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Aloin from *Aloe vera* leaves: A potential natural aluminium detoxificant

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ABSTRACT

Aluminium is a known potent environmental nephrotoxin causing progressive biochemical changes in the kidney. The herb *Aloe barbadensis* is commonly known as *Aloe vera,* belongs to the family of *Liliacea.* It has been widely used in traditional system of medicines and its active compound has many therapeutic potentials. The present study has evaluated the nephroprotective effect of *Aloe vera* and aloin in aluminium sulphate exposed rats for a period of 45, 90 and 180 days. Aloin from *Aloe vera* leaf extract was isolated and characterised by HPTLC methods. Serum creatinine, urea and uric acid levels were found to significantly increased (p<0.05) after treatment of $Al_2(So_4)_3$ in group II compared to control group I animals fed with normal diet. Co treatment with $Al_2(So_4)_3$ and *Aloe vera* extract (group III) and $Al_2(So_4)_3$ and aloin (group IV) showed significant decrease (p<0.05) in creatinine, urea and uric acid. So, our present study has demonstrated that *Aloe vera* and aloin was effective in reducing Al toxicity in kidney. Hence, *Aloe vera* and its active compound aloin can be used as adjuvant therapy for the prevention and management of aluminium sulphate induced renal damage.

KEY WORDS: *ALOE VERA*, ALUMINIUM, CREATININE, UREA AND URIC ACID

INTRODUCTION

Heavy metals exist in our environment both naturally and from pollution. Some of them are very toxic and ranked as human carcinogens. Accordingly, Aluminium (Al) is a systemic toxic metal known for multiple domestic, industrial, medical and technological applications that contribute to its wide distribution in the environment. Aluminium exposure to human beings occurs through different routes. Common routes of exposure include inhalation, oral, and skin. Exposure is more common in people working in Al industries. The promi-

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nent use of Al cookware results in ingestion of some quantity of Al every day. Al is a component of some widely used medications including sucralfate, phosphate binders, and some vaccines. It is also found in preservative, emulsifying agents, colorants, and baking powders. Such widespread use of Al in consumable and non consumable items will eventually lead to entry and deposition in human body, (Gura, 2010; Mitkus *et al*., 2011; Riihimaki and Antero, 2012; Shaw *et al*., 2013; Kramer and Heath, 2014; Sjogren *et al*., 2015; Mahor and Ali, 2015; Exley, 2016; Jakkala *et al*., 2016; Weidenhamer *et al*., 2017; Gouda *et al*., 2018; Mahor and Ali *et al*., 2018;Yan *et al*., 2019).

Aluminium does not have any physiological role in the body but upon ingestion it gets stored in human organs such as liver, lungs, kidney and brain. Due to its atomic size and electric charge similar to important elements of our body like calcium, magnesium and iron, it acts as competitive inhibitors of them and causes severe damage. Additionally, it triggers generation of reactive oxygen species (ROS), and depletes the cellular antioxidant capacity. An imbalance of antioxidant pool affects cellular organelles, antioxidant enzymes, and damages membranes, DNA, proteins, and finally destroys the tissues. Therefore, exogenous administration of antioxidant substances would have a beneficial effect on the cells' antioxidant system to combat aluminium intoxication. In accordance, there are growing interests in using natural compounds to treat aluminium nephrotoxicity, (Garcia *et al*., 2010; Jing *et al*., 2011; Abdel Moneim, 2012; Shaw and Tomljenovic, 2013; Mardani *et al*., 2014; Exley and Mold, 2015; Jakkala and Ali *et al*., 2015; 2016; Tahir *et al*., 2016; Stahl *et al*., 2017; Khan and Strand, 2018; Haese *et al*., 2019 Mahor and Ali 2019).

Aloe vera L. (*Aloe barbendensis* Miller) is an important medicinal plant which belongs to the family *Liliacea*. *Aloe vera* plant grows readily in hot and dry climate but due to its cosmetic demand, it is cultivated on a large scale irrespective of climatic conditions. It is traded in medicinal drug market for an extensive range of therapeutic applications including wound healing effect, reduction of blood sugar, soothing burns, easing intestinal problems, reducing arthritis swelling. Many studies reports protective effect of *Aloe vera* and some of its bioactive compounds especially aloin, also called barbaloin is a bitter tasting yellow crystal found in *Aloe vera*. It is the most important anthraquinone glycoside claimed to be responsible for beneficial effects of *Aloe vera*, (Herrera *et al*., 2010; Yebpella *et al*., 2011; Ali *et al*., 2012; Lad and Murthy, 2013; Jakkala and Ali *et al*., 2015; 2016; Vieira *et al*., 2016; Minjares *et al*., 2017; Yavari *et al*., 2018; Mahor and Ali 2018, 2019; Shi *et al*, 2019).

