ALOE VERA PROTECTS THE ALUMINIUM INDUCED CHANGES IN TESTICULAR ENZYMES ACTIVITY OF ALBINO RATS, RATTUS NORVEGICUS

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ABSTRACT

The current study was carried out to investigate the protective role of Aloe vera plant extract on aluminium induced changes in testicular enzymes of albino rats. Aloe vera is a medicinal plant belonging to the family –Liliaceae, which has a wide range of therapeutic applications such as wound healing, diabetes, burns, for easing intestinal, curing ulcers and arthritic swellings. 30 adult rats were taken and divided into 3 groups 10 (5+5) for each. Control group animals Animals were fed with normal diet and water ad-libitum, as Group I or control group. Group II animals were fed with normal diet and received aluminium in a dose of 98 mg/kg of body weight orally for 30 and 60 days. Group III were fed with normal diet and received aloin (100mg/kg body weight) and aluminium sulphate (98 mg/kg body weight) for 30 and 60 days. On the last day of the experiment animals were sacrificed by cervical dislocation on 30th and 60th days respectively. Testes were removed and homogenized in buffer, homogenate was centrifuged and supernatant was used for further analysis. The results of the present study clearly indicated that aluminium sulphate has significantly altered the normal levels of testicular LPO and testicular GSH of rat testicular enzymes. But after co treatment of rats with aloin the extract of Aloe vera and aluminum sulphate, the levels of testicular LPO, and testicular GSH reached near to normal level, indicating the protective role of aloin against aluminium sulphate toxicity.

KEYWORDS: Aluminium Toxicity, Aloe vera, Albino Rats, estimation of rat testis enzymes, testicular LPO, testicular GSH.
INTRODUCTION
Aluminium (Al), the third most common element approximately 8% of total mineral components in the earth’s crust found combination with oxygen, silicon, fluorine and other elements in the soil, rocks, clays and gems has a significant toxic potential for humans.\[1\] Aluminium enters the human body via food, air, water and drugs and is present in many manufactured foods such as processed cheese, baking powders, cake mixes, frozen dough, pancake mixes\[2\] and pharmaceutical products, especially antacids.\[3\] It has the potential to cause neurological disorders in human and animals, it’s accumulation in the brain has been linked to various neurodegenerative diseases.\[4,5\] Aluminium forms complex with ATP (Al-ATP) as it has strong affinity for phosphate ion, which is 107 times stronger than Mg 2+ ion. Therefore, it can be hypothesized that the synthesis of GSH hindered due to the less availability of ATP by altering the synthesis of g-Glutamylcysteine (g-GluCys) synthetase and glutathione synthetase, enzymes involved in GSH synthesis. Thus decreased activity of glutathione synthetase leads to reduced GSH level.\[6\] Several recent reviews have provided evidence for adverse effects of these metals on certain reproductive parameters like sperm motility, viability and count, histology of testis and epididymis, as well as reproductive hormone levels at various exposure levels.\[7\] Aluminium ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals and causes alterations in histology of testis\[8,9\], deterioration in spermatogenesis and sperm quality, enhancement of free radicals and alterations in antioxidant enzymes\[10,11,12\]; interruption in sex hormone secretion.\[13,14\] The toxic effects of aluminium appear to be mediated, at least in part, by free-radical generation.\[15\]

Plants have been used to treat various diseases and have been an exemplary source of medicine over the years.\[16\] It has been reported that plant extracts detoxify various kinds of environmental pollutant.\[17\] Aloe vera is one such ancient plant it’s medicinal properties have been known since centuries and has wide range of therapeutic applications such as wound healing effect, reduction of blood sugar in diabetes, for soothing burns, for easing intestinal, for curing ulcers and for reducing arthritic swellings.\[18,19\] A. vera gel contains anthroquinones (aloin, aloe-emodin) which may have a variety of properties of anti oxidant agent, including the protective role for heavy metal toxicity.\[20,21,22\] The goal of this study was to investigate the protective role of Aloe vera on aluminium induced changes in testicular enzymes of albino rats.
MATERIALS AND METHODS
Healthy adult male albino rats (*Rattus norvegicus*) weighing 175 ± 5 gm were used for the experiments, procured from Mhow, Bhopal (MP) India, and maintained in our laboratory. The rats were acclimatized in laboratory conditions for two weeks and were maintained at 28 ± 2°C room temperature and relative humidity (60 ± 10%) with a 12 hours light-dark cycle in the animal house of biotechnology laboratory, Saifia Science College, Bhopal. Food and water were provided *ad libitum* throughout the experiment to avoid effects of starvation. No mortality was observed during the acclimatization period and during whole experimentation period up to 60 days.

