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# Assessment of genotoxicity of aluminium acetate in bone marrow, male germ cells and fetal liver cells of Swiss albino mice

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ABSTRACT

Aluminium acetate (AA) has many pharmaceutical applications, which necessitates a thorough evaluation of its toxicity. Dose- and time-dependent genotoxic effects of AA were investigated in Swiss albino mice after exposure via intraperitoneal (i.p.) injection, by employing assays to detect chromosomal aberrations (CA) and micronuclei (MN) in bone marrow, MN in fetal liver, and abnormalities in sperm. Animals were treated with single doses of 50, 100 and 150 mg/kg body weight (bw), and with daily doses of 50 mg/kg bw for seven consecutive days, in order to study the effects of acute and cumulative doses, respectively. Post-treatment sampling was done at 24, 48 and 72 h for bone-marrow CA and MN tests, to study time-dependent effects. Both single and repeated exposures of AA induced chromosomal aberrations, which were dose and time-dependent. The MN test failed to demonstrate genotoxicity after the single-dose exposures, indicating that a higher threshold dose is required for MN induction. Repeated treatment of AA, however, induced MN formation even at the low dose ($P < 0.05$), reflecting genotoxicity following chronic/sub-chronic exposure. A significant reduction in mitotic index and in the P/N (polychromatic/normochromatic erythrocytes) ratio suggests that AA also has a mitodepressive effect in bone-marrow cells. AA-induced germinal genotoxicity was evident from a significant and dose-dependent increase in the percentage of abnormal spermatozoa and a reduction in sperm count. Transplacental exposure of AA resulted in the dose-dependent increase in the frequency of micronucleated erythrocytes in the developing fetus. Thus, the current in vivo study revealed genotoxic effects of AA both on somatic and germ cells of Swiss albino mice.



## 1. Introduction

Aluminium occurs naturally in the soil and makes up about 8% of the Earth's crust, where it constitutes the third most abundant element. Although it does not occur in the free state, aluminium is found in combination with other elements as aluminium oxide, aluminium silicate, aluminium borate, etc. Because of their distinctive physicochemical properties, aluminium compounds and their derivatives are used in the preparation of various commercial products, including therapeutic agents, water purifiers, and as food additives [1]. They are released into the environment both by natural processes and from anthropogenic sources, thus increasing the risk of consumer and occupational exposures [2]. Aluminium induces neurotoxicity in mammals and is a risk factor for Parkinson's and Alzheimer's diseases [3]. The mechanism of aluminium-induced neurotoxicity is not completely understood. However, aluminium forms a complex with the chromatin structure of neurons [4] and induces fragmentation of chromatin in cultured cortical neurons [5]. Obviously, these reports indicate that aluminium can have damaging effects on the genetic material. Aluminium derivatives or salts have been studied for their mutagenicity in bacterial systems [6], in vitro [7,8] in vivo models [9,10], and in plant cells [11]. Earlier studies indicated that aluminium has carcinogenic and mutagenic activities [6,12] and that aluminium salts possess xeno(metallo)-estrogenic properties [13]. Besides their endocrine-disrupting activity, many xeno-estrogens are known to induce genotoxic effects [14]. There are few reports on the exposure of aluminium compounds via the transplacental route [15,16], causing adverse effects on prenatal and postnatal development [2,15,17]. Aluminium-induced reproductive toxicity is a major concern since this metal has been reported to impart adverse effects on germ cells/tissues in mammals [18–20].

Aluminium acetate is a salt produced by the reaction of aluminium hydroxide and acetic acid. It is a general laboratory reagent

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used for many qualitative and quantitative chemical experiments. It is widely used as a drug for the treatment of certain diseases of bone, ear (*otitis externa*), infections in the outer ear canal, as an antiseptic, astringent and as a topical solution to treat severe rashes [21]. Although several reports on the genotoxicity of different derivatives/salts of aluminium are available, there is no report on aluminium acetate in particular. Using Swiss albino mice as the test system, we carried out an invivo study to assess the genotoxic effects of AA in somatic cells, in male germ cells, and during developmental stages after transplacental exposure.

