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**Original Research Article**

# Effects of *Salacia Oblonga o*n aluminium induced changes on membrane ATPases of various organs in *Wistar albino* rats

**Nandhini Ashokan[1], Divyashanthi Chellathambi Malathi[2]*, Nathiya Shanmugam[3], Elango Dhivya[2]**

[1]Department of Pharmacology, Asan Memorial Dental College and Hospital, Chengalpattu, Tamil Nādu, India
[2]Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research, Karaikal, Puducherry, India
[3]Sri. Ramakrishna Dental College and Hospital, Peelamedu, Tamil Nadu, India

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***Correspondence:**
Dr. Divyashanthi Chellathambi Malathi,
Email: dishanthii@gmail.com



## ABSTRACT

**Background:** Aluminium, one of the abundant elements present in nature enters human body through diet, food packaging material and medication. Aluminium can induce toxicity by causing oxidative damage and by altering the function of membrane bound ATPases, *Salacia oblonga*, a woody climbing plant with vast geographical distribution in India possess various biological functions. Previous studies have shown a protective effect of *S. oblonga* on Al induced visceral toxicity. Our aim is to evaluate the effect of *S. oblonga* extract on aluminium induced oxidative organ damage and haematological changes

**Methods:** The experimental animals, Wistar albino rats, were divided into four groups of 6 per group. The four groups are divided into distilled water, Aluminium chloride, Salacia oblonga extract and aluminium and Salacia oblonga. On 37$^{th}$ day animals were sacrificed and organs and blood were collected for assessment of ATPase activity, protein levels and haematological parameters.

**Results:** *Salacia oblonga* increased the ATPase activity in the brain, heart and lungs, improved the protein levels and haematological parameters which were deteriorated by Al toxicity. Though statistically insignificant *S. oblonga* improved the ATPase enzymes and haematological parameters.

**Conclusions:** Our study demonstrated that Aluminium causes membrane damage in various organs by altering the membrane ATPases level as a result of oxidative stress and altered the haematological parameters. We demonstrated that S. oblonga ameliorates Al induced membrane toxicity and also confers hemoprotective effect.

**Keywords:** *Salacia oblonga*, Aluminium toxicity, Oxidative stress, Preclinical study

## INTRODUCTION

Aluminium (Al) is one of the abundant elements present in the nature. Human intake of Al occurs through diet, intake of medication which contains Al, e.g. Antacids, parenteral fluids etc., cosmetics and occupational exposure.[1] Due to the increased bioavailability and potential negative health effects of Al it gained lot of interest among the researchers.[2] The toxic effects of Al is mediated by alteration in the level of vital metals and ions and also causes deposition of insoluble Al in various tissues and organs.[3] Mounting clinical and preclinical reports display that oxidative damage, elevated lipid peroxidation, dysregulated calcium homeostasis, and elevated proinflammatory mediators have been implicated in the Al toxicity.[4,5] Ionic pumps are more needed for the proper cellular functions. Normal ion concentration is needed for

the ionic balance across the membranes, action potential, pH balance and for the regulation of cell volume to mediate the proper functioning of entire physiological system.[6] ATPases are membrane-bound enzymes that orchestrates a pivotal role in many biological functions and act as an energy transducers.[7] Further, it aids the movement of cations across the cell membranes, the maintenance of intracellular functions, and are widely used as markers for ion regulatory changes.[8]

During Al toxicity there has been a significant alteration in cellular concentration of various ions. It has been shown that, Al in its oxidised state, $Al^{3+}$ binds strongly to ATP and phosphate groups and displaces both $Mg^{2+}$ and $Ca^{2+}$ from their enzyme's binding sites.[9] Furthermore, the activity of Na+ K+ -ATPase has been shown to be decreased in brain cortex synaptosomes.[10] Maintenance of normal ionic balance is very essential proper organ functions. Heavy metals mediate their toxic effects by replacing other bivalent cations like $Ca^{2+}$, $Mg^{2+}$, $Fe^{2+}$ and monovalent cations like $Na^{+}$, which ultimately disturbs the biological metabolism of the cell.[11] Chelating agents are widely used for the management of heavy metal toxicity, which generally bind to the heavy metals and accelerates the excretion of toxic elements. Clinically, desferrioxamine (DFO) is the most commonly used chelating for the treatment of Al systemic toxicity.[12] However, DFO therapy displays potential side effects, high economic and exerts its effective antidote action intravenously or subcutaneously with poor oral bioavailability. Natural functional foods contain various potential phytochemicals such as flavonoids and polyphenols which exerts potential benefits in the prevention of heavy metal toxicity in man and animals.[13] *Salacia oblonga* belonging to the family, Celastaceae is woody climbing plant with vast geographical distribution in India and Southeast Asia and possess various biological functions.[14] Previous study shows the protective effect of *S. oblonga* on Al induced toxicity to various visceral organs.[15] In this backdrop, the present study was carried out to evaluate the membrane protective effect of *S. oblonga* extract on aluminium induced oxidative organ damage.

