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# Neuroprotective role of *Salacia oblonga* extract against aluminium chloride induced oxidative stress in rat cortex.

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## ABSTRACT

The present study was designed to investigate the possible neuroprotective and antioxidant effects of hydroalcoholic extract of root bark of *Salaciaoblonga* (SOHE). Neurotoxicity and oxidative stress was induced in Albino rats by administration of aluminiun chloride ($Alcl_3$) (300 mg/kgbody weight oral). The hydroalcoholic extract of *Salaciaoblonga* at a dose of 67mg/kg body weight were orally administered at single dose per day to neurotoxicity induced rats for a period of 36 days.The effect of SOHE on tissue and serum antioxidant markers are investigated. In regards to antioxidant activity, extract exhibits a significant effect ($p<0.05$) showing increased effect of enzymatic and non-enzymatic parameters viz, CAT,SOD,GST, GSH and the LPO level is significantly decreased ($p<0.05$) on treatment with SOHE treatment. The blood and cortex acetylcholine esterase (AchE) level are significantly ($p<0.05$) decreased in SOHE treated rats which indicates the decrease in aluminum induced neurotoxicity. Thus the results indicates that the extract exhibit the neuro-protective and antioxidant activity through correction of oxidative stress.

**Abbreviations :** $Alcl_3$, aluminium chloride; CAT, catalase; SOD, superoxide dismutase; GST, glutathione s transferase; GSH, glutathione reduced; LPO, lipid per-oxidation; AchE, acetly choline esterase.

**Keywords:** SOHE, Oxidative stress, aluminium, Anti oxidant, Lipid per oxidation (LPO).

## INTRODUCTION

*Salaciaoblonga* is also known as Saptrangi, Ponkoranti. It is widely distributed in India and other Southeast Asian countries. The Roots and stems of *Salaciaoblonga* have been used extensively in Ayurveda and traditional Indian Medicine for the treatment of Diabetes. It also possesses an antimutagenic, anti inflammatory and nephroprotective property[1,2]. A recent study shows that the extract of *Salaciaoblonga* enhanced the activities of antioxidant enzymes such as superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx) and restored glutathione reductase (GSH) levels and also reduced the formation of free radicals during oxidative stress [2,3].

Oxidative stress produces reactive oxygen species (ROS) due to electron leakage from enzymes involved in the mitochondrial electron transport chain there by damaging DNA and cytosolic membrane bound macromolecules. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system that significantly delays or inhibits oxidation of that substrate [4]. Antioxidant enzymes catalyze the free radical quenching reactions [5].

Aluminium is the third most abundant metal and makes up about 8% of the Earth's Crust[6]. It is found mainly in food products and drinking water, where it is derived from both natural sources and from treatment methods[7]. Al is confirmed to be a neurotoxin and implicated in several neurodegenerative diseases including Alzheimer's disease (AD), most frequent form of senile dementia, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS)[8,9,10,11].

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A study with paramagnetic resonance spectroscopy showed that higher amount of free radicals burden is seen in post mortem in frontal cortex tissue and another study showed that there is significant rise in lipid per oxidation and superoxide free radical in the temporal cortex brain samples of AD patients[12]. Al salts reported to cause cell depletion in hippocampus, isocortex and degenerates cholinergic terminals in cortical areas[13] .Chronic administration of aluminium chloride resulted in marked oxidative stress as indicated by increase in lipid per oxidation, and decrease in antioxidant activity in rat brain [14,15]. Recent findings suggested that administration of aluminium was found to increase acetyl cholinesterase in mouse and rat brain[15,16].

Hence the present study was an attempt to assess the efficacy of SOHE against aluminium induced alterations in the biochemical parameters including free radicals, AchE enzyme activities, and antioxidant levels in serum and brain cortex of wistar albino rats.

## MATERIALS AND METHODS

### Plant extract

The hydro alcoholic extract of *Salaciaoblonga* 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 extract was dark brown powder and stored in refrigerator at 4°C.

### Chemical

Aluminium chloride was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals and reagents used were of analytical grade.

### Animals

Wistar strain male albino rats of about 120–200 gm are used for this study.

All animal experiments were performed after obtaining prior approval from the Institutional Animal Ethical Committee (IAEC) governed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.

**1. Acute Toxicity Study**

This acute toxicity study was designed as per the OECD Guidelines for Testing of Chemicals, Acute Oral Toxicity (Acute Toxic Class Method), Guidelines 423.

The animals were divided into control and test groups with six animals each. The control group received the double distilled water (1 ml), while the test group got graded doses of the extracts orally and were observed for mortality till 72 h and the dose was calculated.

**Housing**

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 12h 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, each group comprising of six animals used for 36 days of experimental duration.

