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Research Article

Evaluation of antioxidant effect of *Salacia oblonga* against aluminum chloride induced visceral toxicity in albino rats

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ABSTRACT

Background: Aluminum is present in several manufactured foods and medicines and is also used in water purification. It is known that aluminum induces an oxidative stress characterized by an increase in lipid peroxidation and depletion of antioxidants. Therefore, the present experiment was undertaken to determine the effectiveness of hydroalcoholic extract of root bark of *Salacia oblonga* (SOHE) in modulating the aluminum chloride ($AlCl_3$) induced oxidative stress in rats.
Methods: Animals were assigned into four groups: control; $AlCl_3$ 300 mg/kg b.w.; *Salacia* 67 mg/kg; $AlCl_3$ (300 mg/kg b.w.) plus *Salacia* (67 mg/kg b.w.), respectively. Rats were orally administered their respective doses daily for 36 days. The effect of these treatments in influencing the aluminum induced biochemical changes on liver, kidney, lungs, and heart were studied.
Result: The results showed that *S. oblonga* produced significant ($p<0.05$) reduction of malondialdehyde, while the activities of superoxide dismutase, reduced glutathione, glutathione S-transferase and catalase were positively modulated.
Conclusion: Our data demonstrated that *S. oblonga* protects against aluminum-induced oxidative stress, which is an important finding that further reinforces the antioxidant properties of this natural product.

Keywords: Oxidative stress, Antioxidant, Aluminum chloride, *Salacia oblonga*

INTRODUCTION

Aluminum (Al) is the third most abundant element (8%) in the earth's crust. The uptake of aluminum can take place through food, through breathing and by skin contact.[1] Long lasting uptakes of significant concentrations of aluminum can lead to serious health effects; it is a non-essential metal to which humans are frequently exposed. Food ingredients, antacids, buffered vaccines, allergen, injections, food preparations all contain considerable amount of aluminum.[2]

The Agency for Toxic Substances and Disease Registry (ATSDR) reported that aluminum accumulates mainly in bone, liver, testis, kidneys, and brain (ATSDR),[3] 1990. Patients on dialysis tend to accumulate this metal in different organs. The toxicological effects on humans include encephalopathy,[4] bone disease, anemia and skeletal system disease.[5] Aluminum and its salts induce oxidative stress, which is responsible for hepatotoxicity, nephrotoxicity,[4] cardiac toxicity,[6] reproductive toxicity,[5] and also neurodegenerative disease. In a review article, Mohammadirad and Abdollahi recorded a significant increase in lipid peroxidation (LPO) and inhibition of antioxidant enzymes by aluminum in plasma, brain, testes, kidney, renal cortex, serum, erythrocyte, hepatocyte, and liver.[7]

The toxic effect is mediated by free radical generation which results in the oxidative deterioration of cellular lipids, proteins and DNA and also induces changes in the number of tissue antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], etc.).[8]

Hypothetically, since oxidative stress plays a pathogenic role in aluminum toxicity, supplementation with antioxidants should attenuate oxidative stress and improve oxidative stress-mediated damage in aluminum toxicity. Therefore, there is an urgent need to identify effective antioxidants with therapeutic potential to ameliorate aluminum toxicity.

Antioxidants play an important protective role against the (reactive oxygen species) ROS. Plants are the essential and integral part in treatment strategy against metal toxicity as they form secondary metabolites such as proteins, flavonoids, alkaloids, steroids, and phenolics which in turn are used to restore health and heal many diseases.

Salacia oblonga, a woody climbing plant of the family Celastaceae, is widely distributed in India and other Southeast Asian countries. The genus *Salacia* has been used particularly for the treatment of diabetes, gonorrhea, rheumatism, pruritus, and asthma.[9] It is known to have several medicinal properties such as hypoglycemic, hypolipidemic, anti-inflammatory, anti-oxidant, etc.[10] However, the antioxidant capabilities of *S. oblonga* root extracts against aluminum chloride induced oxidative stress on liver, kidney, heart and lungs has not been found in the literature. Hence, the present investigation attempts to evaluate the antioxidant effect of hydroalcoholic extract of roots of *S. oblonga* against aluminum chloride induced visceral (liver, heart, lungs, and kidney) toxicity in Wistar albino rats.

