ABSTRACT

The present study was carried out to investigate the protective role of Aloe vera plant extract on aluminium induced changes in liver 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. Animals were fed with normal diet and water adlibitum, as Group I or control group. Group II animals were fed with normal diet and received aluminium sulphate 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. Liver was 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 elevated the normal levels of liver enzymes such as Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP). But after cotreatment of rats with aloin the extract of Aloe vera and aluminum sulphate, the levels of above mentioned liver enzymes 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 liver enzymes, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP).

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 (Verstraeten et al., 2008). Consumption of processed food and water purified using Al-containing additives is the main route for this metal to enter the human body (Newairy et al., 2009). Chronic exposition to Aluminium can cause alterations in skeletal, nervous, hematopoietic and respiratory systems (Plieth et al., 1999; Afifi, 2002; Chen et al., 2002; Campbell, 2002). Aluminum accumulation in the liver is associated with a number of biochemical changes which include the release of enzyme markers of liver injury, and alteration in the oxidant status (Abubakar et al., 2003, Banasik et al., 2005). Several studies have shown that Al may induce changes in the activity of a number of biological antioxidants such as superoxide dismutase, catalase and glutathione peroxidase/reductase (Moumen et al., 2001). Significant increase in the activity of ALT and AST in the plasma of AlCl3-intoxicated rats due to impaired liver function. Exposure to AlCl3 caused necrosis to the liver with the subsequent release of AST from the injured hepatic cells to the plasma Chinoy and Memon (2001) and El-Demerdash (2004). AlCl3 caused a significant elevation in the activity of ALP Ochmanski and Barabasz (2000) and El-Demerdash (2004). Increase in the activity of ALP attributed severe damage to cell membranes or increased permeability of plasma membrane Esmaeili et al., (2009). Chronic exposure to aluminium is involved in neuro-degenerative disorders, such as Alzheimer’s disease (Flora et al., 2005) dialysis, Parkinson’s dementia (Hirsch et al., 1991) and hepato-toxicity (Yumoto et al., 1998, Muhammad et al, 2014 Crane R.K. 2014).

Plants have been used to treat various diseases and have been an exemplary source of medicine over the years (Ates et al., 2003). It has been reported that plant extracts detoxify various kinds of environmental pollutant (Salt et al., 1998). 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.
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 (Flora et al., 2005; Yadav et al., 2009; Zubaydi et al., 2009). The goal of this study was to investigate the protective role of Aloe vera on aluminium induced changes in liver 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 *Aloe vera* (leaf gel) extract was done according to the method of Arunkumar and Muthuselvam (2009) 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 30th and 60th days respectively. Liver was removed and homogenized in buffer, homogenate was centrifuged and supernatant was used for further analysis.
Liver tissue preparation
Liver tissues were minced and homogenized (10%, w/v) separately in ice cold 1.15% KCl-0.01 M sodium potassium phosphate buffer (pH 7.4) using a polytrone. The homogenate was centrifuged at 10,000 x g for 20 minutes at 4°C and the supernatant was used for enzyme assay.

Enzymes estimation method
Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activity of control group as well as experimental rats were assayed as per the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) level activity was assayed as per the method of Bergmeyer (1963).

RESULTS
Enzyme analysis of rat Liver
In the present investigation, analysis of liver enzymes were done in albino rats subjected to different durations of aluminium sulphate administration. The values of liver enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) of 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 below (table 1 to 6 and figure 1 to 6).

Alanine aminotransferase (ALT)
Alanine aminotransferase is best indicator enzyme to assess liver toxicity. In the present study the level of alanine aminotransferase enzyme was found to be elevated in aluminum sulphate per se treated group, where its level significantly increased from a control value of 45.26 ±1.258 u/lit to 77.94 ± 1.364 u/lit after 30 days treatment (Table 1, Figure 1). In co treatment group (aloin plus aluminium sulphate) ALT levels were decreased and the value found to be 48.16 ±0.7026 u/lit, which is quite low in comparison to aluminum sulphate per se treated group (Table 1, Figure 1).
Table 1: Showing the values of Alanine aminotransferase (ALT, u/l) units of the aluminium sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 30 days along with co treatment with aloin.

<table>
<thead>
<tr>
<th>Variables (N)</th>
<th>Mean ± SE</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.26 ± 1.258</td>
<td></td>
</tr>
<tr>
<td>Alum Per se</td>
<td>77.94 ± 1.364</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Aloin +Alum</td>
<td>48.16 ± 0.7026</td>
<td>0.4670</td>
</tr>
</tbody>
</table>

Figure 1: Showing the values of alanine aminotransferase (ALT, u/l) units of the aluminum sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 30 days along with co treatment with aloin.

When the duration of exposure was increased up to 60 days the level of ALT was further increased in aluminum sulphate per se treated group, where the value was found to be 77.94 ± 1.364 u/lit in comparison to control value 45.26 ±1.258 u/lit (Table 2; Figure 2). In co treated group (aloin plus aluminum sulphate), ALT level was decreased and the value found to be 44.16 ± 0.7026 u/lit which is near to normal the control value, indicating protective action of aloin the extract of Aloe vera against aluminum toxicity.