Collection and preparation of plant materials for experiment
*Aloe vera* plant leaves were used for the present study. Leaves of *Aloe vera* were collected in and around the Bhopal. Preparation of *A. vera* (leaf gel) extract was done according to the method of Arunkumar and Muthuselvam\textsuperscript{[23]} with slight modifications. Skin of the leaves were peeled and the gel inside was used for extraction. 100 gms of the gel was added to 250 mL of ethanol and extracted using the Soxlet assembly. Later on, the solvent of the extracted material was removed at low temperature in a rotary vacuum evaporator and the resulting dried extract was lyophilized in a freeze dryer.

Experimental design
All the experimental animals were divided into three groups as group I, II and III.

**Group I:** - This group of 10 (5+5) animals was fed with normal diet and water *ad libitum*, as control group.

**Group II:** - This group of 10 (5+5) animals was fed with normal diet and aluminium in a dose of 98 mg/kg of body weight orally for 30 and 60 days.

**Group III:** - This group of 10 (5+5) animals were fed with normal diet and received aloin (100mg/kg body weight) and aluminium sulphate (98 mg/kg body weight) for 30 and 60 days. Animals were sacrificed by cervical dislocation on 30\textsuperscript{th} and 60\textsuperscript{th} days respectively. Testes were removed and homogenized in buffer, homogenate was centrifuged and supernatant was used for further analysis.

Reproductive (Testis) tissue preparation
The control and Al with and without *Aloe vera* treated groups were sacrificed and their testes were harvested immediately. The testes was weighed, rinsed with ice-cold de ionized water, and dried with filter paper. Fractions of testes were homogenized in buffer. The homogenate
was centrifuged at 600 × g for 10 min and re-centrifuged at 13,000 × g for 20 min at 4 C to obtain a post nuclear homogenate and post mitochondrial supernatant fractions. The resultant supernatant was used for further analysis.

**Enzyme analysis of rat testes**

The experimental chemicals were obtained from Sigma Chemical Co. USA of analytical grade. The following Testicular enzymes i.e. Testicular LPO and Testicular GSH from rat Testis of aluminium sulphate per se exposed and Aloe vera plus aluminium sulphate exposed and non exposed (Control) was assayed as per the following standard procedures. Testicular LPO activity of control as well as experimental rats were assayed as per the method of Ohkawa et al., (1979)\(^{[24]}\) Testicular GSH activity was assayed as per the method of Flohe and Gunzler., (1979).\(^{[25]}\)

**RESULTS**

In the present investigation, analyses of testicular enzyme were done in albino rats subjected to different durations of aluminium sulphate administration. The values of testicular enzymes (i.e. testicular LPO, testicular GSH) of albino rats exposed to aluminium sulphate per se and in combination with aloin for a period of 30 days and 60 days with well matched controls are reported (See table 1 to 4 and figure 1 to 4).

**Testicular enzyme analysis**

**Testicular LPO**

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. In the present study the value of testicular LPO was found to be 2.298±0.0729 n mol/mg/protein after 30 days of exposure with aluminum sulphate, in comparison to the control value of 0.622 ± 0.0145 nmol/mg/protein found in rats having no toxic exposure (Table 1, Figure 1).

**Table 1: Showing the values of testicular LPO (n mol/mg/protein) of the aluminum sulphate (98 mg/ kg of body weight) per se and in combination with aloin for a period of 30 days with well matched controls.**

<table>
<thead>
<tr>
<th>Variables (N)</th>
<th>Mean ± SE</th>
<th>P-Value</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.622 ± 0.0145</td>
<td></td>
</tr>
<tr>
<td>Alum Per se</td>
<td>2.298 ±0.0729</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Aloin +Alum</td>
<td>1.430 ±0.1277</td>
<td>0.0002***</td>
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</tbody>
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Figure 1: Showing the values of testicular LPO (n mol/mg/protein) of the aluminum sulphate (98 mg/kg of body weight) *per se* and in combination with aloin for a period of 30 days with well matched controls.

When the period of exposure was further increased up to 60 days, the value of testicular LPO was further increased up to 3.298 ± 0.07146 n mol/mg/protein in comparison to the control value of 0.5408 ± 0.1045 n mol/mg/protein.

In an another set of experiment when the rats were treated with aloin the extract of *Aloe vera* after aluminum sulphate intoxication, the value of LPO was found to be reduced up to 1.726 ± 0.1225 nmol/mg/protein in compared to the aluminum *per se* value of 3.298 ± 0.07146 nmol/mg/protein (Table 2 and Figure 2).

Table 2: Showing the values of testicular LPO (n mol/mg/protein) of the aluminum sulphate (98 mg/kg of body weight) *per se* and in combination with aloin for a period of 60 days with well matched controls.

