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

Sr. Prem D'Souza[a], K.K. Vijayalaxmi[a,*], Prashantha Naik[b]

[a] Department of Applied Zoology, Mangalore University, Mangalagangothri 574 199, D.K., India
[b] Department of Post-Graduate Studies and Research in Biosciences, Mangalore University, Mangalagangothri 574 199, D.K., India

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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.

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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

* Corresponding author. Tel.: +91 9449228726.
E-mail address: kkv36@yahoo.co.in (K.K. Vijayalaxmi).

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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 ± 2 °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 ± 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

Table 1
Frequency of chromosomal aberrations induced by aluminium acetate in bone-marrow cells of Swiss albino mice and in controls.

Treatment dose (mg/kg bw)	Time	Mean MI ± SEM*	GS	Iso BS	BS	EXS	RS	FS	MA	CF	St & Pul.	Total** ± SEM
0	24	6.4 ± 0.16	1.4	–	0.4	–	0.6	–	–	–	1.4	3.80 ± 0.24
	48	6.2 ± 0.14	1.8	–	0.4	–	0.2	–	–	–	1.4	3.80 ± 0.24
	72	6.2 ± 0.17	1.4	–	0.6	–	0.2	0.2	–	–	1.0	3.40 ± 0.32
CP-25	24	5.0 ± 0.12[a]	7.9[b]	4.6[b]	13.5[c]	8.9[b]	3.7[a]	2.4[a]	6.8[a]	2.7[a]	3.0	50.10 ± 0.57[c]
	48	5.0 ± 0.09[a]	8.6[a]	6.0[b]	16.0[c]	7.8[b]	4.2[a]	2.2[a]	8.2[b]	2.6[a]	3.6[a]	48.60 ± 0.35[c]
	72	5.7 ± 0.11	9.6[b]	5.6[a]	16.0[c]	7.6[b]	3.6[a]	3.0[a]	8.2[b]	2.4[a]	5.6[a]	44.20 ± 0.56[c]
50	24	6.0 ± 0.15	2.8	–	1.0	0.6	0.8	–	–	0.8	4.8[a]	10.80 ± 0.12[a]
	48	6.2 ± 0.14	3.0[a]	0.2	1.6	0.4	0.2	–	–	1.0	4.6[a]	11.00 ± 0.14[a]
	72	6.2 ± 0.15	2.6[a]	0.2	0.8	0.4	1.0	–	–	0.6	3.2[a]	8.80 ± 0.13
100	24	5.0 ± 0.12[a]	3.0	0.2	1.4	1.2[a]	1.2	–	0.4	0.4	5.2[a]	14.00 ± 0.14[b]
	48	5.6 ± 0.08	3.0	0.6	0.8	1.4[a]	1.4	0.2	–	0.6	3.8[a]	11.80 ± 0.10[a]
	72	5.8 ± 0.13	2.4	0.2	0.8	1.2[a]	1.0	0.2	–	0.6	3.0[a]	9.40 ± 0.12[a]
150	24	5.2 ± 0.14[a]	4.2[a]	0.2	1.4	0.6	2.0[a]	0.4	0.2	1.2[a]	5.8[a]	16.00 ± 0.13[b]
	48	5.4 ± 0.12[a]	4.4[a]	0.2	1.0	0.2	1.0	0.2	0.2	0.6	4.6[a]	12.40 ± 0.06[a]
	72	5.8 ± 0.15	3.0[a]	0.4	0.4	0.4	0.8	0.6	0.2	0.6	3.4[a]	9.80 ± 0.15[a]
50 × 7	CD	5.5 ± 0.99[a]	4.8[a]	0.4	1.4	–	0.6	0.8	0.8	1.2[a]	5.2[a]	15.20 ± 0.17[b]

CD, cumulative dose; GS, gaps; BS, chromatid breaks; Iso BS, isochromatid breaks; EXS, exchanges; RS, rings; FS, fragments; MA, multiple aberrations; CF, centric fusion; St & Pul., stickiness and pulverization; CP, cyclophosphamide; SEM, standard error of the mean.

[a] $P<0.05$.

[b] $P<0.01$.

[c] $P<0.001$ (Dunnett's post hoc test).

* Mitotic index.

** From 2000 cells/animal; 5 animals/group.

control. Cyclophosphamide induced a statistically significant increase in aberrations and a reduction in the MI at different time intervals.

3.2. Bone-marrow micronucleus (MN) test

Table 2 presents the data obtained for the MN test. In the single-treatment groups, there was no statistically significant increase in MN frequency at any of the doses and time intervals, compared with that of the vehicle control. A significant increase ($P<0.05$) in micronucleated PCE compared with the vehicle control was noted in the cumulative treatment group. The P/N ratio was significantly reduced only at the highest single dose (150 mg/kg bw) and after the cumulative dose. The positive control cyclophosphamide induced a highly significant increase in MN frequency and a decrease in P/N ratio at all time intervals ($P<0.001$).

