AMELIORATION OF THE TOXIC EFFECTS OF ALUMINIUM INDUCED HISTOPATHOLOGICAL CHANGES IN TESTIS OF ALBINO RATS BY ALOE VERA

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ABSTRACT
The present study was carried out to investigate the protective role of Aloe vera plant extract of aloin on aluminium induced degenerative changes in male reproductive system 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. In this experiment 30 Adult male rats were divided into 3 groups 10 (5+5) for each. Group I animals were fed with normal diet and water ad libitum, were called 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. While examination of testis sections, Aluminium treated group per se showed abnormal characteristic architecture compared with the control group. Animals treated with aluminium along with the aloin showed recovery of testis tissue structure and was more prominent in long time treatment in comparison to the short time treated group.

KEYWORDS: Aluminium Toxicity, Aloe Vera, Albino Rats, Testis and Histopathology.

INTRODUCTION
Aluminium, the third most abundant element of the Earth’s crust, is a non-essential and toxic metal in humans.[1] Aluminium enters the human body via food, air, water and drugs, aluminium ware and containers and is present in many manufactured foods such as processed
cheese, baking powders, cake mixes, frozen dough, pancake mixes\textsuperscript{[2,3]} and pharmaceutical products, especially antacids.\textsuperscript{[4]} Particulate matters distributed by cement -producing factories contain high amount of Aluminium and populations residing in the vicinity are exposed to the pollution.\textsuperscript{[5,6]} Aluminium has a negative impact on human health.\textsuperscript{[7]} Aluminium causes histopathological responses in liver, kidney, intestine and testis of albino mice.\textsuperscript{[8]} 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.\textsuperscript{[9]} 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 tests\textsuperscript{[10,11]}, deterioration in spermatogenesis and sperm quality, enhancement of free radicals and alterations in antioxidant enzymes\textsuperscript{[12,13,14]}; interruption in sex hormone secretion.\textsuperscript{[15,16]} The toxic effects of aluminium appear to be mediated, at least in part, by free-radical generation.\textsuperscript{[17]} The treatment commonly used in aluminium disorders is desferrioxamine.\textsuperscript{[18]} However, therapy is associated with undesirable side effects, it is very expensive and it is only efficient when applied intravenously or subcutaneously.\textsuperscript{[19]}

Plants have been used to treat various diseases and have been an exemplary source of medicine over the years.\textsuperscript{[20]} It has been reported that plant extracts detoxify various kinds of environmental pollutant.\textsuperscript{[21]} \textit{Aloe vera} is one such ancient plant has a 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.\textsuperscript{[22]} \textit{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.\textsuperscript{[23,24,25]} The goal of this study was to investigate the protective role of \textit{Aloe vera} on aluminium induced degenerative changes in male reproductive system testes of rats.

**MATERIALS AND METHODS**

Healthy adult albino rats (\textit{Rattus norvegicus}) of (60 ± 2 days) age and weighing 175 ± 5 gm were used for the experiments, procured from Mhow, Bhopal (MP) India. The rats were acclimatized in laboratory conditions for two weeks and were maintained at 28 ± 2\textdegree{}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 \textit{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 *A. vera* were collected in and around the Bhopal. Preparation of *A. vera* (leaf gel) extracts was done according to the method of Arunkumar and Muthuselvam\[26\] 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 were fed with normal diet and water *ad libitum*, as control group.

**Group II:** This group of 10 (5+5) animals were fed with normal diet and aluminium sulphate 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. Liver and kidney were isolated and kept in ice cold conditions for experiment.

**Histopathological work**

Samples of Testis of rats both control and treated with aluminium sulphate *per se* treated and aluminium sulphate plus aloin treated rats were immediately excised, fixed in Bouins fixative followed by standard procedures of paraffin embedding and were sectioned at 5-6 microns. Sections were stained with haematoxylin and eosin and mounted with Canada balsam/DPX abd were examined by Olympus light microscope to evaluate histological changes. The experimental chemicals were obtained from Sigma Chemical Co. USA of analytical grade.

**RESULTS**

In the present investigation, histological analyses of testis were performed in albino rats subjected to 30 and 60 days durations of aluminium sulphate *per se* and aluminium sulphate
along with aloin administration. Photomicrographs were taken for histopathology of the testis tissue sections at 10X and 40X magnification.

**Histopathological changes in testis**

Microscopic examinations of the testis in the control group histological sections showed normally arranged seminiferous tubules (ST) with little connective tissue (CT) in the interstitial spaces containing Leydig cells (the cells responsible for testosterone production in the testes). Spermatogonia, spermatocytes and spermatids were regularly arranged with, spermatozoa in the lumen of seminiferous tubules (Photomicrography plate A & D).