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<tr>
<td>Control</td>
<td>0.5408 ± 0.1045</td>
<td></td>
</tr>
<tr>
<td>Alum <em>Per se</em></td>
<td>3.298 ± 0.07146</td>
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Figure 2: Showing the values of testicular LPO (n mol/mg/protein) of the aluminum sulphate (98 mg/kg of body weight) per se and in combination with aloin for a period of 60 days with well matched controls.

The data presented in table 1 and 2 indicated that exposure to the aluminum sulphate led to significant increase in testicular LPO level in both 30 and 60 days treated group of rats as compared to the control group. It is intrusting here to mention that the effect of aluminum sulphate on testicular LPO is more conspicuous in 30 days treated group in comparison to the 60 days treated group. But after co treatment of rats with aloin the extract of Aloe vera and aluminum sulphate the LPO level was get reduced and reached near about the control level.

**Testicular GSH**

Effects of aluminum sulphate alone and in combination with standard aloin the extract of Aloe vera on testicular GSH level in male rat Rattus norvegicus, during 30 days experimental period has shown in table 3 and figure 3. The data obtained from the experiments clearly indicated that after aluminum sulphate intoxication testicular GSH level was decreased drastically in 30 days exposed animals where the value was found to be 7.057 ± 0.3164 n mol/mg/protein which is quite less than the control value of 16.68 ± 0.5766 mol/mg/protein (Table 3 and Figure 3).
Table 3: Showing the values of testicular GSH (n mol/mg/protein) of the aluminum sulphate (98 mg/kg of body weight) *per se* and in combination with aloin for a period of 30 days with well matched controls.

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<tr>
<td>Control</td>
<td>16.68 ± 0.5766</td>
<td></td>
</tr>
<tr>
<td>Alum <em>per se</em></td>
<td>7.057 ± 0.3164</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Aloin +Alum</td>
<td>8.896 ± 0.1860</td>
<td>&lt;0.0001****</td>
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On the other hand, after co-treatment of aloin and aluminum sulphate, decline in testicular GSH level was stopped and slightly increment was observed as compared to the aluminum sulphate *per se* treated group where the value was found to be 8.896 ± 0.1860 n mol/mg/protein, which is higher than the *per se* treated value of 7.057 ± 0.3164 n mol/mg/protein but less than the control value of 16.68 ± 0.5766 n mol/mg/protein. When the period of exposure was increased up to 60 days, more severely decline in GSH level was observed where there the value was found to be 3.147 ± 0.1254 which is more prominent in compared to the 30 days treated group. But when aloin the extract of *Aloe vera* was given to rats after aluminum sulphate intoxication for 60 days the value was elevated up to 8.808 ± 0.5730 n mol/mg/protein (Table 4 and Figure 4).
Table 4: Showing the values of testicular GSH (n mol/mg/protein) of the aluminum sulphate (98 mg/ kg of body weight) *per se* and in combination with aloin for a period of 60 days with well matched controls.

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<th>Variables (N)</th>
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<tr>
<td>Control</td>
<td>16.62 ± 0.2642</td>
<td></td>
</tr>
<tr>
<td>Alum <em>per se</em></td>
<td>3.147 ± 0.1254</td>
<td>&lt;0.0001****</td>
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Figure 4: Showing the values of testicular GSH (n mol/mg/protein) of the aluminum sulphate (98 mg/ kg of body weight) *per se* and in combination with aloin for a period of 60 days with well matched controls.

The above data regarding to the GSH content of rat testis clearly demonstrated that the level of testicular GSH were highly reduced in significant manner after aluminum sulphate treatment for 30 days and 60 days in comparison to the control animals. But the effect of aluminum sulphate on GSH content was more prominent in 60 days of treated group when compared it with 30 days treated group.

In addition to this, the combination treatment of aloin the extract of *Aloe vera* and aluminum sulphate showed less decrease in GSH level in comparison to the aluminum sulphate *per se* treated group. This is also indicated that aloin the extract of *Aloe vera* has properties to minimize the toxic effect of aluminum sulphate.
DISCUSSION

Testicular Lipid Peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids and is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in the cell damage. The disorders of reproduction and hazards to reproductive health and associated functions have become prominent issues in recent decades after reports on adverse effects of certain chemicals and metals used in our daily life. As spermatozoa are rich in polyunsaturated fatty acids they are highly susceptible to membrane lipid peroxide ions (Muanya et al., 2008).[26]

Similar to our present study, increased lipid peroxidation and reduced level of antioxidant capacity of the testis in metal treated rats have earlier indicated an increased free radical generation (Sikka, 1995[27]; 1996.[28] Increased ROS formation due to lipid peroxidation and a compromised antioxidant defence system have also shown to be associated with mid-piece abnormalities and decreased sperm counts in several animal models including human beings (Thiele et al., 1995).[29]

Looking to the importance of this bio marker of reproductive damages, caused by metals and chemicals, the analysis of testicular lipid per oxidation with and without treatment of aluminium sulphate has been done. In the present study the value of testicular LPO was found to be 2.298±0.0729 n mol/mg/protein after 30 days of exposure with aluminium sulphate, in comparison to the control value of 0.622 ± 0.0145 nmol/mg/protein found in rats having no toxic exposure (Table 1 and Figure 1). When the period of exposure was further increased up to 60 days, the value of testicular LPO got further increased up to 3.298 ± 0.07146 nmol/mg/protein in comparison to the control value of 0.5408±0.1045 nmol/mg/protein (Table 2 and Figure 2).