**Group 1:** Rats administered orally with 1.0 ml double distilled water daily till the end of experimental period.
**Group 2:** Rats administered orally with aluminium chloride (300 mg/kg b.w) alone.
**Group 3:** Rats administered orally with *Salaciaoblonga* extract (67mg/kg b.w).
**Group 4:** Rats administered orally with both aluminum chloride and *Salaciaoblonga.*

**Treatment 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 tissue samples for Biochemical analysis**

At the end of the experimental period on 36th day, all the animals were anaesthetized under mild ether anesthesia and blood was collected by retro orbital vein puncture. The animals were subsequently sacrificed by cervical decapitation and the tissue (cortex) was excised quickly. The tissue was washed in physiological saline to remove blood clot and other tissue materials.

**Collection of blood sample and separation of serum**

The blood samples were collected in plain centrifuge tubes and were kept in inclined position to allow complete clotting of blood. The tubes were then centrifuged at 2500 rpm for 30minutes. The resultant clear supernatant was pipette out and preserved in small vials in the freezer for the purpose of biochemical investigations.

**Preparation of tissue homogenate:**

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 minutes and supernatant was used as sample for biochemical investigations.

**Biochemical estimations:**

Melanandialdehyde (MDA), a secondary product of lipid peroxidation, was estimated in the plasma and tissue samples utilizing the colorimetric reaction of thiobarbituric acid (TBA). It gives an index of the extent of progress of lipid peroxidation. Since the assay estimates the amount of TBA reactive substance e.g. MDA, it is also known as TBARS (thiobarbituric acid reactive substance) test[17]. AChE is a marker of the loss of cholinergic neurons in the forebrain. The AChE activity was assessed by the Ellman method[18]. The CAT activity was determined according to the method of Sinha[19]. The SOD activity was determined according to the method of Marklund and Marklund[20]. The GSH activity was determined according to the method of Ellman[21]. The GSH activity was determined according to the method of Ellman[21]. The GST activity was determined according to the method of, Habig[22]. The Protein was determined according to the method of Lowry[23].

**Statistical analysis**

All values were expressed as mean±SD. The data were statistically analyzed using one way ANOVA followed by Tukey'sHSD multiple range test and differences below P<0.05 are considered as significant.

**RESULTS**

**Acute toxicity studies**

During acute toxicity testing of the SOHE, no mortality was found up to 2000 mg/ kg in wistar albino rats by oral administration[1].

**Effects on AchE activity:**

The data obtained in the present study showed that the oral administration of $Alcl_3$ for 36 days significantly (p<0.05) increased the activity of AchE in both serum and brain cortex when compared to the control. In contrast, this

**Table; 1 Effect of *SalaciaOblonga on*enzymatic antioxidants in Aluminium induced neuro toxicity in albino rats.SOD (Super Oxide Dismutase); CAT (Catalase); GST (Glutathione S Transferase); GSH (Glutathione Reduced); AChE: (Acetyl Choline Esterase) & Protein in cortex of albino rats.**

| | Control Mean±SD (n=6) | Aluminum Mean±SD (n=6) | Salacia Mean±SD (n=6) | Alu +SO Mean±SD (n=6) |
|---|---|---|---|---|
| TBARS | 10.76±0.94 | 28.80±1.84[a] | 11.09±1.17 | 13.81±1.16[b] |
| SOD | 11.05±1.05 | 7.73±1.03[a] | 16.59±0.99* | 12.63±0.78[b] |
| CAT_A | 4.22±0.51 | 1.24±0.65[a] | 4.18±0.42 | 2.97±0.25[b] |
| GST | 1.36±0.08 | 0.34±0.21[a] | 1.90±0.55* | 1.52±0.03[b] |
| GSH | 3.47±0.33 | 1.16±0.35[a] | 2.99±0.13* | 2.45±0.19[b] |
| PROTEIN | 37.00±1.13 | 63.30±0.77[a] | 39.90±1.28 | 54.26±0.55[b] |
| AChE | 30.79±0.84 | 69.93±4.08[a] | 31.70±1.92 | 45.74±1.21[b] |

**Table ; 2 Effect of *Salacia Oblonga* enzymeic antioxidants in Aluminium induced toxicity in albino rats. SOD (Super Dismutase); CAT (Catalase); GST (Glutathione S Transferase); GSH (Glutathione Reduced); AChE: (Acetyl Choline Esterase) & Protein in serum of albino rats.**

| | Control n=6 mean ± SD | Aluminium n=6 mean ± SD | *Salacia* n=6 mean ± SD | Alu + SO n=6 mean ± SD |
|---|---|---|---|---|
| TBARS | 1.87±0.23 | 5.23±0.25[a] | 1.96±0.14 | 2.43±0.23[b] |
| SOD | 3.44±0.31 | 1.58±0.24[a] | 4.93±0.23* | 3.59±0.43[b] |
| CAT | 2.85±0.23 | 1.07±0.20[a] | 3.40±0.33 | 2.95±0.19[b] |
| GST | 2.59±0.14 | 0.48±0.06[a] | 3.12±0.19* | 2.49±0.34[b] |
| GSH | 6.35±0.27 | 4.00±0.32[a] | 7.46±0.51* | 5.98±0.24[b] |
| PROTEIN | 100.57±3.27 | 64.83±2.06[a] | 105.00±3.73 | 102.94±2.00[b] |
| AChE | 68.9±1.93 | 91.88±3.14[a] | 66.42±2.46 | 78.28±0.79[b] |