Table 2: Showing the values of Alanine aminotransferase (ALT, u/l) units of the aluminium sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 60 days along with co treatment with aloin.

<table>
<thead>
<tr>
<th>Variables (N)</th>
<th>Mean ± SE</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.26 ± 1.258</td>
<td></td>
</tr>
<tr>
<td>Alum Per se</td>
<td>77.94 ± 1.364</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Aloin +Alum</td>
<td>44.16 ± 0.7026</td>
<td>0.4670</td>
</tr>
</tbody>
</table>

Figure 2: Showing the values of alanine aminotransferase (ALT, u/l) units of the aluminum sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 60 days along with co treatment with aloin.
Aspartate aminotransferase (AST)
Aspartate aminotransferase is also another indicator enzyme to assess liver toxicity. In the present study the level of aspartate aminotransferase enzyme were evaluated. The level of aspartate aminotransferase enzyme was highly elevated after 30 days intoxication of male rat by aluminum sulphate. The value increased form the control value of 79.92 ± 1.618 u/lit to 144.3 ± 2.892 u/lit indicating significant damage in liver tissues by aluminum sulphate (Table 3, Figure 3).

Table 3: Showing the values of aspartate aminotransferase (AST, u/l) of the aluminium sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 30 days along with co treatment with aloin.

<table>
<thead>
<tr>
<th>Variables (N)</th>
<th>Mean ± SE</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>79.92 ± 1.618</td>
<td></td>
</tr>
<tr>
<td>Alum Per se</td>
<td>144.3 ± 2.892</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Aloin +Alum</td>
<td>93.94 ± 1.513</td>
<td>&lt;0.0001****</td>
</tr>
</tbody>
</table>

Figure 3: Showing the values of aspartate aminotransferase (AST, u/l) of the aluminum sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 30 days along with co treatment with aloin.

In another set of experiment when the time of exposure was increased up to 60 days it was found that AST level further increased in aluminium per se treated group and the value was found to be 149.42 ± 1.18 u/lit comparing with control group value 80.11 ± 0.918 u/lit (Table 4, Figure 4).

Table 4: Showing the values of aspartate aminotransferase (AST, u/l) of the aluminium sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 60 days along with co treatment with aloin.

<table>
<thead>
<tr>
<th>Variables (N)</th>
<th>Mean ± SE</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80.11 ± 0.918</td>
<td></td>
</tr>
<tr>
<td>Alum Per se</td>
<td>149.42 ± 1.18</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Aloin +Alum</td>
<td>95.81 ± 1.21</td>
<td>&lt;0.0001****</td>
</tr>
</tbody>
</table>
Figure 4: Showing the values of aspartate aminotransferase (AST, u/l) of the aluminum sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 60 days along with Co-treatment with aloin.

In co treated group (aloin plus aluminum sulphate) for 60 days, AST level was decreased and the value found to be 95.81 ± 1.21 u/lit in comparing with aluminium per se treated group, indicating protective role of aloin against aluminum toxicity towards liver cells.

Alkaline phosphatase (ALP)

Alkaline phosphatase is an important diagnostic tool in diseases like hepatitis, biliary obstruction, hyper parathyroidism. The value of alkaline phosphatase in the present study were found to be elevated in 30 days of aluminum sulphate per se treated group in which the value was increased from the control value of 161.7 ± 1.690 u/lit to 252.6 ± 6.658 u/lit (Table 5, Figure 5). Whereas in co treated group the value was found to be 180.5 ± 2.660 u/lit be which is lying in between control and aluminum sulphate treated group.

Table 5: Showing the values of alkaline phosphatase (ALP, u/l) units of the aluminium sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 30 days along with co treatment with aloin

<table>
<thead>
<tr>
<th>Variables (N)</th>
<th>Mean ± SE</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>161.7 ± 1.690</td>
<td></td>
</tr>
<tr>
<td>Alum Per se</td>
<td>252.6 ± 6.658</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Aloin +Alum</td>
<td>180.5 ± 2.660</td>
<td>0.0001***</td>
</tr>
</tbody>
</table>

Figure 5: Showing the values of alkaline phosphatase (ALP, u/l) units of the aluminum sulphate (98 mg /kg of body weight) per se treated rats and well matched controls for a period of 30 days along with co treatment with aloin.
In another set of experiment when the time of exposure was increased up to 60 days it was found that ALP level further increased in aluminum *per se* treated group and the value was found to be 257.4 ± 3 u/lit of tissue in comparison with control group value 159.18 ± 0.919 u/lit. In co treated group (aloin along with aluminum sulphate) for 60 days ALP level was found to be 195.5 ± 3.815 u/lit (Table 6, Figure 6).