In an another set of experiment when the rats were treated with aloin after aluminium sulphate intoxication, the value of LPO was found to be reduced up to 1.726 ± 0.1225 nmol/mg/protein in compared to the aluminium per se value of 3.298 ± 0.07146 nmol/mg/protein (Table and Figure 1,2).

The data presented in Table and Figure 1,2 indicated that exposure to the aluminium sulphate led to significant increase in testicular LPO level in both 30 and 60 days treated group of rats as compared to the control group. It is interesting here to mention that the effect of
aluminium sulphate on testicular LPO is more conspicuous in 30 days treated group in comparison to the 60 days treated group. But after co treatment of rats with aloin and aluminium sulphate the LPO level was reduced and almost reached near the control level. The data of the present investigation have confirmed that lipid peroxidation plays a significant role in the etiology of defective reproduction due to aluminium intoxication, leading to sperm dysfunctions, as seen in the present work.

**Testicular GSH**

Glutathione (GSH), a tripeptide present in the majority of cells, is responsible for hydrophilic xenobiotics conjugation, it is a master anti oxidant which serves many vital physiological functions including protection of cells from reactive oxygen species (ROS), detoxification of exogenous compounds and amino acid transport (Kojima-Yuasa *et al.*, 2005)[30]; Mendoza-Cózatl *et al.*, 2005).[31] Sulphydryl group of glutathione is essential for its antioxidant activity against some forms of ROS in cells (Cnubben *et al.*, 2001)[32], much of the pathology is associated with the decrease in intracellular GSH concentration (Rouach *et al.*, 1997).[33] Therefore, GSH concentration is important for survival of the cells. It is also a substrate for glutathione peroxidase. Many studies indicate that heavy metals act as catalysts in the oxidative reactions of biological macromolecules therefore the toxicities with these metals might be due to oxidative tissue damage (Stohs and Bagchi, 1993)[34]; Hultberg *et al.*, 1999[35]; Cuypers *et al.*, 1999[36]; Leonard *et al.*, 2004[37]; Flora *et al.*, 2008.[38]

In the present study, important biochemical marker of aluminium pollution has been studied and the effects of aluminium sulphate alone and in combination with standard aloin on testicular GSH level in male rat *Rattus norvegicus*. The data obtained from the experiments clearly indicated that after aluminium sulphate intoxication testicular GSH level got decreased drastically in 30 days exposed animals where the value was found to be 7.057 ± 0.3164 n mol/mg/protein which is quite less than the control value of 16.68 ± 0.5766 mol/mg/protein. On the other hand after co treatment of aloin and aluminium sulphate, decline in testicular GSH lever was stopped and slightly increment was observed as compared to the aluminium sulphate *per se* treated group, where the value was found to be 8.896 ± 0.1860 n mol/mg/protein, which is higher than the *per se* treated value of 7.057 ± 0.3164 n mol/mg/protein but less than the control value of 16.68 ± 0.5766 n mol/mg/protein (Table 3 and Figure 3).
When the period of aluminium exposure was increased up to 60 days, more severely decline in GSH level was observed where the value was found to be 3.147 ± 0.1254 which is more prominent as compared to the 30 days treated group. But when aloin was given to rats after aluminium sulphate intoxication for 60 days the value was elevated up to 8.808 ± 0.5730 nmol/mg/protein (Table 4 and Figure 4). The above data regarding to the GSH content of rat testis clearly demonstrated that, the level of testicular GSH were highly reduced in significant manner after aluminium sulphate treatment for 30 days and 60 days in comparison to the control animals.

The results of the present investigation clearly indicated that aluminium sulphate significantly altered the normal levels of testicular LPO and testicular GSH enzyme of rat testis. The levels of LPO was increased in Aluminium sulphate per se treated group, but in co treated group the elevated levels of LPO reached to near normal level. In another set of experiment the levels of testicular GSH enzyme highly decreased in both treated groups. But in co treatment group of aloin and aluminium sulphate, decline in testicular GSH level was stopped and slightly increment was observed as compared to the aluminium sulphate per se treated group, indicating protective role of aloin the extract of Aloe vera against aluminium sulphate toxicity.

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REFERENCES