3.3. Transplacental MN test

The results obtained for transplacental MN test are presented in Table 3. AA induced a significant increase in the frequency of micronucleated erythrocytes in the fetal liver, in a dose-dependent manner. The P/N ratio was significantly reduced compared with the vehicle control ($P<0.001$). However, this reduction was not found to be dose-dependent.

3.4. Sperm-abnormality assay

AA induced a dose-dependent and statistically significant increase in the frequency of abnormal sperm when compared with the vehicle control (Table 4). There was a dose-dependent reduction in sperm count, which was significant at the medium (100 mg/kg bw) and high (150 mg/kg bw) doses, and body and testis weights were not significantly affected at any of the doses tested (Table 5). The positive control cyclophosphamide induced a significant increase in sperm abnormalities and a significant reduction in sperm count ($P<0.001$).

4. Discussion

Since AA has many consumer applications, particularly as a therapeutic agent, the evaluation of its genotoxic effect is practically relevant to arrive at a safer mode of exposure. In the present study, the doses selected were higher than human exposure levels. In recent years the use of aluminium has increased dramatically. Because of its corrosion resistance and low weight, aluminium is used widely as a building material, thereby increasing occupational exposure. In addition, because of its excellent heat conductivity, aluminium is used for the manufacture of various types of cooking utensils [6], which may also lead to increased human exposure.

In the present study, AA was evaluated for genotoxicity at doses up to 150 mg/kg bw as single exposures, and at 50 mg/kg bw/day for seven days as cumulative exposure. Epstein [39] advocated the need to test chemicals at dose levels higher than those anticipated in humans. While metabolic reactions in any mammalian test animal may differ from those in humans to a greater or lesser extent, the animal experiment has the important advantage that test substances can be applied in much higher doses than are therapeutically safe in humans [40]. Moreover, testing at higher dose may take into account the possible accidental exposure or accumulation of agents/drug during long-term exposure/therapy. The results of the CA test indicate that AA induces dose- and time-dependent genotoxic effects in bone-marrow cells. This observation is in agreement with earlier studies [41–44], where other types of aluminium salt were shown to induce chromosomal aberrations. In the present study, AA-induced aberrations included gaps, breaks, exchanges, centric fusions, rings, and stickiness and pulverization. Aberrations such as breaks, exchanges, centric fusions and rings represent the typical clastogenic effects of test agents. Aluminium compounds induce the formation of DNA adducts [45], which is a basis for clastogenicity due to conformational distortions that inhibit proper DNA replication [46]. Earlier studies have demonstrated that aluminium can disrupt the chromatin structure [47,48]. It is well known that reactive oxygen species (ROS) generated from the metabolism of xenobiotics induce DNA/chromosomal damages. Anane and Creppy [49] and Vota et al. [50] have shown that aluminium induces the production of ROS in animals.

Table 2
Results of the bone-marrow micronucleus (MN) test after i.p. injection of aluminium acetate in Swiss albino mice.

Treatment/dose (mg/kg bw)	Time (h)	% MN PCE* ± SEM	%MN NCE ± SEM	P/N ratio ± SEM
0	24	0.19 ± 0.00	0.09 ± 0.00	1.00 ± 0.00
	48	0.24 ± 0.00	0.16 ± 0.00	1.00 ± 0.00
	72	0.20 ± 0.00	0.14 ± 0.00	1.00 ± 0.00
CP-25	24	1.44 ± 0.07c	0.21 ± 0.03[a]	0.88 ± 0.02
	48	0.91 ± 0.05c	0.33 ± 0.08[a]	0.81 ± 0.01[a]
	72	0.95 ± 0.07c	0.63 ± 0.08[c]	0.76 ± 0.01[a]
50	24	0.12 ± 0.04	0.14 ± 0.02	1.08 ± 0.01
	48	0.08 ± 0.01	0.09 ± 0.03	1.14 ± 0.04
	72	0.06 ± 0.03	0.07 ± 0.03	0.95 ± 0.02
100	24	0.20 ± 0.04	0.03 ± 0.03	0.91 ± 0.02
	48	0.18 ± 0.03	0.04 ± 0.04	0.87 ± 0.03
	72	0.09 ± 0.03	0.04 ± 0.02	0.86 ± 0.01
150	24	0.18 ± 0.04	0.12 ± 0.02	0.83 ± 0.05[a]
	48	0.16 ± 0.02	0.13 ± 0.01	0.82 ± 0.01[a]
	72	0.14 ± 0.03	0.06 ± 0.02	0.86 ± 0.04
50 × 7 CD	24	0.34 ± 0.06[b]	0.15 ± 0.07	0.82 ± 0.06[a]

CD, cumulative dose; PCE, polychromatic erythrocytes; NCE, normochromatic erythrocytes; P/N, PCE/NCE ratio.
[a] $P<0.05$.
[b] $P<0.01$.
[c] $P<0.001$ (paired t-test).
* From 2000 PCE and corresponding NCE/animal; 5 animals/group.

Table 3
Frequency of micronucleated erythrocytes (MN-E) induced by aluminium acetate in fetal liver after 24 h of transplacental exposure.