*ANOVA followed by Tukey Alpha (0.05) multiple range test Values & the results are expressed as mean ± S.D for 6 rats in each group . The values are not sharing a common superscript differ significantly at P < 0.05,*
*a-(p<0.05) control vs. $Alcl_3$, b- (p<0.05) $Alcl_3$ +SOHE vs. $Alcl_3$,*-(P<0.05) control vs. SOHE,*

*LPO- nM of MDA formed/mg protein,CAT-µM of H202 consumed/min/mg protein,SOD-units/ mg protein/ 50% inhibition of pyrogallol/min,GST-µM/MG PROTEIN,GSH-µM of CDNB conjugated /min/mg protein,GST-mg/protein/gram tissue.*

activity was found to be significantly ($p<0.05$) decreased in $Alcl_3$+SOHE treated group when compared to $Alcl_3$ treated group.

**Effects of lipid peroxidation on serum and cortex**

In the tables 1&2, the analysis of variance indicated that TBAR'S level was significantly ($p<0.05$) increased in $Alcl_3$ treated group when compared to the control group. There was a significant ($p<0.05$) decrease in TBAR's level in SOHE + $Alcl_3$ treated group when compared to the $Alcl_3$ treated group.

**Impact of SOHE on serum and cortex antioxidant status:**

The various antioxidant activities expressed as SOD, CAT, GSH, GST and proteins were significantly decreased ($p<0.05$) in $Alcl_3$ treated group when compared to the normal rats as shown in the table 1&2. There is a significant ($p<0.05$) restoration of these enzymatic activities noted in SOHE + $Alcl_3$ treated groups. Likewise the treatment with SOHE significantly raised the antioxidant levels of SOD ($p<0.05$), CAT ($p<0.05$), GST ($p<0.05$), GSH ($p<0.05$). The protein level was significantly increased in cortex ($p<0.05$) of aluminium treated rats when compared to control and it is significantly decreased in treatment group ($p<0.05$) when compared to the aluminium treated group. In serum the protein level was significantly decreased ($p<0.05$) in aluminium group and increased in the treatment group ($p<0.05$).

## DISCUSSION

*Salaciaoblonga* is an ancient herb that has been safely and widely used in ayurvedic medicine for the oral treatment of many diseases and in this study the hydro alcoholic extract of *Salaciaoblonga* was screened for its property on the influence of aluminium.

The reason for selecting brain cortex is because aluminium very severely affects cortex and hippocampus than any other areas of CNS[24,25].

Aluminium was previously found to be a potent pro-oxidant known to enhance lipid peroxides in the cortex .Al uptake in the brain depends on monocarboxylate transporter located in BBB which transports Al from brain interstitial fluid to blood. Aluminum neurotoxicity is due to the neuronal membrane oxidative damage.The membrane oxidation in turn increases aluminum binding, thus, aggravating oxidation[26]. Brain membranes are made up of polyunsaturated fatty acids, phospholipids components and also molecules with a high content of lipid/protein ratio (like e.g., myelin) supporting the fact that ions without redox capacity can stimulate lipid peroxidation by promoting phase separation and membrane rigidification[27]. Mechanism of inhibition of active oxygen species appear to be important for the treatment of disease. Recently many investigators have studied the ability of various drugs or active compounds from plants to eliminate active oxygen in particular.

Our results shows that $Alcl_3$ significantly ($p<0.05$) increased the AchE activity in serum and brain cortex, which is parallel with other studies[28]. The SOHE was able to reduce AchE level significantly ($p<0.05$) in serum and brain cortex of $Alcl_3$ treated rats, which is recorded as a newer finding. Further studies are required to confirm this data.

Our studies also showed a marked increase in LPO in $Alcl_3$ treated rats, which is parallel with other studies[29,30]. This elevation of TBARS level could be attributed to the ability of aluminum salts to accelerate oxidative damage to biomolecules like lipids, protein and nucleic acids[31].SOHE has been shown to exert a potent scavenging action on superoxide anion as well as protective effect against LPO.

As oxidative damage is mediated by free radicals it is necessary to investigate the status of antioxidants which were the first line of defense against free radical damage. SOD and CAT act against oxygen free radicals such as superoxide ($O_2^-$) and hydroxyl (*'OH)* ions and protect the cellular constituents from damage. In the present study we observed that the antioxidant level (SOD, CAT, GSH, and GST) was found to be decreased in the serum and cortex of $Alcl_3$ group, which may be due to the increased production of free radicals and suppression of antioxidant enzymes which contribute in the oxidative stress. Our findings were consistent with results of several investigators[32]. The SOHE treatment decrease the TBARS level and restored the antioxidant levels in aluminium treated animals and the results are recorded in the table1&2.

## CONCLUSION

The results of the present investigation indicate that the hydroalcoholic extract of *Salaciaoblonga* possesses good antioxidant activity. However its clinical usefulness can be investigated by further studies.

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