Examination of sections of testis aluminium sulphate treated rats showed that testis had lost it’s characteristic architecture compared with the control group. There is marked distortion of seminiferous tubules with almost complete disintegration of connective tissue between them (See Photomicrography plate B & E).

In 30 days aluminium sulphate treated animals, disorganization of the spermatogonia to a small extent has been noticed. They have detached from basal lamina of seminiferous tubules causing their distortion and consequent loss of their lumen. Due to desquamation of germ cells, numerous small spaces have appeared inside majority of seminiferous tubules. In the interstitium considerable engorgement of the interstitial blood vessels are noticed.

Histological examination of 60 days treated animals reveal that the characteristic organized architecture of the cells in the seminiferous tubules got disturbed more than that observed in the 30 days treated rats (Photomicrography plate 4). Cells in seminiferous tubules showed nuclear fragmentation, nuclear pycnosis and cytoplasmic disintegration in the varied germ cells. The cytoplasm of spermatogonia, spermatocytes appear paler due to vacuolization.

Sertoli cells have lost their integrity and hence can be identified with difficulty. Interstitial cells show marked reduction in size with nuclear pycnosis. There is a pronounced reduction in sperm amount in the lumen of seminiferous tubules and many tubules are totally devoid of sperms.

In another set of experiment treatment group (aluminium sulphate + aloin), aluminium intoxication showed noticeable improvement in histopathological architecture of testis with significantly reduced toxicological impactions of aluminum. Also, observed there was reversal of the necrotic and atrophic changes of the testis, particularly the histological
structures of the seminiferous tubules and their cell cytoplasm, nuclei, nutritive cells, which were found to become near normal by treatment of aloin (See Photomicrography plate C & F).

**Photomicrograph plate**

Histopathology of the testicular tissue showing  
A. Control,  
B. Aluminium Sulphate *Per se* treated,  
C. Aluminium Sulphate + Aloin co-treated sections at 10X magnification and  
D. Control,  
E. Aluminium Sulphate *Per se* treated,  
F. Aluminium Sulphate + Aloin co-treated sections at 40X magnification.

**DISCUSSION**

In the present study, severe changes were observed in testis of both of the treated groups (aluminium sulphate treated *per se* and aluminium sulphate along with aloin) in comparison to control animals. When the dose was given to animals, moderate degenerative changes in the seminiferous tubules were observed which was more prominent in 60 days aluminium sulphate treated animals in comparison to control and also the 30 days treated animals

Similar to the findings of the presently under taken study with the ameliorating effects of aloin is well supported by Amir Mahdi Imani *et al* (2014), who have examined the efficacy of *Ocimum basilicum* extract, with antioxidant properties, against testicular toxicity induced
by lead. They had observed that lead could induce testicular apoptosis in the germ cells. While concomitant treatment of rats with aqueous basil extract led to an improvement in histological, morphometrical and immunohistochemical changes induced by lead. It’s clearly suggesting that plant extracts do have a positive role in counter effecting the hazardous effects of metals.[27]

Similar to our present work with aloin, Godspower et al (2015), very recently have evaluated the ameliorative potential of methanolic extract of Citrullus lanatus seeds on lead-acetate induced testicular toxicity in adult male albino rats. Their results showed seminiferous tubular derangement in lead-acetate treated rats, when compared with the non treated control rats. The methanolic extract of C. lanatus seed from their results showed a corrective effect as against the lead-acetate treated group in relative to the control group as seen in our study. In conclusion it has been mentioned that that methanolic extract of C. lanatus seed has ameliorative potentials very similar to that of aloin as observed presently to correct the deleterious effects of metal toxicity on male reproductive system. Thus, this study indicates that protective role of aloin treatment against the adverse effects of aluminium on testis of albino rats.[28]

The present results have revealed that aloin ameliorates the toxic effects of aluminium sulphate, where there was reversal of the necrotic and atrophic changes of the testis, particularly the histological structures of the seminiferous tubules and their cell cytoplasm, nuclei, nutritive cells, which were found to become near normal by treatment of aloin for more than 30 days of treatment with aloin i.e. up to 60 days for a significant amelioration of the toxic effects of aluminium by aloin. Thus, this study indicates that protective role of aloin treatment against the adverse effects of aluminium on testis of albino rats.

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REFERENCES
6. A.T. Proudfoot, Aluminium and zinc phosphide poisoning, Clinical Toxicology (Phila), 2009; 47: 89-100.