To our knowledge, this is the first study to evaluate *Aloe vera* effects against aluminium induced nephrotoxicity in rat (*Rattus norvegicus*). Therefore, this study aims to investigate the potential protective effects of *Aloe vera* and its active compound aloin against kidney damage induced by subchronic administration of Aluminium sulphate.

MATERIALS AND METHODS

Chemicals and Drugs

In this study, Al-sulphate $(Al_2So_4)_3$ was purchased from Aldrich chemical Company (St. Lousis mo, USA) and Standard Aloin ($C_{21}H_{22}O_9$) was obtained from Sigma. The diagnostic kits required for enzymatic assays were purchased from Span Diagnostics. All other chemicals used in the experiment were of analytical grade. The dose of Al-sulphate $(Al_2So_4)_3$ was 98.3mg $(Al_2So_4)_3$ /L (1/25 using Probit analysis based LD_{50}). The dose of A.vera extract and Aloin were 100 mg/kg bw.

Collection and identification of plant material

The fresh leaves of *A.vera* (*Aloe barbadensis*) were collected from the Minor Forest Produce Processing and Research Centre (MFP-PARC) Van Parisar, Barkhera Pathani, Bhopal, (M.P.) India. The plant was authenticated by Dr. Zia-Ul-Hassan Head of the Department of Botany at the Saifia College of Science Bhopal, (M.P.) India and the voucher specimen (403/Saifia/Bot/16) has been deposited at the Herbarium of the Saifia Science College, Bhopal, (M.P.) India.

Preparation of extracts

After collection and weighing, fresh leaves of *Aloe vera* were washed with distilled water to remove dirt and dried under shade separately. The extraction of *A. vera* leaves was done according to the method (Kumar and Muthuselvam, 2009). Slight modification, Skin of the leaves were pealed and the gel inside was used for extraction. 100 gm of the gel was added to 250ml of ethanol and extracted using the Soxhlet 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.

Quantitative estimation of aloin in Aloe vera extract

Chromatographic separation of extracts of *A. Vera* was performed on 20 cm x 10 cm aluminium backed HPTLC plates coated with 200 µm layers of silica gel 60F254 (E. Merck, Darmstadt, Germany). Before use, the plates were pre washed with methanol and activated at 110°C for 5 min. Both test and standard samples (5µL each) were applied on to HPTLC plates as 6 mm wide bands and 12 mm apart from middle of bands by spray-on technique along with nitrogen gas supply for simultaneous drying

of bands, by means of a Camag Linomat V auto sample applicator fitted with a 100 µL syringe (Hamilton, Bonaduz, Switzerland).

A constant spot application rate of 150 nL was used. Plates were developed to a distance of 165 mm, in the dark, with 30 mL ethyl acetate, methanol and water (10:1:4:1) for aloin, as mobile phase. Before development the chamber was saturated with mobile phase for 15 min at room temperature (25 ± 2°C) and 50% relative humidity. Chromatography was performed in Camag's twin-trough chamber. Wavelength for detection of aloin was evaluated from complete UV spectrum of aloin. To calculate the concentration of aloin in each sample loaded, following equation was used as developed by Sharma *et al.*, (2012).

Volume made x concentration x total solubility/ weight of dried extract x sample loaded x 1000.

Maintenance of animals and approval of protocol

Healthy adult male albino rats (*Rattus norvegicus*) weighing 120-150g were used for the present investigation. They were housed in a clean polypropylene cage and maintained in an air-conditioned experimental room at 12-hour light: dark cycles. The animals were acclimatized to laboratory condition for one week prior to experiment. Standard pellets were used as a basal diet during the experimental period. The control and experimental animals were provided with purified drinking water *ad libitum*. The animals were maintained in accordance with the "CPCSEA guidelines for laboratory animal facility" (Committee for the Purpose of Control and Supervision on Experiments on Animals) and the approval number is CPCSEA Registration number SSC/06-06-22/CPCSEA, dated 26/10/2006. Before starting the experiment the animals were carefully marked on different parts of their body, which was later used as identification mark for a particular animal, so that the response of a particular mouse prior to and after the administration could be noted separately.

