in Serum of Rats

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ABSTRACT

Introduction: Aluminium is a widely used element which is highly toxic. It has been reported that heavy metals generate oxidants that create a state of oxidative burden which can lead to various pathologies.

Aim: This work is aimed to determine how dose and duration of exposure to aluminium influences the oxidative status in serum of female Wistar rats.

Materials and methods: After 4 and 8 weeks of aluminium chloride administration via oral route at doses of 0, 50, 100, 200 mg/Kg bw respectively, 48 female Wistar rats were sacrificed and serum levels of specific enzymes indicative of prooxidant and antioxidant activity like GSH, LPO, SOD, Catalase, GPx & GR were assayed. The statistics analyzed by the Kruskal-Wallis test and Mann-Whitney test and p value <0.05 was considered significant.



Results:Aluminium treatment lead toraise in the levels of LPO, with concomitant decrease in antioxidant enzymes like GSH, SOD, Catalase, GPx & GR when compared to controls.However, changes were mostly significant only in 100 and 200 mg/Kg bw groups.

Conclusion:This study concludes that aluminium was able to create oxidative imbalancein serum of female Wistar rats, which isindicated by the elevated lipid peroxidation and fall in antioxidant handling capacity. As the changes were mostly significant in aluminium dose of 100 mg/Kg bw and above, in both durations, it can be considered as the minimum dose for further toxicology studies.

Key words: Aluminium, lipid peroxidation , SOD, catalase, glutathione peroxidase , glutathione Reductase , oxidative stress.

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INTRODUCTION

Aluminium (Al) is a metal in that amply present in environment and is third in line to oxygen and silicon in terms of abundance. With the advent of "Aluminum Age" human exposure to this metal is only increasing day by day via air, water and food. Aluminium is liberated into our habitat by both natural means (rock, soil erosion, volcanic eruptions) and human activities like (mining & agriculture).Aluminium compounds are widely found in various industrial and consumer products, like antiperspirants, food additives, infant formulas, cooking utensils and in pharmacological agents like antacids, vaccine adjuvants etc.Food grown in acidic condition (pH<5) is also a route for aluminium transfer to food chain. Hence, aluminum is omnipresent and human exposure to it is inevitable. In spite of its environmental abundance and extensive usage in daily life, up to this time no biological function has been attributed to this metal.¹ Due to its biological inertness aluminium was considered safe, but stemming proof indicates that it may be responsible for causation of various diseases in humans and animals.² Aluminium exposure is linked with various pathological conditions starting from early evidence of dialytic encephalopathy³ to more recent evidences of its role in neurodegenerative diseases like Alzheimer's disease⁴, hepatotoxicity⁵, reproductive toxicity⁶ etc.

Aluminium is a non-redox active element, nevertheless, its strong prooxidant ability can potentiate oxidative damage.⁷ Aluminium may

lead to ROS production via the Fenton-type reaction.⁸Body tries to achieve oxidative homeostasis by gearing up its antioxidant defenses. Failure to counteract the deleterious consequences of ROS, leads to cellular and subcellular damage, leading to a state called oxidative stress.⁹

Hence, our objective is to evaluate the effect of dose and duration of aluminium exposure on the oxidative stress and capacity of antioxidant system in rat serum.

MATERIALS AND METHODS

Chemicals were purchased from Sigma, SRI, SDS, Merck and Himedia and were of analytical grade.48 healthy female Wistar rats, weighing 120-150g, were obtained from NIN, Hyderabad. The experimental protocol was approved by the Institutional Animal Ethics Committee (Lt. No. 47/Chairman-IAEC, NRI Medical College and GH, Chinakakani), all the experiments were conducted following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India).

The study was conducted under hygienic conditions in the Central Animal House of NRI Medical College and General Hospital. Rats were placed in polypropylene cages and were given food and water ad libitum, maintaining 12 h light/dark cycles, 22±2 °C temperature. After 1 week of acclimatization, vaginal opening and regularity of estrus cycle before was checked. The animals were assigned into two sets for different durations of aluminium exposures (4,

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8 weeks). Animals in each set were divided into four groups (n=6) receiving aluminium at a dose of 0, 50, 100 and 200 mg/Kg body weight through daily orogastric gavage. Daily recording of body weights, food intake, and water intake was done. After completion of respective treatment durations, blood samples were collected from overnight fasted animals, through retro-orbital venous plexus and sacrificed by cervical dislocation. Serum level of GSH, LPO, SOD, Catalase, GPX, GR were estimated as described by Nayak.¹⁰ The protein levels of serum were measured by Lowry method.¹¹

All the collected data are presented as Mean \pm SEM of 6 observations. Analysis of variance of the grouped data were carried out by Kruskal-Wallis test. Difference among the groups were evaluated by Mann-Whitney test. All the statistical test was done using PAST statistical software (ver. 4.03; Copyright: Ø. Hammer) and significance was considered at the $p < 0.05$.¹²

RESULTS

As expected, it is observed that higher is the duration of exposure, higher was the effect of toxicity in terms of body weight gain. In addition, the 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

lowest dose of aluminium exposure were significantly different from that of the control in both the 4 weeks and 8 weeks duration studies (Table 1). Serum GSH contents were found to be reduced significantly in response to higher doses (100 mg and 200 mg) of aluminium exposures in both durations (Table 1). Along with that, the levels of LPO were found to be increased in those higher doses of aluminium exposure in both duration studies (Table 1). Similarly, catalase activities were found to be significantly lowered in higher doses and both duration of aluminium exposures (Table 1). However, serum SOD activities of both 4 weeks and 8 weeks studies demonstrated significant decrease in activities only in the case of highest dose (200 mg) of aluminium exposures (Table 1). Nevertheless, the difference in duration of exposure was demonstrated by the GPx and GR

activities of serum. Only in case of 8 weeks of exposure, the 100 mg dose of aluminium could significantly reduce these activities; while, the 200 mg dose of aluminium exposure could significantly reduce the activities of these enzymes in both 4 weeks and 8 weeks durations (Table 1).