## METHODS

The study was performed in Dr. A. L. M. Post graduate institute of medicals sciences during the period of February 2011 to March 2011.

### *Drugs and chemicals*

Aluminium chloride **(**$AlCl_3.6H_2O$) and other reagents used in the study were procured from S.D. fine chemicals, Mumbai, India.

### *Salacia oblonga extract*

The powder extract of *Salacia oblonga* was obtained from the Department of Pharmacology and Environmental Toxicology, Dr. A. L. M. Post Graduate Institute of Basic Medical Sciences (Sekkizhar Campus), Taramani, Chennai. The nature of the powder was dark brown powder. All the extracts were stored in refrigerator at 4°C.

### *Animals*

Wistar strain male albino rats of about 120-200 g were used in the study. All animal experiments were performed after obtaining prior approval from the Institutional Animal Ethical Committee governed by the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines, Government of India. The animals were housed in autoclavable polypropylene cages over husk beddings. The bedding material was changed twice a week under controlled environment (temperature: 23±4 & humidity: 50-70%) and a 12-hr light and dark cycle was maintained. The rats were fed with a commercial pellet diet (M/s Hindustan foods Ltd., Bangalore, India) and water ad libitum.

### *Experimental design*

The experimental animals were divided into four groups (N=6) as follows; Group 1: Rats administered orally with 1.0 ml double distilled water daily until the end of the experimental period for Group 2: Rats administered orally with aluminium chloride (300 mg/kg body weight) alone. The dose of the aluminium chloride was selected based on the study conducted by Nathiya et al.[15] Group 3: Rats administered orally with S. oblonga extract (67mg/kg body weight). Group 4: Rats administered orally with both aluminium chloride (300 mg/kg body weight) and *S. oblonga* extract (67mg/kg body weight).

### *Dosing schedule*

Animals were acclimatized for 15 days under laboratory conditions and treatment was started after the period of acclimatization. The animals were assessed daily for their body weight and behaviour. The extract was weighed, dissolved in double distilled water and made uniform using hand homogenizer

### *Collection of blood and serum*

After the end of experimental period, ie on 37$^{th}$ day the rats were anesthetized using ether and blood was collected by retro-orbital puncture. The collected blood was kept at 37°C for 30 min and centrifuged at 2000 rpm for 15 min to separate serum. The collected serum was stored and used for biochemical estimations.

### *Collection and processing of tissue*

At the end of the experiment, the animals were sacrificed by cervical dislocation and the visceral organs such as liver, heart, kidney, lung and cortex were dissected. The tissues were washed in physiological saline to remove blood clot and other tissue materials. The tissues were

homogenized using 0.1% Triton X-100 buffer (pH 7.4). The homogenate was centrifuged at 12,000 rpm, at 4°C for 30 min and the supernatant was used for the analysis of membrane bound phosphatases.

### *Estimation of membrane bound ATPases*

The tissue levels of $Na^+K^+$ ATPase, $Ca^{2+}$-ATPase and $Mg^{2+}$ ATPase was estimated according to the methods stated by Bonting, Hjerten and Pan and Ohnishi et al respectively.[16-18] The activities of the enzymes were expressed as µmoles of phosphorous liberated/min/mg protein.

### *Statistical analysis*

All values were expressed as mean±SD. The data were statistically analysed using one way ANOVA followed by Tukey's HSD multiple range test. The p value <0.05 was considered as statistically significant.

## RESULTS

### *Effect of S. oblonga and aluminium on $Na^+$ $K^+$ ATPases activity*

In the present study, the $Na^+$ $K^+$ ATPases activity was decreased in heart, lungs, cerebral cortex and increased in liver, kidney of group II Al intoxicated rats as compared to controls. Meanwhile, treatment with S. oblonga extract in group IV rats increased the $Na^+$ $K^+$ ATPases level in heart, lungs, cerebral cortex. Meanwhile, there was no significant alteration in the level of $Na^+$ $K^+$ ATPases in liver and kidney upon treatment with extract. The results were shown in (Table 1).