Acute oral toxicity studies

A.vera extract at the dose range of 100–2500 mg/kg body weight were administered by oral gavage method on different group of mice comprised of 6 rats in each group. Animals were kept under close observation for 4 hours after administering the fraction for behaviour, neurological and autonomic profile and then observed for any change in the general behaviour and physical activities; mortality was recorded within 72 hours. Acute toxicity was determined according to the method of Lorke, (1983).

Animal Grouping /Induction of Toxicity/Experimental design

A total of 24 male (2 months old) Albino rats (*Rattus norvegicus*) weighing 120-150g were used for the present investigation. The animals were divided into four groups (6 rats/ group): Group I:-was kept as control without giving any treatment. Compared to adult controls, Group II: - animals in this group were given 17±6 ml of water supplemented with Al-sulphate to consume, corresponding to 98 mg of Al per day (Jakkala and Ali *et al.*, 2015; 2016) for 45, 90 and 180 days. Group III: - This group animals were fed with normal diet and received aluminium sulphate (98 mg/ kg body weight) and *Aloe vera* extract (100mg/kg body weight) for 45, 90 and 180 days. Group IV: - these group animals were fed with normal diet and received aluminium sulphate (98 mg/kg body weight) and Aloin (100mg/kg body weight) for 45, 90 and 180 days.

Collection of Blood Sample

Blood samples were collected by orbital sinus puncture method (Hui *et al.*, 2007). Serum was separated by following procedure. Blood samples were withdrawn from orbital sinus using non heparinised capillary tubes, collected in dried centrifuge tubes and allowed to clot. Serum was separated from the clot by centrifuged at 3000 rpm for 15 min. at room temperature.

Biochemical Assays

Determination of Serum Creatinine, Urea and Uric acid

Serum Creatinine, Urea, Uric acid levels were assayed using reagent kits purchased from Biosystems (Spain), following methods of Young (1995); Kaplan (1984) and Fossati *et al.*, (1980) respectively.

STATISTICAL ANALYSIS OF DATA

Statistical analysis was performed using Graph Pad Prism 5 software (Graph Pad Software, San Diego, CA). All parameters results were expressed as mean ± standard error (SEM) and all the statistical comparisons were made by means of the one-way ANOVA test, followed by Turkey's test post hoc analysis. A P value <0.05 was considered significant.

RESULTS

In the chromatogram of *Aloe vera* extract, many well resolved spots were observed, out of these spots one spot matched with the Rf value shown by standard aloin (0.76). The results of percentage of aloin found in samples are shown in Table 1.

It was observed that all four groups of rats received the following treatment schedule: shows the significant change in all three parameters discussed here. After 45 days (Group II) showed a significant ($P<0.05$) increase in the level Creatinine, Urea, Uric acid to Al toxicity compared to group I. whereas significant ($P<0.05$) decrease

Table 1. Percentage of aloin found in *Aloe vera* extract

S. No.	Sample	Rf	Amount of sample applied (ng/spot)	Amount of aloin (%)
1	Aloe vera extract	0.76	600	44.41
2	Aloe vera extract	0.76	800	59.31
3	Aloe vera extract	0.76	1000	63.10
4	Aloe vera extract	0.76	1200	65.56

in Creatinine, Urea and Uric acid level was reported in group III and group IV (Fig: 1).

After 90 days (Group II) showed a significant ($P<0.05$) increase in the level of Creatinine, Urea, Uric acid to Al toxicity compared to group I. whereas significant ($P<0.05$) decrease in Creatinine, Urea and Uric acid level was reported in group III and group IV, (Fig: 2).

It was observed that Al toxicity enhances compared to 45, 90 days. It means Al on long term exposure induces toxicity in group II whereas *A. vera* extract and aloin was also effective in reducing Aluminium sulphate toxicity, significant ($P<0.05$) decrease in kidney function test (Creatinine, Urea and Uric acid) studied after 180 days and last study (180 days) show that in group II Creatinine, Urea and Uric acid level significant ($P<0.05$) increase compare to normal (control) group I and whereas group III and group IV showed significant ($P<0.05$) decrease, (Fig.3).