METHODS

Plant extract

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

Chemical

Aluminum 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 g are used for this 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.

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 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, 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 until the end of the experimental period.
Group 2: Rats administered orally with aluminum chloride (300 mg/kg b.w.) alone.
Group 3: Rats administered orally with *S. oblonga* extract (67mg/kg b.w.).
Group 4: Rats administered orally with both aluminum chloride and *S. oblonga.*

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 behavior. 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 sacrificed by cervical decapitation and the tissues (liver, kidney, lungs, and heart) were excised quickly. The tissues were washed in physiological saline to remove blood clot and other tissue materials.

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 min and the supernatant was used as sample for biochemical investigations.

Biochemical estimations

Melondialdehyde (MDA), a secondary product of LPO, was estimated in the plasma and tissue samples utilizing the colorimetric reaction of thiobarbituric acid. It gives an index of the extent of progress of LPO.[11] The CAT activity was determined according to the method of Sinha.[12] The SOD activity was determined according to the method of Marklund and Marklund.[13] The glutathione (GSH) activity was determined according to the method of Ellman.[14] The glutathione S-transferase (GST) activity was determined according to the method of, Habig et al.[15] The Protein was determined according to the method of Lowry et al.[16]

Statistical analysis

All values were expressed as mean ± standard deviation. Data were statistically analyzed using one-way ANOVA followed by Tukey's HSD multiple range test and differences below $p<0.05$ are considered as significant.

RESULTS

The effects of aluminum chloride and *Salacia* on the selected biochemical parameters in the liver, kidney, lungs, and hearts of rats are represented in Table 1-4.

The MDA levels in liver, kidney, heart, and lungs of Al treated rats were significantly ($p<0.05$) increased when compared to control groups. Coadministration of *Salacia* and aluminum significantly ($p<0.05$) lowered the MDA levels when compared to aluminum treated groups.

In the liver and kidney, the antioxidant levels (SOD, CAT, GST, GSH) of aluminum treated rats were significantly decreased ($p<0.05$) when compared to control. Concurrent administration of both *salacia* and aluminum groups shows a significant increase in the antioxidant level ($p<0.05$) when compared to the aluminum treated group.

In the heart, the antioxidant levels were significantly decreased in aluminum group when compared to control group (CAT, GST, GSH-[$p<0.05$], SOD – [$p>0.05$]). However, in the treatment group the antioxidant levels were significantly increased when compared to aluminum group (SOD-$p<0.05$), CAT, GST, GSH-($p>0.05$).

In lungs the level of antioxidant decreased in aluminum group (GSH-[$p<0.05$], SOD, CAT, GST-$p>0.05$). In the treatment group, the antioxidant levels were significantly increased when compared to aluminum treated rats (GSH-[$p<0.05$], SOD, CAT, GST-$p>0.05$). There is no significant difference was noted between control and *Salacia* treated group and its show that *Salacia* doesn't produce any harm to rats.

DISCUSSION

Aluminum has many adverse effects on human health. ATSDR reported that Al accumulates mainly in liver, kidney, testis, brain, and bone causes oxidative stress. It is mainly due to the pro-oxidant effect of Al. The treatment commonly used in aluminum disorders is desferrioxamine, which is a chelator

Table 1: Effect of SOHE on LPO and antioxidant levels in liver of control and experimental rats.

	Control	Aluminum	Salacia	$Alcl_3$ + Salacia
TBARS	2.68±0.42	5.18±0.76[a]	2.54±0.47	3.39±0.37[b]
SOD	3.27±0.57	1.65±0.63[a]	4.3±0.54	3.00±0.10[b]
CAT	9.36±0.67	5.22±0.68[a]	10.59±0.39	6.85±0.75[b]
GST	2.51±0.51	1.84±0.29[a]	2.78±0.67	2.09±0.40[b]
GSH	8.84±0.79	6.67±0.74[a]	8.94±0.46	7.84±0.40[b]

ANOVA followed by Tukey Alpha (0.05) multiple range test values & the results are expressed as mean±SD 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 versus $Alcl_3$, [b]$p<0.05$ $Alcl_3$, + SOHE versus $Alcl_3$. LPO - nM of MDA formed/mg protein, SOD - units/mg protein/50% inhibition of pyrogallol/min, CAT - µM of H202 consumed/min/mg protein, GST - µM/mg protein, GSH - µM of CDNB conjugated/min/mg protein

Table 2: Effect of SOHE on LPO and antioxidant levels in kidney of control and experimental rats.