## 2. Materials and methods

### 2.1. Chemicals

Aluminium acetate (AA; $C_6H_9AlO_6$) (basic) (CAS No. 142-03-0; Batch No. 1049), a white, light hygroscopic powder obtained from Rolex chemical industries, Mumbai, India, was used for the experiments. Cyclophosphamide (CP, CAS No. 6055-19-2; Batch No. G106) Endoxan, Asta Medica AG Germany, marketed by German Remedies Ltd., Ponda, India) was used as the positive control. Colchicine ($C_{22}H_{25}NO_6$; CAS No. 64-86-8; Batch No. T 823279) was purchased from Super Religare Laboratories (SRL) Ltd., Mumbai, and used as the mitotic arrestant for chromosome preparation. Hank's balanced salt solution (HBSS; Batch No. 11461.3) and fetal calf serum (FCS: Batch No. 1112) were procured from Hi-media Laboratories Pvt. Ltd., Mumbai, India.

### 2.2. Animals

Swiss albino mice (*Mus musculus*) were bred and maintained in the departmental animal house. Care and handling of the animals were in accordance with the guidelines of CPCSEA, India [22]. Animals were maintained in a good hygienic condition, at a temperature of $23 \pm 2\,^{\circ}C$ and on a 12-h light/dark cycle. Commercial food pellets (Amrutha feeds, Bangalore) and water were provided *ad libitum*. Five mice (8–10 weeks old; average body weight, $25 \pm 2$ g) were used in each experimental and control group.

### 2.3. Dose and treatment schedule

The oral LD50s of aluminium nitrate, chloride, and sulfate in mice and rats range between 200 and 1000 mg of aluminium/kg/bw [23]. However, no substantial report is available on the lethal dose of AA when given intraperitoneally to Swiss albino mice. Therefore, for selection of test doses, the median lethal dose (LD50) was determined by employing Log-probit analysis [24]. The analysis revealed an LD50 value of 1150 mg/kg bw at a post-exposure observation period of 24 h, after i.p. administration of AA. Sub-lethal doses of AA, i.e. 50, 100, 150 mg/kg bw were selected for the present genotoxicity assessment, based on previous reports on aluminium salts with special reference to their lowest-observed-adverse-effect-level (LOAEL) in animal studies. The LOAEL for aluminium lactate-induced neurotoxic effects is reported to be 160 mg/kg bw/day both in CD mice [25] and in Swiss-Webster mice [26]. According to the panel on safety of aluminium from dietary intake of the European Food-Safety Authority, the LOAELs for neurotoxicity, testicular toxicity, embryotoxicity, and effects on the developing nervous system are 52, 75, 100, and 50 mg aluminium/kg bw/day, respectively [27]. The LOELs for aluminium in a range of different dietary studies in mice, rats and dogs were in the range of 50–75 mg/kg bw/day [28]. As far as human exposure is concerned, the daily intake of aluminium through food and beverages ranges between 2.5 and 13 mg for adults. Drinking water may contribute 0.4 mg/day. Pulmonary exposure may contribute up to 0.04 mg/day. In some circumstances such as during occupational exposure and upon antacid use, more than 500 mg of aluminium may be consumed in two average-size antacid tablets [2].

Double-distilled water and cyclophosphamide (CP) (25 mg/kg bw for CA and MN test; 50 mg/kg bw for the MN test in fetal liver and the sperm-abnormality assay) were used as the vehicle and positive controls respectively. All were administered to experimental animals intraperitoneally in a volume of 0.2 ml.

To assess time-dependent effects, bone-marrow samplings were done at 24, 48 and 72 h after the treatment with the single doses. The cumulative-dose experiment was performed with a dose of 50 mg/kg bw, which was administered daily on seven consecutive days at 24-h time intervals. Bone marrow was sampled 24 h after the last administration of the test chemical.