Treatment/dose (mg/kg bw)	% PCE	% NCE	% MN-E ± SEM	P/N ratio ± SEM
0	93.26	6.74	0.12 ± 0.02	13.84 ± 0.46
CP-50	82.17	17.83	4.14 ± 0.07[c]	4.61 ± 0.13[c]
50	89.29	14.71	0.22 ± 0.04[a]	6.07 ± 0.26[c]
100	87.05	12.95	0.34 ± 0.05[b]	6.72 ± 0.01[c]
150	85.44	10.56	0.48 ± 0.04[c]	8.09 ± 0.11[c]

Three pregnant females/group, 500 PCE/fetus and 4 fetuses/animal. PCE, POLYCHROMATIC erythrocytes; NCE, normochromatic erythrocytes; MN-E, micronucleated erythrocytes; P/N, PCE/NCE ratio.
[a] $P<0.05$.
[b] $P<0.01$.
[c] $P<0.001$ (paired t-test).

Table 4
Frequency of different types of abnormal sperm induced in Swiss albino mice after five weeks of treatment with aluminium acetate.*

Treatment/dose (mg/kg bw)	A	B	H	F	DH	DT	Total Abnormal	% Abnormal sperms ± SEM
0	33	08	31	26	02	07	107	1.07 ± 0.26
CP-50	182	43	149	100	12	25	511	5.11 ± 0.59[c]
50	89	28	46	36	06	12	217	2.17 ± 0.31[a]
100	115	62	48	57	05	25	312	3.12 ± 0.76[b]
150	121	52	54	79	22	23	351	3.51 ± 0.81[b]

A, amorphous; B, banana shaped; H, hookless, F, folded; DH, double headed; DT, double tailed.
[a] $P<0.05$.
[b] $P<0.01$.
[c] $P<0.001$ (paired t-test).
* 2000 sperm/animal; 5 animals/group.

Table 5
*Effect of aluminium acetate on body weight, testis weight and sperm count.

Treatment/dose (mg/kg bw)	Initial body wt. (g)	Sacrifice body wt. (g)	Testis wt. g ± SEM	Sperm count/epididymis $\times 10^6$ ± SEM
0	27.00	32.20	0.26 ± 0.01	8.70 ± 0.52
CP-50	24.00	27.20	0.19 ± 0.03[b]	5.52 ± 0.24[b]
50	29.24	30.30	0.26 ± 0.02	8.20 ± 0.33
100	25.18	27.08	0.24 ± 0.03	6.47 ± 0.34[a]
150	24.44	28.60	0.25 ± 0.01	6.39 ± 0.51[a]

[a] $P<0.05$.
[b] $P<0.01$(paired t-test).
* Five animals/group.

Further, aluminium has been reported to enhance the permeability of the lysosomal membrane [51] and to inhibit the lysosomal proton pump [52]. Both these actions result in release of DNAse, whose passage into the nucleus leads to DNA fragmentation. Aly et al. [53] demonstrated that DNase introduced into the cytoplasm by electroporation results in severe damage to the genetic material. Thus, the observed clastogenic effect of AA may be due to any one of the above-mentioned mechanisms, or to a combination of these.

The major type of AA-induced chromosomal abnormality as noted in the present study is stickiness. According to Heddle [54], the first chromosomal abnormality seen at mitosis is chromosome stickiness, other aberrations occur only later, but the reason for this is not known. Although, Savage [30] considered chromosomal stickiness as an unconventional aberration, he suggested that an increase in its frequency after chemical treatment clearly indicates that it is produced due to the effect of the test agents. The AA-induced stickiness observed in the present study is in agreement with earlier reports of Manna and Das [41] and Roy et al. [43] who also demonstrated the induction of chromosome stickiness and bizarre configuration in bone-marrow cells of mice and rats. Several mechanisms have been hypothesized for chemical-induced stickiness [55–57]. McGill et al. [58] analyzed mitotic cells that showed chromosome stickiness by means of an electron microscope, which revealed the inter-chromosomal and inter-chromatid connections both in metaphase and anaphase. From these studies the authors gave a mechanistic explanation, which states that, 'When the chromosome fibers fail to condense properly in the preparation of mitosis, they may be trapped and tangled with the fibers of other chromosomes, thus becoming physically connected. Such abnormal sub-chromatid connections cause many chromosomes to adhere to one another resulting in stickiness'. Extreme stickiness leads to clumping and disintegration of chromosomes resulting in cell death [59] or reduced mitotic division [60].

Concerning time-dependent effects, there was a decrease in the frequency of cells with chromosomal aberrations with increasing time interval after exposure. It can be hypothesized that if the exposure time increases, the damaged cells may be eliminated by cell death, hence the decrease in chromosomal aberrations at later time points after exposure. Fulda et al. [61] emphasized on how the cell-death programs are activated to eliminate the damaged cells from the organism if cellular defense mechanisms and pro-survival strategies fail during the course of time. Another possible reason is that AA or its metabolites may not be present in the animal at later time intervals following the treatment to cause genetic damage. The half-life of aluminium has been studied in various animals like rat, rabbit and dog, which ranges from 80 to 280 min, indicating the fast clearance from the body [6].