DISCUSSION

Aluminium is one of the trace elements with toxic effect on living organism. However, in recent years, increased attention is being focused on possible adverse effects of aluminium on human health. The present study reveals that the administration of aluminium sulphate significantly ($P<0.05$) enhanced the levels of creatinine, urea and uric acid. Significantly ($P<0.05$) elevated creatinine, urea and uric acid were observed in aluminium sulphate fed rats (group II) when compared with control (group I). The rise in creatinine, urea and uric acid may indicate aluminium toxicity in kidney function. This is in

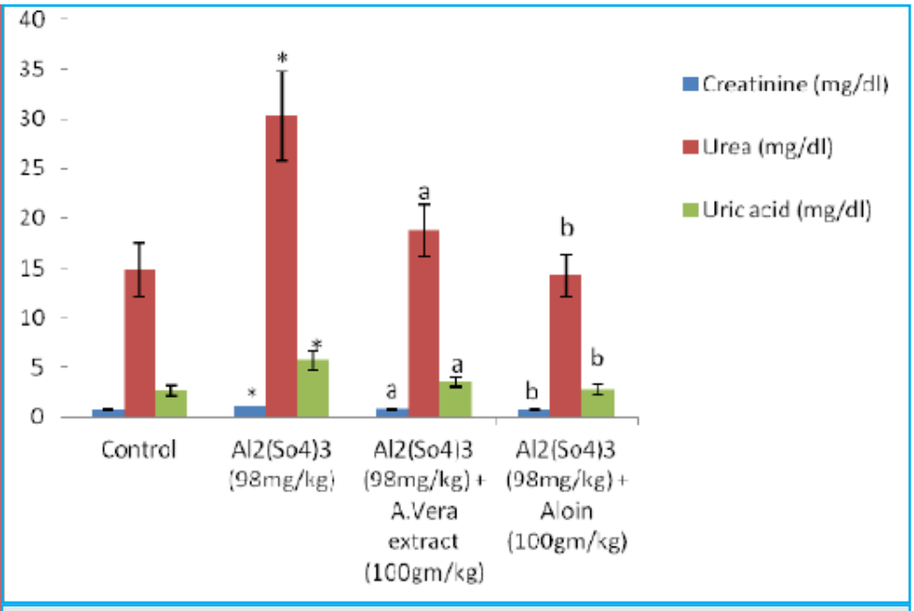

FIGURE 1. Graph showing variation in different parameters level of Creatinine, Urea and Uric acid against Aluminium sulphate (98mg/kg/bw) induced toxicity after 180 days.
Results are expressed as mean ± S.E., $P<0.05$ was considered to be statistically significant.
* Significantly different form control group.
a Significantly different form Aluminium sulphate treated group.
b Significantly different form Aluminium sulphate + *Aloe vera* extract treated group.

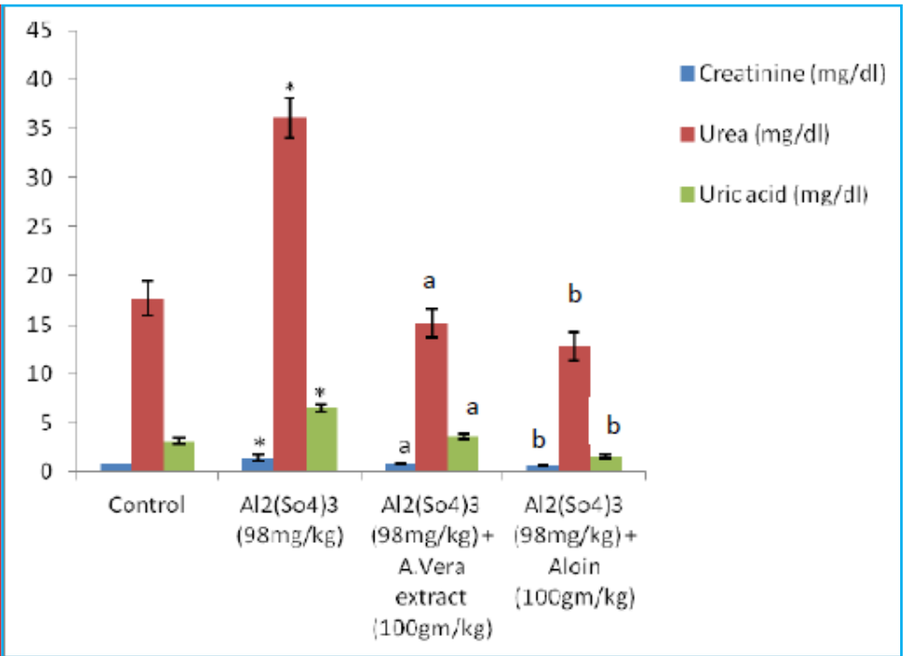

FIGURE 2. Graph showing variation in different parameters level of Creatinine, Urea and Uric acid against Aluminium sulphate (98mg/kg/bw) induced toxicity after 180 days.
Results are expressed as mean ± S.E., $P<0.05$ was considered to be statistically significant.
* Significantly different form control group.
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b Significantly different form Aluminium sulphate + *Aloe vera* extract treated group.