	Control	Aluminum	Salacia	$Alcl_3$ + Salacia
TBARS	1.81±0.25	3.98±0.39[a]	1.83±0.24	3.07±0.33[b]
SOD	4.12±0.34	2.67±0.07[a]	4.2±0.85	3.05±0.14
CAT	8.57±0.09	5.26±0.39[a]	9.80±0.56	7.08±0.64[b]
GST	4.11±0.15	2.60±0.44[a]	4.56±0.39	3.32±0.31[b]
GSH	5.41±0.39	2.84±0.28[a]	5.17±0.24	3.98±0.17[b]

ANOVA followed by Tukey Alpha (0.05) multiple range test values & the results are expressed as mean±SD 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 versus $Alcl_3$, [b]$p<0.05$ $Alcl_3$, + SOHE versus $Alcl_3$. LPO - nM of MDA formed/mg protein, SOD - units/mg protein/50% inhibition of pyrogallol/min, CAT - µM of H202 consumed/min/mg protein, GST - µM/mg protein, GSH - µM of CDNB conjugated/min/mg protein

Table 3: Effect of SOHE on LPO and antioxidant levels in lung of control and experimental rats.

	Control	Aluminum	Salacia	$Alcl_3$ + Salacia
TBARS	2.57±0.27	3.50±0.33[a]	2.65±0.20	3.37±0.34
SOD	0.96±0.43	0.566±0.22	0.96±0.31	0.65±0.17
CAT	1.37±0.43	0.85±0.27	1.50±0.37	0.96±0.22
GST	1.80±0.49	1.39±0.50	2.35±0.81	1.60±0.49
GSH	5.40±0.39	2.83±0.28[a]	8.98±0.46	3.97±0.17[b]

ANOVA followed by Tukey Alpha (0.05) multiple range test values and the results are expressed as mean±SD 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 versus $Alcl_3$, [b]$p<0.05$ $Alcl_3$, + SOHE versus $Alcl_3$. LPO - nM of MDA formed/mg protein, SOD - units/mg protein/50% inhibition of pyrogallol/min, CAT - µM of H202 consumed/min/mg protein, GST - µM/mg protein, GSH - µM of CDNB conjugated/min/mg protein

Table 4: Effect of SOHE on LPO and antioxidant levels in the heart of control and experimental rats.

	Control	Aluminum	Salacia	$Alcl_3$ + Salacia
TBARS	2.37±0.31	2.98±0.36[a]	2.35±0.40	2.21±0.37[b]
SOD	1.09±0.62	0.60±0.32	1.10±0.53	0.69±0.20
CAT	2.06±0.488	1.34±0.41[a]	2.41±0.47	1.61±0.41
GST	1.51±0.28	0.78±0.15[a]	2.23±0.26	1.03±0.41[b]
GSH	5.98±0.60	5.03±1.16[a]	6.06±0.67	5.70±0.40

ANOVA followed by Tukey Alpha (0.05) multiple range test values and the results are expressed as mean±SD 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 versus $Alcl_3$, [b]$p<0.05$ $Alcl_3$, + SOHE versus $Alcl_3$. LPO - nM of MDA formed/mg protein, SOD - units/mg protein/50% inhibition of pyrogallol/min, CAT - µM of H202 consumed/min/mg protein, GST - µM/mg protein, GSH - µM of CDNB conjugated/min/mg protein

that decreases the aluminum body burden by increasing its excretion in the urine.[17] However, it is only efficient when it is applied intravenously or subcutaneously and it has been shown that desferrioxamine therapy has side-effects that are often not well tolerated and seen to be expensive treatment. Thus, its application limits the success of this therapy.[18]