4 WEEKS						
Animal Groups	GSH	LPO	SOD	Catalase	GPx	GR
Al-0	6.67 \pm 0.36	51.54 \pm 2.94	42.93 \pm 1.94	16.73 \pm 0.99	3.21 \pm 0.21	4.09 \pm 0.29
Al-50	6.59 \pm 0.27	58.35 \pm 1.85	41.07 \pm 1.69	15.28 \pm 0.90	3.31 \pm 0.24	4.02 \pm 0.28
Al-100	3.73 \pm 0.13*	97.44 \pm 6.04*	33.80 \pm 3.34	12.23 \pm 0.73*	2.51 \pm 0.26	3.52 \pm 0.25
Al-200	2.81 \pm 0.27*	128.62 \pm 9.36*	25.94 \pm 1.67*	9.34 \pm 0.65*	1.86 \pm 0.13*	2.73 \pm 0.16*
8 WEEKS						
Al-0	6.68 \pm 0.39	55.12 \pm 4.03	49.11 \pm 2.53	18.77 \pm 1.37	3.67 \pm 0.17	4.20 \pm 0.33
Al-50	6.22 \pm 0.22	68.72 \pm 5.72	47.59 \pm 2.70	17.62 \pm 1.05	3.18 \pm 0.27	3.76 \pm 0.20
Al-100	4.48 \pm 0.48*	105.57 \pm 3.05*	37.93 \pm 3.40	13.32 \pm 0.74*	2.38 \pm 0.25*	3.47 \pm 0.11*
Al-200	3.56 \pm 0.20*	144.10 \pm 7.97*	27.13 \pm 1.79*	9.32 \pm 0.78*	1.68 \pm 0.06*	2.70 \pm 0.11*

* $p < 0.05$; GSH (mole/g protein); LPO (nmol MDA/100mg protein); SOD (unit/100mg protein); Catalase (nmol H₂O₂ decomposed/hr/100mg protein); GPx (nmol NADPH oxidized/hr/100mg protein); GR (nmol NADPH oxidized/hr/100mg protein)



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

DISCUSSION

When the body's ability to remove reactive oxygen species is hampered, it results in a state of oxidative stress, which can lead to damage at cellular and subcellular level involving various organ systems.¹³ Normally the antioxidant defense system eliminates this risk effectively and guards our body against oxidative damage.¹⁴

GSH is an intracellular thiol-based antioxidant, which acts as an electron donor and helps keep redox sensitive sites on various enzymes in a reduced state.¹⁵ In the present study, serum 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 serum GSH is an indicator of distress of systemic antioxidant capacity. This is in line with the works reported by Gunfer *et al.*¹⁶ in mice treated with aluminium sulphate. The reduction of hydrogen peroxide, using GSH, to water and oxygen and thus limiting the formation of hydroxyl radical is carried out by the GPx. As the GSH level of serum is lowered, it is expected that concomitant decrease in GPx activity should also be there. Serum GPx activities were significantly decreased in Al-100 and Al-200 groups in the 8 weeks study. Similar alterations are also observed in another enzyme GR activity. Nonetheless, GR catalyzes the formation of GSH from oxidized glutathione (GSSG). Thus, exposure to aluminium is altering the GSH metabolism – both formation and utilization. However, the decrease in GPx and GR was significant only in the highest aluminium group (200mg/Kg) in the 4 weeks study, which hints that GPx and GR activity is influenced by the duration of aluminium exposure. Shrivastava *et al.*¹⁷ observed a similar decrease in GPx and GR levels in serum and brain on administration of aluminium at a dose of 27 mg/kg/d i.p. for 60 days. On the other

hand, opposing results regarding GPx and GR were observed in geriatric rats who were treated with aluminium sulphate.¹⁸ Lipid peroxidation was significantly higher in 100 and 200 mg/Kg doses of aluminium in both durations, indicating the pro-oxidant ability of aluminium.^{19,20} Catalase activities were found to be significantly lowered in higher doses and both duration of aluminium exposures which is in agreement with the findings of Sharma *et al.*²¹ Contrary to our findings male Wistar rats treated with aluminium chloride showed increased SOD activity in serum with increased aluminium dose.²² However, the decrease in SOD was significant only in the highest aluminium group (200 mg/Kg) in both durations.

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CONCLUSION

In the present study we observed that Aluminium intoxication in rats induced a state of oxidative imbalance in female Wistar rat's serum which was significant starting from a dose of 100mg /Kg /day. The oxidative imbalance is evidenced by an increase in lipid peroxidation and decrease in antioxidant enzymes.

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