MN formation is a biomarker for genotoxicity manifested by two mechanisms, i.e. clastogenic and spindle-damaging actions. Surprisingly, in the present study, the MN test did not reveal a clastogenic and/or spindle-damaging effect of AA at any of the single-dose exposures, although in the CA test, it induced clastogenicity in terms of gaps, breaks, exchanges, centric fusions and rings. These differential results may be due to the sensitivity of the endpoints and/or the threshold dose of AA required for the induction of a positive response in a particular endpoint. In the CA test, all types of structural aberration are observed, while in the MN test only lagging chromosomes and acentric fragments are shown as MN [14]. Induction of MN after repeated exposure indicated the cumulative effect of AA. Maier and Schmid [62] justified the usefulness of multiple-treatment schedules in the MN test; some chemicals show a positive response only after repeated exposures rather than after a single exposure. It has been demonstrated that certain agents are more sensitive to induce MN in multiple treatments than after a single exposure [63], as revealed from the present cumulative data. The current observation of induction of genotoxicity after repeated exposure even at the lower dose, but no effect in single/acute exposure even at the higher doses of the same agent alerts about the higher risk of prolonged exposure to chemicals.

The ratio between the number of cells in mitosis and the total number of non-dividing cells represented as MI is a measure of the proliferation status of a cell population. A decrease of the ratio of PCE to NCE (P/N) in the micronucleus test is an indicator of bone-marrow toxicity [64]. In the present study, AA-induced toxic effect on cell proliferation in bone marrow was confirmed by the significant reduction in MI and P/N ratio in CA and MN tests, respectively. This mito-depressive effect was observed only at the higher doses of AA, i.e., 100 and 150 mg/kg bw. At the lower dose (50 mg/kg bw) there was no such effect at any time interval. However, a multiple exposure to AA at a lower dose induced a mito-depressive effect as indicated by the significant reduction in MI and P/N ratio. These observations coincide with the earlier studies reporting the cytotoxic effect of aluminium compounds in different cell types [7,43,50,11]. As specified earlier, aluminium produces ROS upon bio-transformation, and one of the toxic effects of ROS is cytotoxicity/mitodepression [65]. Further, it is well known that cytotoxicity is a direct consequence of DNA/chromosomal damage.

Mutation during the prenatal period poses a hazard as it may stimulate important neoplastic alterations, and/or induce manifestation of other diseases, in postnatal life [66,67]. Furthermore, there is a link between mutagenicity and teratogenicity [68,69]. Considering some reports on transplacental exposure of the fetus to aluminium [70,71], it is appropriate to evaluate its possible genotoxicity during the developmental stage. The transplacental micronucleus assay developed by Cole et al. [34] is a recommended method for assessment of genotoxicity during prenatal life. In the present study, a significant dose-dependent increase in the frequency of micronucleated fetal erythrocytes indicated the transplacental genotoxic potency of AA. This observation is in line with earlier studies where some aluminium compounds displayed genotoxicity [9,72,73], including the induction of MN in the fetus [41,42,46]. It is interesting to note that in adult animals AA did not induce the formation of MN in bone-marrow erythroblasts even at high dose, but it induced MN in fetal erythroblasts at a significant level, even at low dose. This is indicative of the high sensitivity of fetal cells to the genotoxicity of AA. Similar results were obtained with certain other agents that induced a higher frequency of MN in fetal cells, but no or a relatively low frequency in cells of adults [74,75]. According to Cole et al. [74], since fetal liver possesses a greater metabolic potential than adult bone marrow, the transplacental tests respond to genotoxic agents not detected by the adult bone-marrow system. This type of result exemplifies age-related chemosensitivity [75].

A report of the US Environmental Protection Agency Gene-Tox Program based on the review of the literature proposes that the mouse sperm morphology test is highly sensitive to germ-cell mutagens: 100% of the known mutagens were correctly identified as positive in the sperm morphology test [76]. AA induced dose-dependent damaging effects in male germ cells, which are manifested in the form of an increased frequency of abnormal sperm and a decreased sperm count. The positive response obtained in the present study is comparable with the reports of Llobet et al. [19] and Guo et al. [77], who also demonstrated the adverse effects of aluminium on the male reproductive system in the mouse. Oxidative stress induced by xenobiotics is a major cause of sperm abnormality [78]. It is known that free radicals/oxidative stress induce DNA damage in meiotic chromosomes [79] and also interfere with the differentiation process during spermatogenesis [80]; both mechanisms are involved in the production of abnormal sperm. AA-induced oxidative stress mediated by free radicals has been demonstrated in brain cells of albino mice [81].