S. oblonga is a perennial herb grown in some areas of India and Sri Lanka. For at least 4000 years, *Salacia* plants have been used in the traditional Ayurvedic system of medicine to manage several common ailments. It also knows to have several medicinal properties such as hypoglycemic, hypolipidemic, anti-inflammatory, anti-oxidant, etc.[10]

Therefore, the present study was undertaken to determine whether hydroalcoholic extract of root bark of *Salacia oblonga* (SOHE) can prevent Al-induced oxidative stress by examining different biochemical parameters of oxidative damage in the liver, kidney, heart, and lungs in rats.

Our result showed that aluminum administration enhanced the LPO in liver, kidney, heart, and lungs, but caused a significant decline in the antioxidant such as SOD, CAT, GST, and GSH levels. SOHE treatment decrease the TBARS level and restored the antioxidant levels in the aluminum treated animals.

Gibanananda & Hussain observed that the improper balance between reactive oxygen metabolites and antioxidant defense results in "oxidative stress".[19] Oxygen derived free radicals generated in excess in response to various stimuli could be cytotoxic to several tissues. Under normal physiological conditions, low concentrations of lipid peroxide are found in plasma and tissues. The most abundant oxidative free radicals generated in living cells are superoxide anions and hydroxyl radical which induces peroxidation of the cell membrane lipids. The enzymatic antioxidant defense system such as SOD and CAT serves as a first line of defense against oxidative stress and protect the cellular constituents against oxidative damage. SOD is extensively used as a biochemical indicator in pathological condition associated with oxidative stress.[20] GSH serves as a cofactor for GST, which helps to remove drugs, chemicals and reactive molecules from cells.

In the present study, we observed that oral administration of $Alcl_3$ elevate the LPO in liver, kidney, heart and lungs, of rats. Our results are in agreement with other authors that a significant increase in TBARs in the liver and kidney after aluminum intoxication.[21,22] This elevation of LPO in the liver and kidney was evident by the increased production of TBARS, which suggests participation of free-radical induced oxidative cell injury in mediating the toxicity of Al.[21] Abubakar et al., ascertained that even small quantities of aluminum in hepatocytes associated with an increase in ROS and peroxidation.[23]

In the present study, $AlCl_3$ induce the formation of free radicals and also inhibit the enzyme involve in the antioxidant defense. Participation of iron in Fenton reaction *in vivo*, leading to production of more reactive hydroxyl radicals from superoxide radicals and H_2O_2 results in increased LPO.[24] This might be one of the reasons for significant alteration in LPO and significant changes in the activity of antioxidant enzymes, observed in the present study.

In our result, the level of antioxidant such as SOD, CAT, GSH, and GST levels were found to be decreased in aluminum treated group. This is due to the accumulation of free radicals, which causes failure of antioxidant defense system and result in tissue injury. Our result where in parallel with other studies.[1,21] Experimental animal models and cell culture studies reveal that aluminum affects the expression of SOD, CAT, GSH peroxidase and GSH possibly leading to membrane fragility as a consequence.[25]

SOHE treatment decrease the TBARS level and restored the antioxidant levels in Liver, kidney, lungs and heart of aluminum treated animals. Kumaran & Karunnakaran, 2007 reported that, alkaloids have also been reported to strongly inhibit LPO induced in isolated tissues through its antioxidant activity.[26] The protection offered by the extract could have been due to the presence of alkaloids. Thus, the extract activates the antioxidant enzymes and thereby protects the tissues from oxidative damage induced by aluminum chloride.

The above finding supported our hypothesis that SOHE could have both antioxidant activity and free radical scavenging property which could be tested in humans as newer drug molecule.

CONCLUSION

Our results reported that $AlCl_3$ capable of causing oxidative damage and inhibited the activities of antioxidant enzymes. SOHE has been shown to exert a potent scavenging action on free radicals, protective effect against LPO, restore the antioxidant level and hence possess good antioxidant effect. Attention should pay toward the use of Al in foods, water, and medical drugs.

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