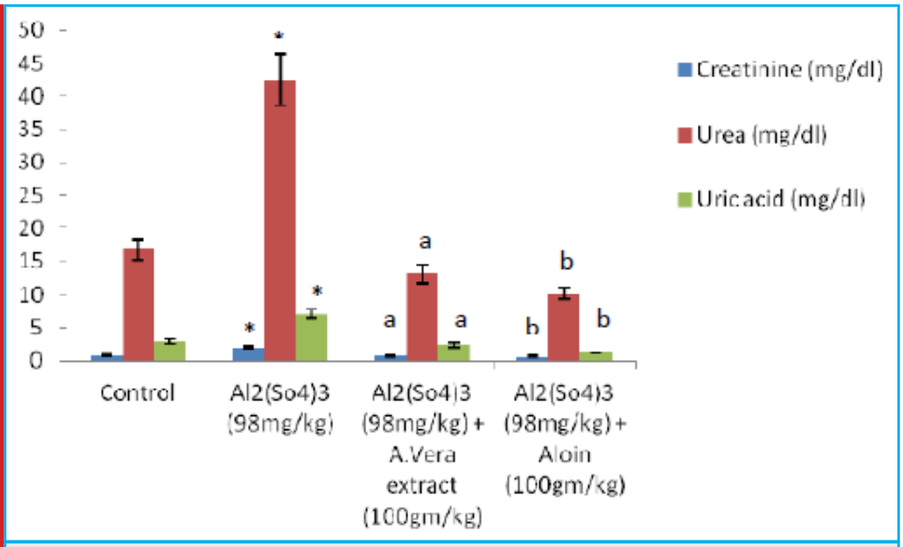

FIGURE 3. Graph showing variation in different parameters level of Creatinine, Urea and Uric acid against Aluminium sulphate (98mg/kg/bw) induced toxicity after 180 days.
Results are expressed as mean ± S.E., $P<0.05$ was considered to be statistically significant.
* Significantly different form control group.
a Significantly different form Aluminium sulphate treated group.
b Significantly different form Aluminium sulphate + *Aloe vera* extract treated group.

consonance with the recent investigation of Ajibade *et al.*, (2019) and Yousef *et al.*, (2019) in which there were biochemical changes observed in the kidney of adult Wistar rats when fed with aluminium chloride. Significant increase ($p<0.05$) in serum urea and creatinine were observed.

In the present study, significant decreased ($p<0.05$) level of creatinine, urea and uric acid were observed in aluminium sulphate and *Aloe vera* extract fed rats (group III) when compared to group II animals. These results are in agreement with the study of Belaid-Nouira *et al.*, (2013; Miraj *et al.*, 2015) using other plant, they have found that fenugreek seeds showed effectiveness in restoring normal plasma values of urea, creatinine in the kidney injured by aluminium chloride. Several other studies using *Aloe vera* plant, also support our findings concluded that *Aloe vera* extract showed nephroprotective effect against heavy metal toxicity (Iftikhar *et al.*, 2015; Hussain *et al.*, 2016). In our study, administration of aloin showed significant decreased ($p<0.05$) level of creatinine, urea and uric acid when compared to group III animals. This is analogous to the study of Al Dera, (2016) who has proposed that standard resveratrol when administered with aluminium chloride showed significant ($p<0.05$) decreased in serum creatinine and urea. In our previous study it has been showed that aloin also significantly ($p<0.05$) reduced in Total cholesterol, triglyceride, HDL and LDL (Mahor and Ali, 2018).

In agreement with previous studies, results from this study revealed that aluminium induced nephrotoxicity is indicated by significant ($p<0.05$) increase in creatinine, urea and uric acid. But, co treatment of aluminium sulphate and *Aloe vera* extract and Aluminium sulphate and aloin showed significant ($p<0.05$) reduction in creatinine, urea and uric acid. This suggests that *Aloe vera* and its active compound aloin is very potent in preventing aluminium toxicity in kidney.

CONCLUSION

Based on the findings of present work, it can be concluded that the *A. vera* extract and aloin was effective in reducing Al toxicity in kidney function tests (Creatinine, Urea and Uric acid). Deeper study is needed using histological analysis for gaining better pharmacological information and intervention.

CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

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