**Table 1: Estimation of $Na^+$-$K^+$- ATPase activity in *Wistar albino* rats.**

| Name of the Tissues | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| **Liver** | 0.46±0.403 | 1.56±0.540 | 0.8±0.546 | 0.84±0.559 |
| **Heart** | 0.86±0.537 | 0.74±0.470 | 1.31±0.510 | 1.72±0.652 |
| **Lung** | 1.52±0.526 | 0.85±0.400 | 1.65±0.436 | 1.94±0.392 |
| **Kidney** | 1.21±0.694 | 1.44±0.570 | 1.42±0.640 | 1.73±0.680 |
| **Cortex** | 0.83±0.647 | 0.56±0.195 | 1.54±0.481 | 1.07±0.312 |

n=6, Mean Group I Control; Group II $AlCl_3$; *p<0.05 ±SD, Group III *Salacia oblonga*; Group IV *Salacia oblonga*+$AlCl_3$

**Table 2: Estimation of $Ca^+$-ATPase activity in *Wistar albino* rats.**

| Name of the tissues | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| **Liver** | 0.56±0.55 | 1.36±0.43 | 0.40±0.06 | 0.67±0.34 |
| **Heart** | 1.06±0.87 | 0.86±0.56 | 1.05±0.58 | 1.40±0.64 |
| **Lung** | 1.15±0.60 | 1.23±0.82 | 1.67±0.80 | 2.10±0.83 |
| **Kidney** | 1.26±0.87 | 0.98±0.58 | 1.57±0.47 | 1.68±0.49 |
| **Cortex** | 0.66±0.56 | 0.40±0.27 | 1.18±0.76 | 1.13±1.19 |

n=6, Mean Group I Control; Group II $AlCl_3$; *p<0.05 ±SD, Group III *Salacia oblonga*; Group IV *Salacia oblonga*+$AlCl_3$

**Table 3: Estimation of $Mg^{++}$ ATPase activity in *Wistar albino* rats.**

| Name of the tissues | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| **Liver** | 0.07±0.039 | 0.21±0.109 | 0.12±0.072 | 0.14±0.126 |
| **Heart** | 0.76±0.329 | 0.18±0.078 | 0.28±0.243 | 0.33±0.251 |
| **Lung** | 0.22±0.138 | 0.17±0.073 | 0.34±0.210 | 0.30±0.201 |
| **Kidney** | 0.87±0.468 | 0.15±0.064 | 0.26±0.127 | 0.38±0.350 |
| **Cortex** | 0.122±0.09 | 0.070±0.04 | 0.174±0.13 | 0.188±0.22 |

n=6, Mean Group I Control; Group II $AlCl_3$; *p<0.05 ±SD, Group III *Salacia oblonga*; Group IV *Salacia oblonga*+$AlCl_3$

### *Effect of S. oblonga and aluminium on $Ca^{2+}$ATPases activity*

In our study, in group II Al intoxicated rats, there was a substantial decrease in the level of $Ca^{2+}$ATPase in heart, kidney, cerebral cortex and increase in liver and lung tissue homogenates as compared to the control rats. Meanwhile, treatment with S. oblonga extract in group IV rats increased the level of $Ca^{2+}$ATPase in heart, kidney, cerebral cortex and lungs and decreased in liver tissue as compared to group II Al intoxicated rats. The data were shown in (Table 2).

### *Effect of S. oblonga and aluminium on $Mg^{2+}$ATPases activity*

In our study, in group II Al intoxicated rats, there was a substantial decrease in the level of $Mg^{2+}$ATPase in heart, kidney, cerebral cortex, lungs and increase in liver tissue homogenates as compared to the control rats. Meanwhile,

treatment with *S. oblonga* extract in group IV rats increased the level of $Mg^{2+}$ATPase in heart, kidney, cerebral cortex and lungs and decreased in liver tissue as compared to group II Al intoxicated rats. Compared with Control Group I, $Mg^{+}$ activity in other Groups didn't show any statistically significant change however there was an increase of liver, cortex and serum level but in other tissues shows a decreased activity. There is no statistically significant ($p<0.05$) shown in any other group when compared with control.