Intraperitoneal administration of AA induced lipid peroxidation and changes in oxidative stress enzymes in different tissues, including testis in albino mice [82]. A similar mechanism may be a plausible explanation for the induction of the testicular toxicity observed. Among the abnormal sperm, the frequency of head abnormalities was found to be high. The Y-chromosome plays an important role in determining the shape of sperm heads, and any damage here may lead to malformations of the sperm head [83,84]. However, Y-chromosome specific damage induced by aluminium and/or its salts has not been reported. Further studies on this particular aspect will give insight into the link between Y-chromosome damage and AA-induced sperm head abnormalities. Although AA did not show any effect on testis weight, its cytotoxic effect was clear in terms of a significant reduction in sperm count, with the minimum threshold-dose required for the effect being 100 mg/kg bw. Other forms of aluminium salts also induced a reduction in epididymal sperm count in mice [19] and in Wistar rats [85]. Thus, from our observation and from earlier reports it can be deduced that aluminium salts and/or their metabolic products interfere with the production or maturation or storage of sperm in the epididymis.

From the current study it can be concluded that despite the failure of AA to induce MN after a single exposure, its genotoxic effect in adult mice is evident from chromosomal and sperm parameters. Induction of MN in fetal erythrocytes suggests that fetal tissues are more sensitive than those of adults to the toxic effects of AA. Under the test conditions of this study, AA at doses ≥50 mg/kg bw was genotoxic in mice. Further studies should be conducted to identify a safe dose level for human exposure. AA was also found to have a mitodepressive/cytotoxic activity in somatic and germ cells. These toxic responses alert us for the health hazards from overexposure and/or chronic/sub-chronic exposure to aluminium salts, with special reference to AA. Therefore, care should be taken to ensure a safe use of these compounds.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