### 2.4. Chromosomal aberration (CA) test

The experimental animals were injected i.p. with colchicine (2 mg/kg bw), 1.5 h prior to sacrifice to arrest the cells at the metaphase stage. Chromosomal preparations were prepared from the bone marrow according to the method described by Tjio and Whang [29]. Flame-dried slides were stained with buffered Giemsa (pH 6.8) and coded. The frequency of chromosomal aberrations in bone-marrow cells was determined by scoring 100 well-spread metaphases, all with 40 chromosomes ($2n = 40$) from each animal following standard criteria [30,31].

The percentage of total aberrations was calculated as follows: [cells with chromosomal aberration(s) ÷ total number of cells scored] × 100. For the mean value, the standard error of the mean (SEM) was applied for the data obtained from five animals. Comparison was done between cells with intact chromosomes and cells with chromosomal aberrations for statistical analysis. Mitotic indices (MI) were also determined by scoring the number of dividing cells in a total of 2000 bone-marrow cells/animal, and then calculated with the formula: MI = [number of mitotic cells ÷ total number of cells scored] × 100.

### 2.5. Micronucleus (MN) assay

The MN assay was performed according to the method described by Schmid [32], modified by Seetharam Rao et al. [33]. Bone-marrow smears were stained with May Gruenwald-Giemsa and scored for the presence of MN in polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs). Two thousand PCEs and NCEs in the corresponding field of observation were scored per animal. The ratio of PCE and NCE (P/N) was calculated for all treated and control groups.

### 2.6. Transplacental MN assay

The MN assay in fetal liver tissue was performed following the method of Cole et al. [34]. Eight-week-old virgin female mice were placed with a virgin male of the same age group, overnight. Successful mating was confirmed by the presence of a copulatory plug the following morning. The day of appearance of the vaginal plug was designated as gestation day zero. The pregnant mice were treated with the test agent on day 14 of gestation. Three pregnant females were included in each treated and control group. On day 15 of gestation, i.e. 24 h after the treatment with the test chemical, the animals were sacrificed and four fetuses from each animal were used. A total of 2000 PCE and NCE present in the same field of observation were scored from each fetus to determine the frequency of micronucleated erythrocytes and the P/N ratio.

### 2.7. Sperm-abnormality assay

The same three doses of AA as mentioned above were selected for the sperm-abnormality test. Eight-week-old virgin male mice ($n = 5$) were used in each experimental and control group. Post-treatment sampling was done after five weeks considering the duration required for spermatogenesis, and for sperm reaching the *cauda epididymis*. Sperm suspensions were made in phosphate-buffered saline (pH 7.2) from both *caudae* and stained with 1% eosin-Y. A total of 2000 sperm per animal were scored to determine the frequency of sperm abnormalities, according to the criteria of Wyrobek and Bruce [35], which include hookless, amorphous, banana-shaped, folded, double-headed and double-tailed. The frequency of abnormal sperm was expressed as percentage, calculated with the formula: [number of abnormal sperm ÷ total number of normal and abnormal sperm scored] × 100. In addition, testes weight and sperm count per epididymis [36] were determined in these animals.

### 2.8. Statistical analysis

Statistical analysis of the data was performed by means of one-way ANOVA with Dunnett's post hoc test for the chromosomal aberrations [37], and the paired *t*-test for MN, transplacental MN and sperm abnormalities [38]. A $P$-value $< 0.05$ was considered to correspond with statistical significance.

## 3. Results

### 3.1. Chromosomal aberration test

Results of the chromosomal aberration test are presented in Table 1. The single exposures to AA induced a significant increase in total aberrations at different time intervals compared with the vehicle control, except for the dose of 50 mg/kg bw at 72 h. There was a decrease in chromosomal aberrations with longer time after exposure. Cumulative dose treatment also induced aberrations at a significant level. The observed aberrations include gaps, breaks, exchanges, ring chromosomes, centric fusions, stickiness and pulverization. Compared with the vehicle control, there was a significant reduction in the mitotic index at higher doses in AA-treated groups ($P < 0.05$). In cumulative dose treatment, AA induced a higher frequency of cells with chromosomal aberrations ($P < 0.01$) compared with the vehicle control. There was a dose-dependent decrease in MI in treated groups (100 mg/kg bw at 24 h, and 150 mg/kg bw at 24 and 48 h) compared with the vehicle