Table 6: Showing the values of alkaline phosphatase (ALP, u/l) units of the aluminium sulphate (98 mg /kg of body weight) *per se* treated rats and well matched controls for a period of 60 days along with co treatment with aloin.

<table>
<thead>
<tr>
<th>Variables (N)</th>
<th>Mean ± SE</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>159.18 ± 0.919</td>
<td></td>
</tr>
<tr>
<td>Alum <em>Per se</em></td>
<td>257.4 ± 3.829</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>Aloin +Alum</td>
<td>195.5 ± 3.815</td>
<td>&lt;0.0001***</td>
</tr>
</tbody>
</table>

Figure 6: Showing the values of alkaline phosphatase (ALP, u/l) units of the aluminum sulphate (98 mg /kg of body weight) *per se* treated rats and well matched controls for a period of 60 days along with Co-treatment with aloin.

It was observed that in co treated groups for 30 days and 60 days experiment (aloin plus and aluminum sulphate) ALP level was found to be decreased comparing with aluminum sulphate *per se* treated group, indicating protective role of aloin the extract of *Aloe vera* against aluminum sulphate toxicity.

**DISCUSSION**

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels in liver

Aminotransferases are a group of enzymes that catalyze the process of biological transamination forming major enzymes. They represent a link between carbohydrate, fat and protein metabolism providing a source of keto acids for Kreb's cycle and gluconeogenesis.
Aminotransferases importance in amino acid metabolism and gluconeogenesis was reported by Knox and Greengard (1965).\(^{[28]}\) In the present investigation, both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are very good indicator enzymes for assessing liver toxicity in response to aluminum treatment per se as well in combination with aloin in rats for both 30 and 60 days of exposure.

The data obtained from the 30 days and 60 days experiments clearly indicate that after aluminum sulphate intoxication, the level of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels in liver increased. In co treated groups (aloin plus aluminium sulphate) for 30 days and 60 days, it was observed that ALT and AST levels were restored in both short and long term treatments with aloin and got reduced almost coming near the control levels (Table & Figure 1 to 4 & 1 to 4).

Mohamed et al., (2010)\(^{[29]}\) reported the protective effect of neem (Azadirachta indica) leaves extract which has proven to be an antioxidant to protect against mercury-induced oxidative stress and hepatotoxicity in male albino Sprague Dawley rats. Hepatotoxicity was assessed by these workers by measuring the level of marker enzyme AST and ALT. They have observed that mercuric chloride (HgCl\(_2\)) in 2 mg/kg body weight concentration caused significant elevation in level of AST and ALT which was further reduced and reached to control level when rats were treated with neem extract (Mohammad et al., 2010).\(^{[29]}\)

The findings of the recent study of Tantaway (2015)\(^{[30]}\) have also demonstrated that, lead induced toxicity in male rats significantly increases serum ALT and AST activities as compared to their corresponding controls. His finding that administration of Spirulina supplement caused a significant decrease in serum ALT, AST activities, reversing the toxic effects of lead gives full support to our present data, where it has been demonstrated that aloin administration reverses the increased levels of ALT and AST.

**Alkaline Phosphatase (ALP) level in liver**

In the present study, the data obtained from the 30 days and 60 days experiments clearly indicate that after aluminum sulphate intoxication, the level of ALP increased. In co treated groups (aloin plus aluminium sulphate) for 30 days and 60 days, it was observed that ALP level was restored in both short and long term treatments with aloin and got reduced almost coming near the control levels (Table & Figure 5,6).
Our findings are well supported by Mohamed et al., (2010)\cite{29} reported the protective effects of neem (*Azadirachta indica*) leaves against mercury-induced oxidative stress and hepatotoxicity in male albino Sprague Dawley rats. Similarly, Anuradha and Krishnamoorthy (2012)\cite{31} have observed protective effects of the methanolic extract of *Pongamia pinnata* flowers against lead acetate induced hepatotoxicity in rats. The above referred workers reported that the elevated ALP level was reduced and reached control level after administration of plant extracts. John et al., (2014)\cite{32}, who have reported that treatment with aqueous extract of *Coriandrum sativum* L of 100 and 200mg/kg of body weight produced a well pronounced protective effect on cadmium intoxicated rats, where they had observed that administration of cadmium showed an elevated level of serum marker enzyme ALP, which was due to the severe hepatic damage, as seen in the present study in relation to aluminum in the present work.

The results of the present study clearly indicated that aluminium sulphate *per se* treated has significantly elevated the normal levels of liver enzymes such as Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels in liver and Alkaline phosphatase (ALP). But after co treatment of rats with aloin the extract of *Aloe vera* and aluminum sulphate, the levels of above mentioned liver enzymes reached near to normal level, indicating the protective role of aloin against aluminium sulphate toxicity.

**ACKNOWLEDGEMENT**

Authors are grateful to Principal Saifia College of Science Bhopal and Secretary, Saifia Education Society Bhopal, for providing necessary facilities and encouragement.

**REFERENCES**