[1] NTP (National Toxicological Program). Technical Report on the Carcinogenesis Aluminium. Tech. Rept. No. TR-215, NTP-80-35, NIH Publ. No. 82-1771, 2005.
[2] EHC, 194 (Environmental Health Criteria, 194), Aluminium, International Program on Chemical Safety, 1997.
[3] J. Campdelacreu, Parkinson disease and Alzheimer disease: environmental risk factors, Neurologia (2012) (in press).
[4] G.Y. Wen, H.M. Wisniewski, Histochemical localization of aluminum in the rabbit CNS, Acta Neurophathol. 68 (1985) 175–184.
[5] H.J. Fu, Q.S. Hu, Z.N. Lin, T.L. Ren, H. Song, C.K. Cai, S.Z. Dong, Aluminum induced apoptosis in cultured cortical neurons and its effect on SAPK/JNK signal transduction pathway, Brain Res. 1 (2003) 11–23.
[6] A. Leonard, G.B. Gerber, Mutagenicity, carcinogenicity and teratogenicity of aluminium, Mutat. Res. 196 (1988) 247–257.
[7] A. Banasik, A. Lankoff, A. Piskulak, K. Adamowska, H. Lisowska, A. Wojcik, Aluminium-induced micronuclei and apoptosis in human peripheral blood lymphocytes treated during different phases of the cell cycle, Environ. Toxicol. 20 (2005) 402–406.
[8] P.D.L. Lima, D.S. Leite, M.C. Vasconcellos, B.C. Cavalcanti, R.A. Santos, L.V. Costa-Lotufo, C. Pessoa, M.O. Moraes, R.R. Burbano, Genotoxic effects of aluminium chloride in cultured human lymphocytes treated in different phases of cell cycle, Food Chem. Toxicol. 45 (2007) 1154–1159.
[9] A. Balasubramanyam, N. Sailaja, M. Mahboob, M.F. Rahman, S. Misra, S.M. Hussain, P. Grover, Evaluation of genotoxic effects of oral exposure to aluminum oxide nanomaterials in rat bone marrow, Mutat. Res. 676 (2009) 41–47.
[10] F. Geyikoglu, H. Turkez, T.O. Bakir, M. Cicek, The genotoxic, hepatotoxic, nephrotoxic, haematotoxic and histopathological effects in rats after aluminium chronic intoxication, Toxicol. Ind. Health 29 (2013) 780–791.
[11] B. Chakravarty, S. Srivastava, Toxicity of some heavy metals in vivo and in vitro in *Helianthus annuus*, Mutat. Res. 283 (1992) 287–294.
[12] A. Leonard, E.D. Leonard, Mutagenic and carcinogenic potential of aluminium and aluminium compounds, Toxicol. Environ. Chem. 23 (1989) 27–31.
[13] P.D. Darbre, Aluminium, antiperspirants and breast cancer, J. Inorg. Biochem. 99 (2005) 1912–1919.
[14] P. Naik, K.K. Vijayalaxmi, Effects of xenoestrogens on animals and human health, in: Genotoxicity of Xenoestrogens and Genoprotection by Ascorbic Acid, LAP Lambert Academic Publishing, Germany, 2012, pp. 1–49.
[15] Z. Gonda, K. Lehotzky, A. Miklósi, Neurotoxicity induced by prenatal aluminum exposure in rats, Neurotoxicology 17 (1996) 459–469.
[16] R. Anane, M. Bonini, E.E. Creppy, Transplacental passage of aluminum from pregnant mice to fetus organs after maternal transcutaneous exposure, Hum. Exp. Toxicol. 16 (1997) 501–504.
[17] J. Borak, J.P. Wise Sr., Does aluminum exposure of pregnant animals led to accumulation in mothers or their offspring? Teratology 57 (1998) 127–139.
[18] J.L. Domingo, Reproductive and developmental toxicity of aluminum: a review, Neurotoxicol. Teratol. 17 (1995) 515–521.
[19] J.M. Llobet, M.T. Colomina, J.J. Sirvent, J.L. Domingo, J. Corbella, Reproductive toxicology of aluminum in male mice, Fundam. Appl. Toxicol. 25 (1995) 45–51.
[20] R. Kutlubay, E.O. Oğuz, B. Can, M.C. Güven, Z. Sinik, O.L. Tuncay, Vitamin E protection from testicular damage caused by intraperitoneal aluminium, Int. J. Toxicol. 26 (2007) 297–306.
[21] Medicine Net. "Acetic acid/aluminum acetate-otic (Domeboro) Side Effects, Medical Uses, and Drug Interactions" MedicineNet. N.p., n.d. Web. 08 October 2012. http://en.wikipedia.org/wiki/Aluminium_acetate#cite_ref-2