### *Effect of S. oblonga and aluminium on tissue protein level*

In Group II Al intoxicated rats, there was marked decrease of protein level in liver, heart, lungs and kidney as compared to the group I control rats. However, treatment with S. oblonga extracts in group IV rats substantially decreased the protein level in liver, heart and lungs as compared to the group II Al intoxicated rats.

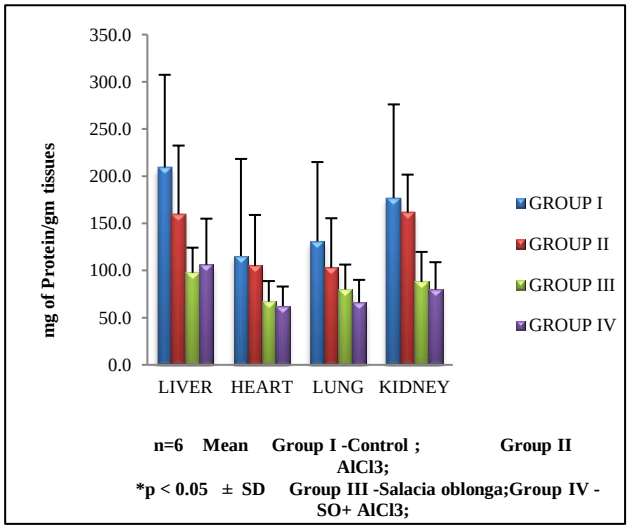


**Figure 1: Estimation of protein in various tissues of *Wistar albino* rats exposed to AlCl3, SO and their combination for 36 days.**

## DISCUSSION

The noxious effects of Al is mainly as a result of pro-oxidant activity which leads to oxidative stress, free radical attack and oxidation of cellular proteins and lipids.[19] In the present study, Al intoxication leads to decreases activity of $Na^{+}$ -$K^{+}$ -ATPase, $Mg^{2+}$-ATPase, and $Ca^{2+}$-ATPase, indicating that $AlCl_3$ exposure induced the intracellular accumulation of $Na_{+}$ and $Ca^{2+}$, leading to the cytotoxic effect and impairment of the cells in the various visceral organs such as heart, lungs, kidney, except the hepatic tissues . Al can directly interfere with –SH groups of enzyme at the active site, thus prevents the -SH groups from functioning in certain chemical reactions.[20] The –SH groups are involved in the maintenance of the membrane-bound Na+ -K+ ATPase oligomeric structure.[10] It indicates that Al lowers the activities of Na+ -K+ -ATPase, Ca2+-ATPases, and Mg2+-ATPases by the reduction of –SH groups. In addition, Al perturbed the structure and functions of cell membranes through regulating apical Cl− secretion and ATPase inactivation in class of lipids.[21] $Na^{+}$ -$K^{+}$ -ATPase enzyme presents the cell membrane of all the animals. Thus, $AlCl_3$ can decrease the activity of Na+ -K+ -ATPase by disruption of the membrane. Calcium (Ca) is actively transported into intracellular organelles and out of the cytoplasm by $Ca^{2+}$/$Mg^{2+}$- ATPases located in the endoplasmic reticulum and plasma membranes.[22] A recent investigation showed that disintegration of endoplasmic reticulum was observed when young gerbils were injected intraperitoneally with $AlCl_3$ for 5 weeks.[23] Thus, the decrease of $Ca^{2+}$/$Mg^{2+}$-ATPases attributed to the disintegration of endoplasmic reticulum induced by Al. Al binds to the plasma membrane phospholipids, alters the lipid protein interaction, and modifies the activities of the transporters. $Al^{3+}$ accelerates $Ca^{2+}$ release from mitochondria and strongly inhibits $Ca^{2+}$-ATPase activity.[24] These results indicate that $AlCl_3$ can induce ionic disorders by altering ATPases activity and contribute to multiorgan dysfunction during intoxication. Previous reports conducted in our laboratory show the membrane protective effect of S. oblonga in Al induced oxidative brain damage.[25]

### *Limitations*

Limitations of current study were; this study was carried out in a small group of animals for a short duration. Further clinical research in this area would prove the effects of the plant extract on Aluminium induced visceral toxicity.

## CONCLUSION

In conclusion, the present study suggests that Al causes membrane damage by altering the membrane ATPases level as result of oxidative stress as evident in the enzyme results of various organs. Thus, we conclude that the extracts of Salacia oblonga exerts membrane protection by inducing changes on ATPase enzyme levels of various organs. Further studies in this particular area would help the researchers to understand more about the viscero protective effect of Salacia oblonga in aluminium toxicity.

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