[22] CPCSEA, India CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals), India, http://envfor.nic.in/divisions/awd/cpcsea laboratory.pdf.
[23] WHO, Aluminium, World Health Organization, International Programme on Chemical Safety, Geneva, 1997 (Environmental Health Criteria 194).
[24] R.A. Fisher, F. Yates, Statistical Tables for Biological, Agricultural, and Medical Research, 6th ed., Hafner, New York, 1963.
[25] P. Zatta, T. Kiss, M. Suwalsky, G. Berthon, Aluminium (III) as a promoter of cellular oxidation, Coord. Chem. Rev. 228 (2002) 271–284.
[26] M.S. Golub, C.L. Keen, M.E. Gershwin, Neurodevelopmental effect of aluminum in mice: fostering studies, Neurotoxicol. Teratol. 14 (1992) 177–182.
[27] EFSA, Safety of aluminium from dietary intake: scientific opinion of the panel on food additives, flavourings, processing aids and food contact materials (AFC), EFSA J. 754 (2008) 1–34.
[28] WHO, WHO Technical Report Series, Evaluation of Certain Food Additives and Contaminants – Sixty-seventh Report of the Joint FAO/WHO Expert Committee on Food Additives, 2010, pp. 33–40, http://www.mhlw.go.jp/stf/shingi/2r98520000034tf3-att/2r98520000034tms_2.pdf.
[29] J.H. Tjio, J. Whang, Direct chromosome preparation of bone marrow cells, Stain Tech. 37 (1962) 17–20.
[30] J.R. Savage, Classification and relationships of induced structural changes, J. Med. Genet. 12 (1975) 103–122.
[31] I.D. Adler, Cytogenetic tests in mammals, in: S. Venitt, J.M. Parry (Eds.), Mutagenicity Testing – A Practical Approach, IRL Press Ltd., Oxford, England, 1984, pp. 275–306.
[32] W. Schmid, The micronucleus test, Mutat. Res. 31 (1975) 9–15.
[33] K.P. Seetharam Rao, M.A. Rahiman, S.P. Koranne, Bovine albumin as a substitute for fetal calf serum in the micronucleus test, in: Int. Symp. on Recent Trends in Med. Genet., Madras, 1983.
[34] R.J. Cole, N.A. Taylor, J. Cole, C.F. Arlett, Transplacental effects of chemical mutagens detected by the micronucleus test, Nature 277 (1979) 317–318.
[35] A.J. Wyrobek, W.R. Bruce, Chemical induction of sperm abnormalities in mice, Proc. Natl. Acad. Sci. U. S. A. 72 (1975) 4425–4429.
[36] S.G. Vega, P. Guzman, L. Garcia, J. Espinosa, C.C. de Nava, Sperm shape abnormality and urine mutagenicity in mice treated with niclosamide, Mutat. Res. 204 (1988) 269–276.
[37] C.W. Dunnett, New tables for multiple comparisons with a control, Biometrics 20 (1964) 482–491.
[38] T.H. Hassard, Understanding Biostatistics, Mosby Year Book, St. Louis, 1991.
[39] S.S. Epstein, Use of the dominant-lethal test to detect genetic activity of environmental chemicals, Environ. Health Perspect. 6 (1973) 23–26.
[40] W. Schmid, G.R. Staiger, Chromsome studies on bone marrow from Chinese hamsters treated with benozdiazipine tranquilisers and cyclophosphomide, Mutat. Res. 7 (1969) 99–100.
[41] G.K. Manna, R.K. Das, Chromosome aberrations in mice induced by aluminium chloride, Nucleus 15 (1972) 180–186.
[42] A.K. Roy, G. Talukdar, A. Sharma, Effects of aluminium sulphate on human leukocyte chromosomes in vitro, Mutat. Res. 244 (1990) 179–183.
[43] A.K. Roy, A. Sharma, G. Talukdar, Effects of aluminium salts on bone marrow chromosomes in rats in vivo, Cytobios 66 (1991) 105–111.
[44] H. Dhir, A.K. Roy, A. Sharma, G. Talukdar, Modification of clastogenicity of lead and aluminium in mouse bone marrow cells by dietary ingestion of *Phyllanthus emblica* fruit extract, Mutat. Res. 241 (1990) 305–312.
[45] A. Wedrychowski, W.N. Schmidt, L.S. Hnilica, The in vivo cross-linking of proteins and DNA by heavy metals, J. Biol. Chem. 261 (1986) 3370–3376.
[46] H. Bartsch, DNA adducts in human carcinogenesis: Etiological relevance and structure–activity relationship, Mutat. Res. 340 (1996) 67–79.

[47] P.R. Walker, J. LeBlanc, M. Sikorska, Effects of aluminum and other cations on the structure of brain and liver chromatin, Biochemisty 28 (1989) 3911–3915.
[48] Bharathi, K.S. Jagannatha, R. Stein, First evidence on induced topological changes in supercoiled DNA by an aluminium D-aspartate complex, J. Biol. Inorg. Chem. 8 (2003) 823–830.
[49] R. Anane, E.E. Creppy, Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase, catalase and vitamins E and C, Hum. Exp. Toxicol. 20 (2001) 477–481.
[50] D.M. Vota, R.L. Crisp, A.B. Nesse, D.C. Vittori, Oxidative stress due to aluminum exposure induces eryptosis which is prevented by erythropoietin, J. Cell Biochem. 113 (2012) 1581–1589.
[51] G.B. van der Voet, A.E. Brandsma, E. Heijink, F.A. de Wolff, Accumulation of aluminium in rat liver: association with constituents of the cytosol, Pharmacol. Toxicol. 70 (1992) 173–176.
[52] P. Zatta, M. Ibn-Lkhayat-Idrissi, P. Zambenedetti, M. Kilyen, T. Kiss, In vivo and in vitro effects of aluminum on the activity of mouse brain acetylcholinesterase, Brain Res. Bull. 59 (2002) 41–45.
[53] M.S. Aly, A. Wojcik, C. Schunck, G. Obe, Correlation of chromosomal aberrations and sister chromatid exchanges in individual CHO cells pre-labelled with BrdU and treated with DNaseI or X-rays, Int. J. Rad. Biol. 78 (2002) 1037–1044.
[54] J.A. Heddle, Revelling in cytogenetics, Environ. Mol. Mutagen. 23 (1994) 35–38.
[55] M.E. Gaulden, Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomeraseII and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations, Mutagenesis 2 (1987) 357–365.
[56] S.K. Shukla, V.P. Singh, Chemical mutagen induced chromosomal stickiness in *Lens culinaris* (Medik), Plant Arch. 11 (2011) 49–53.
[57] G. Renault, C. Malvy, W. Venegas, A.K. Larsen, *In vivo* exposure to four ellipticine derivatives with topoisomerase inhibitory activity results in chromosome clumping and sister chromatid exchange in murine bone marrow cells, Toxicol. Appl. Pharmacol. 89 (1987) 281–286.
[58] M. McGill, S. Pathak, T.C. Hsu, Effects of ethidium bromide on mitosis and chromosomes: a possible material basis for chromosome stickiness, Chromosoma 47 (1974) 157–166.
[59] A.B. Mendes-Bonato, M.S. Pagliarini, C.B. Valle, M.I.O. Penteado, A severe case of chromosome stickiness in pollen mother cells of *Brachiaria brizantha* (Hochst) Stapf (Gramineae), Cytologia 66 (2001) 287–291.
[60] D.C. Curle, M. Ray, T.V. Persaud, Methylmercury toxicity: in vivo evaluation of teratogenesis and cytogenetic changes, Anat. Anz. 153 (1983) 69–82.
[61] S. Fulda, A.M. Gorman, O. Hori, A. Samali, Cellular stress responses: cell survival and cell death, Int. J. Cell Biol. 2010 (2010) 1–23.
[62] P. Maier, W. Schmid, Ten model mutagens evaluated by the micronucleus test, Mutat. Res. 40 (1976) 325–337.
[63] K.I. Yamamoto, Y. Kikuchi, Studies on micronuclei time response and on the effects of multiple treatments of mutagens on induction of micronuclei, Mutat. Res. 90 (1981) 163–173.
[64] Y. Suzuki, Y. Nagae, J. Li, H. Sakaba, K. Mozawa, A. Takahashi, H. Shimizu, The micronucleus test and erythropoiesis. Effects of erythropoietin and a mutagen on the ratio of polychromatic to normochromatic erythrocytes (P/N ratio), Mutagenesis 4 (1989) 420–424.
[65] N. Sasaki, N. Baba, M. Matsuo, Cytotoxicity of reactive oxygen species and related agents toward undifferentiated and differentiated rat pheochromocytoma PC12 cells, Biol. Pharm. Bull. 24 (2001) 515–519.
[66] J.A. Swenberg, A. Koestner, W. Wechsler, R.H. Denlinger, Quantitative aspects of transplacental tumor induction with ethylnitrosourea in rats, Cancer Res. 32 (1972) 2656–2660.
[67] L.A. Rollins, S. Leone-Kabler, M.G. O'Sullivan, M.S. Miller, Role of tumor suppressor genes in transplacental lung carcinogenesis, Mol. Carcinog. 21 (1998) 177–184.
[68] T. Nomura, Transgenerational effects from exposure to environmental toxic substances, Mutat. Res. 659 (2008) 185–193.
[69] W.X. Du, Y.L. Wang, Y.H. Sun, F. Xie, Z.Q. Chang, Y.F. Xing, Y.W. Zhang, Study on mutagenicity and teratogenicity of ammonium dinitramide, Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 29 (2011) 843–845.
[70] S. Yumoto, H. Nagai, H. Matsuzaki, T. Kobayashi, W. Tada, Y. Ohki, S. Kakimi, K. Kobayashi, Transplacental passage of Al from pregnant rats to fetuses and Al transfer through maternal milk to suckling rats, Nucl. Instrum. Methods Phys. Res. B 172 (2000) 925–929.
[71] D. Krewski, R.A. Yokel, E. Nieboer, D. Borchelt, J. Cohen, J. Harry, S. Kacew, J. Lindsay, A.M. Mahfouz, V. Rondeau, Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide – review, J. Toxicol. Environ. Health B: Crit. Rev. 1 (2007) 1–269.
[72] K. Nashed, Preparation of peritoneal cell metaphases of rats, mice and Chinese hamsters after mitogenic stimulation with magnesium sulphate and/or aluminium hydroxide, Mutat. Res. 30 (1975) 407–416.
[73] R.K. Bhamra, M. Costa, Trace elements aluminum, arsenic, cadmium, mercury and nickel, in: M. Lippmann (Ed.), Environmental Toxicants – Human Exposure and their Health Effects, VanNostrand Reinhold, New York, 1992, pp. 575–632.
[74] R.J. Cole, J. Cole, L. Henderson, N.A. Taylor, C.F. Arlett, T. Regan, Short-term tests for transplacentally active carcinogens. A comparison of sister-chromatid exchange and the micronucleus test in mouse fetal liver erythroblasts, Mutat. Res. 113 (1983) 61–75.
[75] A. Fucic, D. Markovic, Z. Herceg, M. Gamulin, J. Katic, R. Stojkovic, Z. Ferencic, B. Mildner, A.M. Jazbec, T. Dobranic, Developmental and transplacental genotoxicology: fluconazole, Mutat. Res. 657 (2008) 43–47.
[76] A.J. Wyrobek, L.A. Gordon, J.G. Burkhart, M.W. Francis, R.W. Kapp Jr., G. Letz, H.V. Malling, J.C. Topham, M.D. Whorton, An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals. A report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutat. Res. 115 (1983) 1–72.
[77] C.H. Guo, Y.F. Lu, G.S. Hsu, The influence of aluminum exposure on male reproduction and offspring in mice, Environ. Toxicol. Pharmacol. 20 (2005) 135–141.
[78] R.J. Aitken, M.A. Baker, D. Sawyer, Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease, Reprod. Biomed. Online 7 (2003) 65–70.
[79] R.J. Aitken, A.J. Koppers, Apoptosis and DNA damage in human spermatozoa, Asian J. Androl. 13 (2011) 36–42.
[80] A.M. Shostia, Role of reactive oxygen species in regulation of spermatogenesis and fertilization in mammals, Ukr. Biokhim. Zh. 81 (2009) 14–22.
[81] N.J. Sushma, U. Sivaiah, N.J. Suraj, K.J. Rao, Aluminium acetate induced oxidative stress in brain of albino mice, J. Pharm. Toxicol. 1 (2006) 579–584.
[82] N.J. Sushma, U. Sivaiah, N.J. Suraj, S. Kishore, K.J. Rao, Lipid peroxidation and some oxidative stress enzymes in aluminium acetate intoxicated albino mice, Toxicol. Int. 16 (2009) 1–4, http://www.toxicologyinternational.com/article.asp?issn=0971-6580;year=2009;volume=16;issue=1;spage=1;epage=4;aulast=Sushma.
[83] H. Krzanowska, Inheritance of sperm head abnormality types in mice and the role of the Y chromosome, Genet. Res. 28 (1976) 189–198.
[84] J. Styrna, H.T. Imai, K. Moriwaki, An increased level of sperm abnormalities in mice with a partial deletion of the Y chromosome, Genet. Rev. Camb. 57 (1991) 195–199.
[85] A.A. Buraimoh, S.A. Ojo, J.O. Hambolu, S.S. Adebisi, Effects of aluminium chloride exposure on the sperm count of adult male Wistar rats, Asian J. Exp. Biol. Sci. 3 (2012) 439–442.